Problems and prospects in the theory of gel electrophoresis of DNA

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I. INTRODUCTION

Electrophoresis of DNA through gels of agarose or polyacrylamide (PA) has been one of the most widely used techniques of molecular biology during the past decade, serving both analytical and preparative purposes. The molecular theory of this process has been developing slowly over the same period of time as the result of the efforts of a small but expanding group of people. Initially simple, the theory has grown in ways that no one anticipated at the beginning, partly in response to unexpected experimental discoveries. In this review we describe its current state, including both solved and unsolved problems.

For the theorist interested in polymer-chain dynamics, gel electrophoresis of DNA presents problems that are in some ways simpler but in others more challenging than those of the more familiar fields of polymer physics. In electrophoresis attention is focused on only one moving probe chain surrounded by the anchored chains of the gel; this simplifies the problem. Complicating factors are: The energies resulting from the experimental electric fields range from

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List of Abbreviations: bp, base pair(s); kbp, 1000 base pair(s); PA, polyacrylamide.



Fig. 1. Electrophoretic mobility of double-stranded DNA in 1% agarose gel as a function of DNA length and for different electric fields. General form of the graphs from McDonell *et al.* (1977), and data points from Hervet & Bean (1987). Field strengths in V/cm, from top down, 0.8, 0.5, 0.3, 0.15, 0.05.

small to large in comparison to $k_B T$. The electric fields may be either stationary or varying in time. The mesh size of the experimentally used gels ranges by a hundredfold from tightly constraining to very loose, while the lengths of the DNA probes vary over an even greater range.

We refer to experimental papers only when required in connection with discussions of theoretical problems or results; the extensive experimental literature on agarose gels and pulsed-field electrophoresis has been reviewed by Dawkins (1989), and by Lai *et al.* (1989). Since Nordén *et al.* (1991) have recently reviewed the literature on orientation of the DNA as detected by optical means, we make only passing reference to activity in this field.

When we write DNA in this review we shall mean double-stranded DNA, unless specifically noted otherwise. (In fact other highly charged polyelectrolytes behave in similar ways, for example, polystyrene sulphonate (Smisek & Hoagland, 1989, 1990).) The observed steady-field electrophoretic mobility of DNA in an agarose gel as a function of the DNA chain length and electric field is shown in Figs. 1 and 2. The straight section in the middle of the graph in Fig. 1 justifies the common use of this semi-log plot to correlate data. At the short-chain, highmobility end the curves approach a common limit, the free-solution value, which is a constant practically independent of length (Ross & Scruggs, 1964*a*, *b*; Olivera *et al.* 1964). Complications arise at the other end of the graph, where a dependence on field strength appears and the dependence on molecular length disappears; this region has inspired substantial theoretical activity, some of which we shall discuss. Figure 2 shows the effect of field in more detail.



Fig. 2. Electric-field dependence of mobility of DNA in 1 % agarose, data points from Hervet & Bean (1987), curves merely to guide the eye. Numbers on curves are DNA lengths in bp.

The mobility also shows remarkable changes when the electric field is not steady in time. Unsteady fields have become popular with molecular biologists because the different behaviours of the different lengths is practically useful in overcoming the loss of length dependence in steady fields seen at the right of Fig. 1. Figure 3, adapted from Kobayashi *et al.* (1990), illustrates the effect of a field that is periodically reversed in an asymmetrical cycle with more time in one direction than in the other. The pronounced dip in mobility at one cycling frequency has been called the 'anti-resonance'. The phenomenon has inspired several computer simulations, which we shall also discuss.

All current theories of electrophoresis are based on simplified models, and the nature of the most appropriate model depends on the size of the DNA and the nature of the gel. The two most common gels differ widely in 'pore size', this being in the neighbourhood of 3 nm for PA and 100 nm for agarose (more about this below in Section 7). Since double-stranded DNA has a helix diameter of about 2.5 nm and single-stranded only somewhat less, it seems that DNA of almost any length must be closely constrained in PA. Relevant here are two other characteristic lengths of the DNA. The first of these is the contour length; for the B-form, the usual form in dilute solution, this is 0.34 nm times the number of base pairs. Also important is the 'persistence length', a measure of stiffness in bending; pieces shorter than the persistence length do not bend freely from thermal motion. The persistence length has a value for the B-form between 50 and 80 nm, depending on the salt concentration, and is much greater than the pore size of PA and comparable with that of agarose.

In free solution the electrophoresis of DNA is remarkable mainly in that the



Fig. 3. Mobility of three different sizes of double-stranded DNA in periodically reversing electric fields, data from Kobayashi *et al.* (1990), curves merely to guide the eye. The field of 15.4 V/cm was applied in one direction for the period shown on the abscissa, immediately reversed for a time one third as long, and these cycles then repeated indefinitely. Gel was 1% agarose. Numbers on curves are the lengths in kbp.

mobility is practically independent of the DNA length (Ross & Scruggs, 1964a, b; Olivera et al. 1964). This independence is easily understood when one remembers that in electrophoresis the macromolecule moves through a sheath of counterions moving in the opposite direction. The thickness of this sheath is approximately the Debye-Hückel screening length, which is less than about 10 nm for the salt concentrations commonly used. The result is that hydrodynamic interactions between parts of the macromolecule separated by more than the screening length are effectively cut off by the streaming counterions, so no mechanism exists to generate a dependence of velocity on length on a scale greater than about 10 nm (Schellman & Stigter, 1977). Electrophoresis is different from sedimentation or diffusion, where the counterions are not driven in the opposite direction and longranged hydrodynamic interactions are important. In other words, the friction coefficient for DNA in electrophoresis is not at all the same as in sedimentation, as is sometimes supposed. An important practical consequence is that free solution electrophoresis is useless for analysis of DNA because there is no dependence on molecular length; this is the reason for using gels.

In agarose, DNA shorter then 100 nm (about 300 base pairs, or bp) should be able to move in any orientation among the gel fibres, though its motions would be impeded by collisions with the gel. Motion of this kind is frequently called sieving, and is probably similar to the motion of globular proteins through PA, a point of view accepted by Slater & Noolandi (1989). A theory for the sieving of globular proteins was outlined by Rodbard & Chrambach (1970) based on the concept of volume exclusion introduced by Ogston (1958) (see also Giddings *et al.* 1968). In this theory the migrating particle is assumed to move freely in the spaces between the gel fibres, but to be greatly slowed whenever it is in contact with a gel fibre. Thus the average mobility is a function, usually assumed to be a simple proportionality, of the fraction of the space that is not excluded to the centre of the particle by the gel fibres. Ogston's theory assumes a random array of gel fibres and finds this fraction to have an exponential dependence on the radius of the particle, assumed spherical, similar to the probability of no collision in a Poisson distribution of random collisions, so that a plot of the logarithm of the mobility against the gel concentration becomes linear. Such plots, called Ferguson plots, are in fact approximately linear for small DNA's over a considerable range of agarose concentrations (Serwer & Allen, 1984; Stellwagen, 1985b). Regrettably, little has been done to work out the details of a theory of such plots for a fibrous molecule like DNA.

When the DNA chains are substantially longer than the pore size, a widely used model called the reptation model, which was originally introduced into polymer physics by de Gennes (1971, 1978) and Doi & Edwards (1978), becomes attractive. In this model the moving chain, here DNA, is assumed to move among the chains of the gel only by creeping along its axis, like a snake through a dense thicket of bamboo; de Gennes coined the word 'reptation' to describe the motion. The model is appropriate for PA and DNA longer than about 50 bp, and for agarose and DNA longer than about 1 kbp, in view of the lengths presented above, and in much of this review we restrict the discussion to DNA in this size range. The transition between the so-called Ogston regime and the reptation regime has been examined by Slater & Noolandi (1989), who consider it to occur when the mobility is approximately $\frac{1}{3}$ of the free-solution mobility. See also Slater *et al.* (1988, 1989) for relevant experiments.

In most of the theoretical work in this field the gel is assumed to be an isotropic, rigid, inert matrix. There is evidence, however, that this is true only to a first approximation. In the work discussed in Section 5 it was necessary to assume that the fibres in a PA gel were elastically deformable. Jonsson *et al.* (1988) found that a band of DNA in an agarose gel in an electric field compressed the gel at the leading edge of the band and stretched it at the trailing edge. Stellwagen & Stellwagen (1989) found that the gel developed orientation when the field was applied, while Holmes & Stellwagen (1989) found that the gel was setting, and that the fibre orientation affected the mobility of DNA in a subsequent electrophoresis experiment.

2. TIGHT-TUBE REPTATION MODEL

By this title we refer to a model in which the moving DNA chain is assumed to be so tightly confined by the gel that it can move only along its axis as though confined to a tube, like a worm in its burrow. The concept in a more relaxed form

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was introduced by Doi and Edwards, and is thoroughly described by them (Doi & Edwards, 1978, 1986). As Doi & Edwards present the model, the chain is allowed to fold within the tube, but extensive excursions of loops of chain between gel fibres, that is, through the walls of the tube, are assumed to be forbidden. The contour length of the tube is assumed to be effectively constant with minor fluctuations. In the tight-tube model that we discuss here no folding of the chain back on itself is allowed, so that contour length of the tube is the same as that of the chain.

With this model it is easy to derive an expression for the mobility as a function of chain length, using the method of Lumpkin & Zimm (1982). (Lerman & Frisch, 1982, had previously obtained the same result as an extension of the Doi & Edwards (1978) reptation-theory formula for the diffusion constant.) We take the contour length of the tube to be L and the total effective charge on the chain to be Q. Assume an electric field **E** in the x-direction, and consider a short segment of tube represented by a vector Δs . The electric force driving the chain in this segment along the tube axis, the so-called axial force, is the charge on a segment, $(Q/L)\Delta s$, times the component of the field along the axis, or

$$\frac{Q}{L}\mathbf{E}.\Delta s.$$
 (1)

The total axial force on the whole chain is then

$$\frac{Q}{L}\Sigma \mathbf{E} \cdot \Delta \mathbf{s} = \frac{QE}{L}\Sigma \mathbf{i} \cdot \Delta \mathbf{s} = \frac{QEh_x}{L},\tag{2}$$

where **i** is a unit vector in the x-direction and h_x is the x-component of the endto-end vector of the tube and chain, equal, of course, to the sum of the xcomponents of all the segments Δs . If the friction coefficient for translational motion of the whole polymer along the axis of the tube is ζ , then the axial velocity is

$$\dot{s} = \frac{QEh_x}{\zeta L}.$$
(3)

However, in the usual experiment the observable quantity is the velocity of the centre of mass, not the velocity along the tube axis. The centre-of-mass velocity, which on the average is along the field direction, x, is related to \dot{s} by

$$\dot{x}_{cm} = \dot{s}h_x/L,\tag{4}$$

a relation which can be obtained as follows. The definition of the position of the centre of mass, \mathbf{R}_{cm} , is

$$M\mathbf{R}_{cm} = \sum m_i \,\mathbf{r}_i,\tag{5}$$

where M is the total mass of the chain and m_i and \mathbf{r}_i are the mass and position of the *i*th segment of the chain. Using the mass per unit length we can write

$$m_i = M\Delta s_i / L. \tag{6}$$



Fig. 4. Plot of reciprocal of mobility in arbitrary units against molecular weight of DNA, adapted from Southern (1979). Only a few of the data points of the original figure are shown. Numbers on lines are concentrations of agarose in percent.

Since we assume that the chain motion is always along the axis of the tube, the velocity of the ith piece of chain must be

$$\dot{\mathbf{r}}_i = \dot{s}\mathbf{t}_i,\tag{7}$$

where \mathbf{t}_i is the unit vector tangent to the tube at the *i*th segment. Introducing (6) and (7) into the time derivative of (5) we get

$$\mathbf{R}_{cm} = (\dot{s}/L) \sum \mathbf{t}_i \Delta s_i = (\dot{s}/L) \,\mathbf{h},\tag{8}$$

where \mathbf{h} is the end-to-end vector of the tube. Equation (4) is the component of equation (8) parallel to the electric field.

Combining (3) and (4) and averaging, we get the electrophoretic mobility

$$\frac{\langle \dot{x}_{cm} \rangle}{E} = \frac{Q \langle h_x^2 \rangle}{\zeta L^2}.$$
(9)

If we assume that both Q and ζ are proportional to L, as seems reasonable, then (Q/ζ) is independent of L. Further, if the tube is a simple random walk, as it must be when the chain is sufficiently long and the electric field sufficiently small, then $\langle h_x^2 \rangle$ is proportional to L, so the electrophoretic mobility is proportional to L^{-1} . Thus we get a simple explanation for the experimental observation that the mobility decreases with increasing chain length, since we have assumed that the length of the tube is proportional to the chain length, N, measured in base pairs. In fact, experiments by Southern (1979), Fig. 4, had already demonstrated the reciprocal relation between N and mobility before the development of the theory.

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Thus the qualitative result of the theory is correct. Quantitative testing of the theory is more difficult; comparison of the absolute magnitudes of the mobility from equation (9) or its high-field extensions to experiment is not a useful test, mainly because an independent value of the resistance coefficient ζ is not available for motion through a gel. The gel is heterogeneous, reflecting the random growth processes that gave rise to it, and even though the average structure can be deduced from electron micrographs (Waki *et al.* 1982; Attwood *et al.* 1988), the moving chain is very likely to select favourable environments whose structure may be far from average. It is true, however, that the mobility varies as the reciprocal of the viscosity of the solvent (West, 1987b; Hervet & Bean, 1987), as would be expected from the reptation model. We should also note that the value of the charge Q is not its formal chemical-structure value because of screening by counter-ions (Schellman & Stigter, 1977; Stigter, 1991).

3. PROBLEMS WITH THE SIMPLE REPTATION MODEL

Despite the qualitative success just quoted, problems at both ends of the range shown in Fig. 1 were apparent even as the above simple model was published. At the short-chain end the model should merge with the free-electrophoresis regime. In free electrophoresis the mobility is independent of chain length, as discussed in Section 1. The model can be forced to merge with this regime by assuming that the DNA becomes too stiff to bend at very short lengths. If the DNA does not bend, then it is not a random walk, and $\langle h_x^2 \rangle$ is proportional to L^2 rather than L^1 , and the mobility by equation (9) is independent of L. Unfortunately the degree of stiffness required is unrealistically great (Hervet & Bean, 1987). The problem no doubt arises because the reptation model is inappropriate when the DNA is shorter than the pore size of the gel. Diwan & Schuster (1989) have made a formal extension of the reptation model to connect it with this regime; see also Slater & Noolandi (1989). As mentioned above, this is a situation for which more theoretical work would be welcome.

In Fig. 1 complications are also apparent at the long-chain end in the form of a dependence on electric field and a loss of molecular-length dependence at the higher fields. These effects are easy to explain qualitatively by recognizing that the tube segments should have some orientation induced by the field because the field biases the direction of the leading end of the chain as it moves to form an extension of the tube. According to the model the rest of the chain follows the leading segment, so eventually all the segments of the tube have been formed by the leading segment, and all have become somewhat oriented. Actually, the orientation can be measured by optical techniques, as described in the review by Nordén et al. (1991). The tube is now a biased random walk, and its mean-square end-to-end vector acquires a term in the x-component that varies as L^2 as well as the random-walk term that varies as L. When introduced in equation (9), this new term leads to a length-independent term in the mobility, equation (9), a term which grows in relative importance as either L or E is made larger, in agreement with the experimental situation as shown on the right of Figs. 1 and 2. The ratio of these length-independent and length-dependent terms can be brought close to

the experimental values by a reasonable choice of parameters (Lumpkin *et al.* 1985). The dependence on field strength is qualitatively correct, though the experiments show more rapid saturation of the mobility with increasing field than the theory predicts.

Qualitatively this length-independent term is easy to understand. Consider the hypothetical extreme case where the DNA chain is oriented perfectly parallel to the field direction. Then the driving force is proportional to the total charge, Q, which is proportional to L, while the resistance coefficient, ζ , is also proportional to L. The mobility is proportional to the driving force over the resistance coefficient, and the length factors cancel out in this extreme case. On the other hand, when the chain has the form of a random walk, the local effective force and the local direction of motion are aligned with the tube axis and not with the field, and the mobility depends on the projections of these quantities on the x-axis, as expressed by the factor $\langle h_x^2 \rangle / L^2$ in equation (9). The dependence of this factor on L varies from L^{-1} to L^0 , depending on the degree of bias in the random walk.

Parenthetically we should note that the extreme case of a chain oriented perfectly parallel to the field is not achieved in practice. If such orientation were achieved, the mobility would be Q/ζ by equation (9), and would be the same for the shortest and longest chains. Clearly this is nowhere near the case in Figs. 1 and 2, so we must conclude that the greatest degree of orientation obtained in those experiments was much less than perfect.

There remain several theoretical problems with the tube model as described above. The first is the question of how to do the averaging over h_x^2 in equation (9), or more precisely, what weighting factor to use. At sufficiently low fields where the system is close to thermodynamic equilibrium, the weighting is that of the usual canonical ensemble (Lerman & Frisch, 1982; Lumpkin & Zimm, 1982; Lumpkin et al. 1985; Slater & Noolandi, 1986), but Slater et al. (1987) found that at the fields (several V/cm) usually encountered in practice it is necessary to use lifetime weighting, which is distinctly different from equilibrium weighting. In this kind of weighting the weight is proportional to the average lifetime that a particular conformation has in the moving chain. Under these conditions the mobility is predicted not to be a monotonic function of chain length (Doi et al. 1988; Slater & Noolandi, 1989; Lumpkin et al. 1989; Déjardin, 1989), instead showing a minimum at a particular length with both larger and smaller chains moving faster. This prediction was verified experimentally under some conditions (Noolandi et al. 1987; Slater et al. 1988, 1989). Actually, the effect had been discovered before by Bell & Byers (1983) in work mainly devoted to the electrophoresis of branched DNA, but had been generally overlooked. The non-monotonicity, usually called 'band inversion', seems to appear only in the most dense (high-concentration) agarose gels. Slater et al. (1987) and Slater & Noolandi (1989) attribute this to the overlapping of the reptation regime with the sieving (Ogston-theory) regime in the more dilute gels, with the minimum in mobility being overrun in the transition. West (1987c) has pointed out other quantitative discrepancies between the theory and experiment in the effect of gel concentration.

Another question is how to reconcile the simple derivation given above with the fact that Brownian motion must be greatly perturbing the system at any instant.

This concern led to studies of the 'biased reptation model' by Slater & Noolandi (1985, 1986, 1989), Slater *et al.* (1987), in which they explicitly introduced Brownian motion. In effect, however, the same formulas came out as previously. Presumably the reason is that the effects of Brownian motion, being random, produce displacements of the DNA that grow as the square root of time, and thus eventually become negligible compared to the electrophoretic displacement, which grows as the first power of time. The same reasoning explains the narrowness of the moving bands in the usual experiment, a narrowness which is one of the features of gel electrophoresis that makes it so useful for separation and analysis. The transition from motion dominated by diffusion to motion dominated by the electric field has been carefully analysed by Adolf (1987).

There are two other serious disagreements with experiment: (1) long molecules in agarose gels show a remarkably large change of mobility when the field is switched in strength or direction, and (2) short DNA fragments in PA gels shows an unexpectedly large sensitivity to the sequence of base pairs. To elucidate these has required new concepts, and we shall discuss these separately below.

4. SWITCHED FIELDS; NEW APPROACHES REQUIRED

Until recently all DNA gel electrophoresis was done with a steady dc field. This method works well for small DNA molecules, but, as we have seen, its power to resolve different chain lengths becomes less and less as the chain length gets larger and chain orientation sets in, and above about 50 kb it is practically useless. In 1983 Schwartz and Cantor introduced a technique in which the field was periodically switched in direction, and were able to separate the large DNA's from the various yeast *S. cerevisiae* chromosomes (hundreds of kb), a feat which had previously been impossible (Schwartz *et al.* 1983; Schwartz & Cantor, 1984). Several variations of this technique have now been developed which are referred to by various acronyms, such as OFAGE (Orthogonal-Field Agarose Gel Electrophoresis) and FIGE (Field-Inversion Gel Electrophoresis). The biased reptation model predicts that periodically switching the field through 90 degrees (OFAGE) should disorganize the growth of orientation induced by the field (Lumpkin *et al.* 1985; Slater *et al.* 1987), and thus can account for at least some of the increased length resolution that this technique yields.

On the other hand, this model is unable to explain a change in mobility produced by simple periodic reversal of the field (FIGE), since only even powers of the field enter the mobility expression. The FIGE technique was first described by Carle *et al.* (1986), who obtained a separation of the *cerevisiae* chromosomal DNA's by applying the field in one direction for a few seconds and then applying the field in the opposite direction for a time one third as long; this cycle was then repeated indefinitely. Figure 3 from a recent paper by Kobayashi *et al.* shows typical results obtained in this way. At a certain length of period the mobility drops to a minimum value that is less than the mobility at longer or shorter periods. The effect is minimal with short DNA's, but becomes dramatic with larger ones. When the period is suitably adjusted, DNA's too large for steady-field methods can still be efficiently separated.



Fig. 5. Successive conformations of DNA moving through a bed of obstacles, from Deutsch & Madden (1989), by permission. This is a two-dimensional simulation. The electric field is directed downward, and each dot represents one obstacle. The times (in arbitrary units) for each frame are: (a) 100, (b) 105, (c) 110, (d) 112, (e) 114, (f) 116, (g) 118, (h) 119.

The inability of the simple reptation model or its biasing extensions to account for the dramatic field-inversion phenomena exposed serious deficiencies of this model. Jamil & Lerman (1985), Fesjian *et al.* (1986), and Jamil *et al.* (1989) also showed that the model was not adequate to describe experiments with intermittent fields. Much of the theoretical work of the last few years in this area has been devoted to finding and studying these deficiencies. Computer simulations by Deutsch (1987, 1989), Zimm (1988, 1991 *a*, *b*), Duke (1989, 1990 *a*, *b*), and Shaffer & Olvera de la Cruz (1989) have agreed in showing that the DNA does not occupy a tube of fixed length in the gel when the molecule is long and the field is high. Instead the chain alternately curls up and extends. When the frequency of alternation between these extremes matches the frequency of field inversion in FIGE, then the mobility seen in the simulations drops, similar to the experiments. Thus under these conditions the tube concept has to be modified or even abandoned.

The computer simulation by Deutsch (1988; Deutsch & Madden, 1989) is probably the most impeccable methodologically and at the same time the simplest conceptually of these simulations. Fig. 5 is from this work. The gel is represented by a two-dimensional bed of regularly spaced obstacles, the dots in the figure, while the DNA is represented by a chain of beads connected by freely hinged rods. The chain is moved according to a Langevin equation in which the friction coefficient of a bead times the velocity of the bead is set equal to the total force on the bead, a force which is the sum of the tension in the rods, the electric field, a repulsion from any nearby obstacles, and a random impulse representing Brownian motion. The rods are represented by linear-elastic springs, but a special procedure continually adjusts the tensions to keep the lengths of the rods constant, so in effect they are inextensible. The repulsive forces between the beads and the obstacles prevent the chain from crossing over any obstacles, so the chain motion is entirely between the obstacles. This Langevin equation is integrated numerically by a Runge-Kutta algorithm.

The eight panels, (a-h), in Fig. 5 show the chain conformation at successive times midway through the simulation. The electrostatic force is directed downward. Starting from a conformation, (a), in which the trailing end is 'hooked' over an obstacle, the chain pulls free in (b) but soon piles up on its leading end in (c) and (d). From the resulting coil three 'pseudopods' extend in (e) and (f), the one on the right being a chain loop (also called a kink) and the other two the chain ends. The loop is pulled out by the rest of the chain in (g), and in (h) the longer arm is pulling the shorter arm over an obstacle in a conformation similar to (a) in which the sequence began. This cycle repeats with minor variations.

While the conformations in (a-b) and (g-h) could be thought of a chain in a tube, at least three events in this cycle violate the simple tube model: (1) the piling up in (c) and (d), (2) the extension of both ends at once in (e), (3) the extrusion of a loop in (e). In (c) the chain appears to be still in a tube, but the tube is much shorter than in (b), violating the constant-length assumption. The loop formation in (e) violates the assumption that the chain does not move sideways between the obstacles. This assumption is reasonable when the field is small because of the reduction in entropy that must occur when the two sides of the loop leave their separate spaces and move into the same space between obstacles, but the electrostatic energy of the loop in a higher field can overcome this barrier (Deutsch, 1987).

Deutsch & Madden (1989) analyse the growth of a loop, or 'kink', by the diagram of Fig. 6. Here the chain is draped over an obstacle with the part ABC forming the loop, and the electrostatic force again pulling downward. The part AB moves with the electric force just balanced by friction, so its resulting velocity is

$$v_{AB} = qE/\xi,\tag{10}$$

where q and ξ are the charge per unit length and friction coefficient per unit length respectively. The parts BC and CD are being pulled in opposite directions by the field, so their velocity is determined by the force imbalance, and is

$$v_{BC} = \frac{qE(CD - BC)}{\xi(CD + BC)},\tag{11}$$

where BC and CD represent the lengths of the corresponding part. The rate of growth of the loop is the difference of these two velocities, and is

$$v_{AB} - v_{BC} = \frac{2qEBC}{\xi(CD + BC)}.$$
(12)



Fig. 6. A loop, or 'kink', near the trailing end of a chain moving through a bed of obstacles. The electric field is directed downward, and each circle represents one obstacle. Adapted from Deutsch & Madden (1989).

This is always positive, so such a loop, once formed by surmounting the initial entropy barrier, grows until it 'swallows' the trailing end, A. Thus the system is unstable against this kind of loop formation when E > 0. When several loops are present, the above analysis applies only to the one nearest the trailing end, and then only if the trailing end is not hooked over an obstacle. In the more general case a loop may grow at first but then decay later as the conformation of the rest of the chain changes; compare Fig. 5(e-g).

Other simulations have produced similar results, even though the details of the methods differ considerably. Duke (1989, 1990 a, b) based his on the 'repton' theory of Rubinstein (1987). This theory is an extension of reptation theory in which motions within the tube of loops of chain, called reptons, are explicitly considered. In the absence of a field the reptons diffuse by random jumps. Duke considers that when a field is applied the reptons jump preferentially in the direction of the field, and thus the chain tends to drift down field. Using this repton motion as the elementary dynamic process, he simulates the motion of chains of various lengths in field inversion, and finds curves that look very much like the experimental ones in Fig. 3. The chain conformations resemble those of Deutsch (1988); because of the small sample published it is not clear whether the apparent differences in detail are significant. At low fields Duke recovers the biased-reptation formulas.

With admirable caution Duke explicitly points out three assumptions of his model and procedures: 'that the motion is governed by local detailed balance; that

there is no free sliding of the chain around gel fibres; and that there are no lateral excursions of the chain through the sides of the tube. This limits its validity to low field strengths. We expect the dynamics to follow from local detailed balance provided that the chain is never far from equilibrium'.

Certainly, the caution about detailed balance is important but sometimes forgotten in 'dynamic Monte Carlo' simulations. The assumption is that the relative rates of various local processes, such as up-field and down-field jumps, are governed by the Boltzmann factor of their respective energy changes. As Deutsch & Madden (1989) and Deutsch & Reger (1991) have pointed out, this assumption can lead to very unrealistic dynamics when forces are strong. (In work in press as this is written Duke & Viovy (1991) have attempted to extend the repton method to higher fields by introducing long-ranged jumps of the reptons.)

Deutsch & Maden (1989) also examined the chain conformations predicted by Rubinstein's repton theory. They found that the reptons, representing chain folds, tended to concentrate at the tail end of the chain at high fields, but in their simulations folds tended to concentrate at the leading end, as in Fig. 5c, while the trailing end was frequently dominated by a 'hair-pin' conformation, as in Fig. 5g.

Shaffer & Olvera de la Cruz (1989) used a two-dimensional square lattice of obstacles with the DNA chain interspersed between the lattice points. The chain moved according to Langevin equations of motion. The obstacles were considered to be points, so it was not possible to prevent the chain crossing them by a method like Deutsch's; instead, trial moves were made according to the Langevin formula including the random-force term, and whenever the chain crossed an obstacle the move was rejected. The authors express the well-founded concern that this procedure does not allow chain tension to be transmitted properly between the two arms of a hairpin at high fields; nevertheless, the chain conformations and motions found were similar to those of the previously mentioned simulations.

In a series of important papers Baumgärtner & Muthukumar (1987), Muthukumar & Baumgärtner (1989a, b) have shown by simulation and by analysis that the zero-field behaviour of polymer chains in fields of obstacles that are randomly distributed is different from that in regular arrays. The chain tends to coil up in the larger spaces between the obstacles, and its motion is impeded by the entropy barriers it encounters when it squeezes between obstacles in course of diffusion. It is not yet clear to what extent these effects persist when typical electrophoretic fields are applied. We discuss this further in Section 7 below.

One of us has attempted to reduce the requirements of the above models for extensive computation by introducing a more coarse-grained model incorporating some analytic theory (Zimm, 1988, 1991 a, b). A model is proposed in which the chain can coil freely in spaces between the gel fibres but is constrained by occasional narrow gates between fibres; this picture is related to the Baumgärtner & Muthukumar random-medium studies just described. We call the three-dimensional open spaces 'lakes' and the constraining gates 'straits'. A succession of such lakes and straits in which the DNA lies replaces the tube. Since the chain can coil in the lakes, the length of the succession of lakes is always less than the length of the DNA chain, and may at times be very much less, unlike the tight-tube model. To distribute the chain among the lakes we balance three kinds of forces, the electric field and hydrodynamic friction, as in equations (1) and (2), and, third, tension in the chain. This latter is assumed to arise ultimately from Brownian motion, and is obtained by calculating the change of the entropy of the chain when segments are transferred from one lake to another; the result is a tension function that describes the average pull (or push) exerted on the segment in a strait by the segments in an adjoining lake. This function, which is closely related to the kinetic-elasticity function of an ideal rubber, depends on the size of the lake, on the number of segments in the lake, and on the temperature.

The zero-field, or equilibrium, limit of this model shows the DNA chain of contour length L' occupying a chain of lakes whose length, L, is shorter than L'by the factor b/a, where b is the length of the 'Kuhn segment' of DNA and a is the diameter of a lake. (The Kuhn segment is the length of an individual flight in a chain of random flights that has the same contour length and the same meansquare end-to-end length as the real chain. The Kuhn segment is equal to two persistence lengths.) Since b for DNA is approximately 100 nm and a for agarose, as judged from electron micrographs (Waki et al. 1982; Attwood et al. 1988), is about three times larger, a is set equal to 3b for simplicity. Requiring that the tensions all be in balance leads to each lake being occupied by the same amount of chain, which is equal to $(a/b)^2$, or about 9, segments. Thus if we equate the tube to the chain of lakes, the tube is shorter than the chain by the factor b/a. This is in accord with the form of the tube model originally presented by Doi & Edwards (1978, 1986), in which they allowed the chain to fold within the tube, as opposed to the tight-tube reptation model discussed in Section 2. However it was not immediately clear how to apply the electric field to the original Doi-Edwards model, since the local directions of the chain and the tube are not the same.

To find out how to apply the field in the lakes-straits model it was necessary to examine by simulation the motion of the chain through a single lake and to find the axially effective value of the driving force. In equation (9) this is simply the projection of the electric force on the tube axis, but the simulation showed that in a lake much of this force is wasted in pushing segments into collision with the gel fibres represented by the 'shores' of the lake. In the case of electric fields too small to drive the number of segments in any lake far from the equilibrium value, the current of chain flowing through all straits must be nearly the same; in effect, the chain moves as an inextensible and incompressible string, just as was assumed in the strict-reptation model. The result for the mobility is

$$\frac{\langle \dot{x}_{cm} \rangle}{E} = \frac{Q}{\zeta} \langle h_x^2 / LL' \rangle \tag{13}$$

which is the same as equation (9) except that L is now a variable that must be included in the averaging operation, and one of the factors L in the denominator is replaced by L'. The physical origin of the latter difference is in the calculation of the axially effective driving force. Nevertheless, the scaling mobility with chain length remains the same, since the average L is proportional to L'.

The picture changes at higher fields, where the electrostatic forces can



Fig.-7. Mobility as a function of cycle period in the lakes-straits model, adapted from Zimm (1991 a). Numbers on curves are the chain lengths measured in Kuhn segments (in parentheses, measured in kbp). The unit of time is approximately 0.8 ms.

overwhelm the tension function and force the chain to bunch up. For illustration we can use the Gaussian approximation for a lake containing n Kuhn segments; the expression for the tension function is then very simple,

$$f = \frac{3k_B T}{2nb} \left(\frac{a^2}{nb^2} - \mathbf{I} \right),\tag{14}$$

where f is the average force on a segment in a strait due to the tension function. This function has a very asymmetrical shape, going to indefinitely high positive values when the chain is stretched (n small), but being able to support only a limited amount of compressive force before allowing the chain to collapse to an indefinitely large value of n. In fact, the maximum negative (pushing) value of f occurs at $n = 2(a/b)^2$, and is only

$$f_{\min} = -\frac{3bk_B T}{8a^2}.$$
(15)

Compressive forces generated in the loops of a long chain by modest fields can easily exceed this limit, with the result that large numbers of segments temporarily accumulate in some lakes. (These correspond to the bunching seen in the Deutsch simulations, Fig. 5c, d.)

These accumulations favour another process, 'loop overflow', that is, the flow

of a loop of chain out between the fibres constituting the side of a lake. As discussed in connection with Deutsch's simulation, such flow is inhibited by an entropy barrier, but the barrier can be overcome by the field, given enough time. Loop overflow is unlikely when the tension in a lake is positive, as it is when the lake contains only a small number of segments, but becomes much more likely when the tension is negative, that is, when the lake is under compression, as it is when $n > (a/b)^2$. Thus overflows frequently spill out of lakes with large accumulations of segments, in analogy to Fig. 5*e*, *f*. Once started, an overflow tends to grow until it swallows the trailing end of the chain. The eventual result is a configuration like Fig. 5*h*, where two arms of the chain are pulling against each other and attempting to go down separate paths.

Just as in the Deutsch simulation, in steady field the chain cycles repeatedly through a series of conformations from bunched up to hung up to extended. However, if the field is cycled as in FIGE, the mobility can be markedly decreased if the period of field cycling matches that of the spontaneous bunching cycle. Figure 7 shows data from a number of computer runs of this lakes-straits model of chains of various lengths. The similarity to the experimental data of Kobayashi *et al.* (1990) in figure 3 is noteworthy. Just as in the data, a minimum in the mobility appears at a certain cycle period, and the minimum gets deeper as the chains get longer. See also similar experimental data by Heller & Pohl (1989), and by Crater *et al.* (1989).

Including the loop-overflow process made the minima in the mobility deeper and more dramatic, but minima were nevertheless obtained even without loop overflows, thus agreeing with Duke's simulations. Apparently the loop-overflow process exaggerates tube-length fluctuations that arise in any case from the inability of the chain to support strong compressive forces.

While at these fields the chain is clearly not moving as an inextensible and incompressible string, still the corresponding formula for the mobility, equation (13), works remarkably well if the actual values of h_x^2 and L observed during the simulation are used (Fig. 15 in Zimm, 1991*a*). This formula thus seems to describe the average dynamics in spite of the large fluctuations in chain conformation. To use this formula to predict mobility, however, we would have to find some way of predicting the average of the ratio of h_x^2 and L, since these quantities can be far from their equilibrium values at high fields.

This model relieves another problem encountered with the biasing extensions of the simple-reptation model. In those models orientation of the chain came only from orientation of the leading segment as it entered new spaces between gel fibres, with the result that the amount of orientation increased with increasing field but was independent of chain length; this was in contradiction to experiment. In the lakes-straits model the chain in any lake can be oriented by tension applied through the chain in the adjoining straits, so the amount of orientation in this lake is sensitive to the pull of the field on other pieces of chain. The average amount of such pulls increases with chain length, leading to an increase of average orientation with chain length, as was observed in linear dichroism experiments by Holzwarth *et al.* (1987) and by Jonsson *et al.* (1988).

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Because the overflow process is delayed by the entropic barrier, there is usually an accumulation of segments at the downfield end of the chain. This accumulation is actually visualized in some remarkable micrographs by Schwartz & Koval (1989), Smith *et al.* (1989), and Gurrieri *et al.* (1990). In this procedure the DNA in the gel is labelled by a fluorescent dye and illuminated with light of frequency in the excitation band of the dye. (For an overview of the technique see Bustamante, 1991.) Because of the limited resolution of the visible-light microscope only details larger than about $\frac{1}{4}$ of a micrometer can be seen, but this is sufficient to show many interesting things with DNA molecules longer than about 50 kb (about 25 μ m). Among these is the cycling between bunched and extended forms as predicted by the simulations.

Other preliminary theoretical and simulation approaches to the cycling problem were made by Viovy (1988) and by Noolandi *et al.* (1989).

While the simulations are very informative, a quantitative and complete analytical theory has so far been lacking. There have been several approaches. Deutsch (1989) proposed a simple model in which the chain cycles between straight, bunched up, and U-shaped conformations according to some simple quantitative rules. He called this type of motion 'geometration', in analogy with the looping motion of the larvae ('inchworms') of geometrid moths. The conformations, of course, were inspired by the simulations. When this model was subjected to computer simulation in inverting fields (FIGE) it showed the same deep minimum in mobility as the full simulation, and as seen in Figs. 3 and 7. The model seems simple enough to allow an analytical theory, but this is not yet available. Deutsch & Madden (1989) proposed a differential equation that incorporates a friction coefficient that increases where the chain becomes bunched up, and found that the solutions of the equation showed a bunching instability like the simulations. The mobility could only be obtained by numerical solution. Lumpkin (1989) introduced an abstract chain model consisting of three beads connected by two springs. The unique feature of the model was making the friction coefficients of the beads depend on the amount of stretch in the adjacent springs, becoming large when the springs were short and small when they were stretched, much the same physical idea as in the Deutsch & Madden (1989) theory just mentioned. While analytical formulas could be developed for steady field and for very rapid field reversals in FIGE, it was necessary to resort to numerical solutions of the equations for intermediate switching periods. The mobility showed a deep minimum as a function of cycle period, again like the experiments and simulations.

In summary, the various simulations are in remarkably good agreement with the field-inversion mobility experiments, the field-inversion orientation experiments, and the microscope images at high fields. (When we use the phrase 'high fields', it must be understood that the important quantity is really the product of the field and the charge Q; the fields can actually be very modest, of the order of a Volt per centimetre, since the charge on the DNA molecules of interest is thousands or even millions of electron equivalents.) From the point of view of reptation theory, the main lesson is the need to take account of the great fluctuations in tube length

that occur with long chains at high fields, and the dominance in this case of distinctive conformations, extended, bunched up, and hung up, that are very different from the typical random walk, differences that are more than just biasing of individual steps. Simple theoretical models incorporating these conformations are successful in showing the same qualitative behaviour as the simulations and the experiments.

Relatively little theoretical work has been done so far to explain the OFAGE experiments, though we should mention a simple model put forward by Southern *et al.* (1987), and a recent computer simulation by Duke & Viovy (private communication).

5. BENT DNA AND TIGHT GELS

Polyacrylamide (PA) gels are routinely used to separate double-stranded DNA fragments in the size range from approximately 50 bp to 1000 bp and singlestranded DNA fragments from about 4 bp up to 300 or 400 bp. As is the case with typical separations in the useful molecular weight range of agarose gels, the mobility of DNA is observed to be approximately a decreasing linear function of the logarithm of the molecular weight (McDonell et al. 1977; Stellwagen, 1985b). There are important exceptions to this rule, however. Marini et al. (1982a, b)observed that some DNA fragments isolated from the kinetoplast body of unicellular parasites migrated in native PA gels with dramatically lower mobilities than electrophoretic standards of similar molecular weight. This anomalous behaviour was more pronounced in PA gels of high percentage than in less concentrated PA gels and was apparently absent in 2 percent agarose gels. Further physical characterization of these fragments showed that the anomalous fragments undergo accelerated rotational diffusion in solution relative to electrophoretically normal DNA molecules of identical molecular weight, implying that the anomalous fragments have an unusually compact conformation in solution. This behaviour was best explained by postulating the presence of an intrinsically bent region of helical structure in the kinetoplast DNA molecules, a conclusion that has been borne out by a considerable body of experimental evidence since then (for review, see Hagerman 1990).

The feature of the DNA sequence responsible for the anomalous behaviour has been identified as a repeating motif of a tract of oligo (dA)-oligo (dT), dA5-6/dT5-6, repeated in phase with the helical repeat of DNA (Koo *et al.* 1986). Experiments in which the centre of this sequence element was permuted with respect to the ends of the molecule, yielding a set of DNA fragments of identical molecular weight but with differing average dimensions in solution, showed that the reduction in mobility is correlated with the reduction in molecular dimensions (Wu & Crothers, 1984; Levene *et al.* 1986). Thus molecules with an intrinsic bend near one end have nearly normal electrophoretic properties, whereas those fragments with the bend located near the centre of the fragment have the lowest mobilities relative to standards. The principle that reduced mobility correlates with a reduction in molecular dimensions among a set of circularly permuted isomeric fragments has been used extensively as a basic assay for the presence of intrinsic bends, due either to structural polymorphism in DNA or induced by the binding of specific proteins (for example, see Gartenberg & Crothers, 1988).

Explanations for the effect of circular permutation of intrinsically bent regions on mobility often invoke the proportionality of mobility to $\langle h_x^2 \rangle$ as predicted by simple tube-reptation models, equation (9). This logic leads to serious contradictions, however. Because the conformation of the tube is assumed to be rigidly fixed in space and determined entirely by the random fluctuations in the orientation of the leading segment independently of the orientation of all other segments, the presence of an intrinsic bend near the centre of the chain should not have any effect on the chain's mobility. In fact, the only position in which an intrinsic bend would be expected to have any effect according to this model would be at the position of the leading segment. Thus, new concepts need to be introduced in order to explain the anomalous mobility of intrinsically bent DNA fragments. We have introduced (Levene & Zimm, 1989) a modified tube-reptation model that takes the elastic energy of the chain into account and predicts effects of an intrinsic bend on the mobility even when the bend is located near the centre of the chain. This model accounts semi-quantitatively for the effects of PA concentration on the relative mobilities of intrinsically bent DNA fragments.

Measurements of the mobilities of spherical proteins in PA gels suggest that the 'mean pore diameter' ranges from approximately 1 to 8 nm and depends on the concentration of acrylamide monomer and cross-linking reagent in the gel (Chrambach & Rodbard, 1971). The characteristic length that measures the stiffness of double-stranded DNA is the persistence length P, which is known to be about 50 nm in solutions above 10 mM monovalent ion concentration. These values imply that the mean length of a tube segment in the reptation picture is anywhere from slightly more than one-sixth to as little as one-twentieth of the DNA persistence length, with the lower limit of the length of a tube segment assumed to be equal to the diameter of the DNA double helix, or 2.5 nm. This situation stands in contrast to that in agarose gels, where the mean interfibre spacing is similar to or greater than P. In a tight gel in which the interfibre spacing is appreciably smaller than P, the DNA chain is subject to elastic deformations as it moves around the obstacles in the gel. The new tube conformations that are generated by the succession of random fluctuations in the orientation of the leading segment depart in varying degrees from the chain conformation of minimum elastic energy; thus the elastic energy of the chain is expected to fluctuate during electrophoresis in tight gels.

We calculate by the 'dynamic Monte Carlo method' (Baumgärtner, 1984) the effect of the chain's elastic energy on the motion of the chain undergoing reptation in a tube. Since the original publication was necessarily condensed (Levene & Zimm, 1989), we describe the methods in some detail here. Reptation is treated as a series of discrete steps of length *a* along the tube axis subject to the electric field force, bending forces, and Brownian motion. The conformation of a chain confined to a tube of N segments of length *a* is specified by N-1 pairs of angles, θ_i and ϕ_i , the usual polar and azimuthal angles between the tube segments *i* and

i-1, respectively. If the chain is isotropically elastic, then the energy of the chain confined in the tube, U_{el} , is given by the Hooke's law expression

$$U_{el} = B \sum_{i=1}^{N-1} (\theta_i - \theta_i^0)^2,$$
(16)

where B is one-half the bending-force constant for the chain and θ_i^0 is the equilibrium value of the bend angle between segments i and i-1. The value of the force constant is related to the intrinsic flexibility of the chain through the ratio of the tube segment length, a, to the persistence length, P through the equation (Schellman, 1974)

$$a/P = \mathbf{I} - \langle \cos \theta \rangle, \tag{17}$$

where $\langle \cos \theta \rangle$ is the Boltzmann average of the cosine of θ for a pair of adjacent segments with an equilibrium bend angle of zero. The value of *B* is determined numerically by computing $\langle \cos \theta \rangle$ using an initial estimate for *B*, substituting this value into equation (17), and then using a root-locating procedure (such as the false-position method) iteratively until the desired value of a/P is obtained within a specified tolerance. With the expression for the elastic energy of the chain in the tube completely specified, it is possible to use the algorithm described below to simulate the conformation and position of the tube as a function of time.

During a single step in the simulation, the chain is moved by creating a new leading segment of the tube and removing the trailing segment. The new leading segment is chosen from a distribution of orientations that is random with respect to the field but has the Gaussian distribution of polar angles with respect to the previous orientation of the leading segment that is characteristic of the wormlike chain (Schellman, 1974). The azimuthal orientation of the new leading segment is chosen from a uniform distribution over the range o to 2π . The use of the Gaussian distribution to generate the new value of θ_i instead of a uniform distribution from o to π improves the efficiency of the calculation enormously, but leads to a bias in the distribution of conformations that must be removed. This is a common approach in Monte Carlo simulations, called importance sampling. The bias is removed by calculating the statistical weight corresponding to a given configuration and multiplying the results from each step by the reciprocal of this statistical weight. This procedure was checked by comparing even moments of the tube end-to-end length calculated with the simulation in the absence of a field and comparing the results with exact values calculated from the Porod-Kratky wormlike chain formulas (Flory, 1969).

We now consider the motion of a characteristic point on the chain. For convenience, this point is chosen to be the end of the leading segment of the chain, although we could choose any fixed point on the chain contour. At time t = 0, the position of the chain end inside the tube segment, s, is uniformly distributed inside the segment boundaries, namely over the interval $-a/2 \le s \le a/2$. The direction of motion, either with or against the field, is chosen with equal probability in the simulation. The configuration of the new leading segment is chosen and a trial move is executed. The chain is considered to have advanced a distance a along the tube when the end of the chain passes one of the segment boundaries at s = -a/2 or a/2. Following the approach taken in dynamic Monte Carlo simulations (Baumgärtner, 1984), the transition probability, the probability that the chain undergoes the trial move in unit time, is calculated. The transition probabilities for a downfield move, d, or upfield move, u, in time t are given by

$$\begin{aligned} u_{d}^{u} &= \frac{\sqrt{Dt}}{a} \left[\frac{a \pm vt}{2\sqrt{Dt}} \operatorname{erfc}\left(\frac{a + vt}{2\sqrt{Dt}}\right) - \frac{1}{\sqrt{\pi}} \exp\left(\frac{-(a \pm vt)^{2}}{4Dt}\right) \\ &+ \frac{vt}{2\sqrt{Dt}} \operatorname{erfc}\left(\frac{\pm vt}{2\sqrt{Dt}}\right) + \frac{1}{\sqrt{\pi}} \exp\left(\frac{-(vt)^{2}}{4Dt}\right) \right], \quad (18) \end{aligned}$$

where D is the diffusion coefficient of the chain along the tube axis, v is the instantaneous drift velocity of the chain, and erfc is the complement of the error function. We outline the derivation of these equations in what follows.

We begin with the solution of the one-dimensional diffusion equation in a frame moving with velocity v,

$$C(s,t;s_0) = \frac{1}{2\sqrt{\pi Dt}} \exp\left(\frac{-(s-vt-s_0)^2}{4Dt}\right),$$
(19)

where $C(s, t; s_0)$ is the fraction of chains initially at s_0 at time t = 0 that arrive at sat time t. The initial position, s_0 , is uniformly distributed between -a/2 and a/2. We wish to find the fraction of chain ends that have left the tube segment; the fraction of chains that have moved out of the leading tube segment in the downfield direction, g_+ , and upfield direction, g_- , are found by integrating equation (19) over the intervals $a/2 \leq s \leq \infty$ and $-\infty \leq s \leq -a/2$, respectively. This yields

$$\begin{cases} g_{-} \\ g_{+} \end{cases} = \frac{1}{2} \operatorname{erfc}\left(\frac{a/2 \pm x_{0} \pm vt}{2\sqrt{Dt}}\right).$$
 (20)

The distribution of chain ends is uniform at t = 0, but becomes Gaussian with time. The peak of this distribution moves gradually downfield. Equation (20) gives the fractions of chains moving in either direction that were initially located at s_0 , but because we want the solution for chain ends that are uniformly distributed in the leading segment at time t = 0; we average g_+ and g_- over the initial uniform distribution, obtaining

Evaluating the integrals leads to the expressions given in equation (18). We now consider the form of the drift velocity, v, and the value of the time increment in the simulation.

We make the usual assumption of steady-state motion, that is, we are interested in processes that occur on a time scale that is much slower than the relaxation of the chain's velocity. The velocity is then given by $v = F/\zeta$, the ratio of the instantaneous force acting on the chain, F, to the friction coefficient for translation along the tube, ζ . F is composed of the force due to the electric field, $QE|h_x|/L$, from equation (2), and an additional force that is derived from the change in the chain's elastic energy, $\Delta U/a$. The velocity v is therefore given by

$$v = \frac{I}{\zeta} \left(\frac{QE|h_x|}{L} + \frac{\Delta U}{a} \right).$$
(22)

If we restrict our treatment to relatively low-molecular-weight DNA fragments, molecules with small values of the Coulombic charge, the electric field is a small perturbation, and the dynamics of the chain can be treated in terms of weakly biased diffusion. It is therefore convenient to take as the unit of time in the simulation the time τ_D for the chain to diffuse an average distance *a* in the absence of electrical and bending forces. This diffusion time is given by $\tau_D = a^2/2D$, where *D* is the diffusion coefficient of the chain along the tube axis. The friction coefficient for diffusion, equal to $k_B T/D$, is probably similar to but not identical to the value for electrophoresis, ζ ; however for simplicity we assume that these values are the same. Substituting v and τ_D into equation (18), we obtain expressions for the probability that the chain makes an upfield or downfield step of length *a*, one interfibre distance along the tube, in the time τ_D driven by an electric field and under the influence of bending forces,

where $V = QEa/k_B T$, $X = |h_x|/L$, ΔU is the change in the elastic energy of the chain in units of $k_B T$ and the upper and lower signs correspond to the expressions for u and d, respectively.

In the simulation a trial step in either the downfield (with the field) or upfield (against the field) direction is chosen with probability $\frac{1}{2}$. The trial step is accepted if the transition probability d or u is greater than a random number selected at that point from a uniform distribution between \circ and I; otherwise the step is rejected.

Figure 8 shows the calculated effect of an intrinsic bend on the mobility of a chain as a function of the intrinsic bend angle at the centre of the chain. The mean-square end-to-end length, $\langle h^2 \rangle$, is also plotted and shows less dependence on bend angle than the calculated mobility. With decreasing values of the reduced interfibre spacing, a/P, the mobility of the bent chain diverges more from that of unbent chain, implying that the mobility reduction of a bent chain relative to an unbent control will be larger in a concentrated gel than in a dilute gel. This behaviour reproduces an observed property of bent DNA molecules: these molecules migrate anomalously in concentrated gel systems, such as 8% polyacrylamide, but have normal mobilities in dilute gels such as 1.5% agarose. The gel-concentration effect is due to the coupling of the motion to the elastic energy in this model; the transition probabilities given by equation (23) depend approximately exponentially on the elastic energy and thus are quite sensitive to



Fig. 8. Calculated results for bent chains; mobility (solid line) and h_x^2 (dashed line) as function of bend angle. From Levene & Zimm (1989).

the value of the bending elasticity modulus B appearing in equation (16). This constant increases linearly with the value of P/a in the limit of large P/a.

Following the moment-by-moment progress of the chain and the chain conformation revealed distinct differences between the mechanisms of motion of the bent and unbent chains (Levene & Zimm, 1989). In the case of the unbent chain, the velocity fluctuated rapidly in time and large jumps in the centre-of-mass position generally coincided with large values of h_x^2 . This response contrasted with that of the intrinsically bent chain, where the centre-of-mass velocity was weakly correlated with h_x^2 . The intrinsically bent chain experienced long periods where there was little net motion despite relatively large instantaneous values of h_x^2 ; these intervals corresponded to situations in which the chain was trapped in local freeenergy minima. The tendency for the chain to fluctuate about favourable tube conformations accounts for the major difference in the behaviour of intrinsically bent and intrinsically straight chains in this model. A single intrinsic bend imposes a large barrier to the motion of a chain if the tube conformation is close to the minimum free-energy conformation of the chain.

When the mobilities calculated using this model are compared with measured values for a set of circularly permuted kinetoplast DNA fragments, the mobility as a function of the position of the centre of the intrinsic bend agrees quite well with the data, provided that the value of B is made much smaller than the value estimated from a/P based on the mean interfibre spacing in polyacrylamide gels and the free-solution values of P. This low apparent value of B can be rationalized as an artifact of our highly idealized model of the gel as an array of rigidly fixed

obstacles. Polyacrylamide gels are elastic media; we have assumed that the network forces the DNA molecule to bend, but the DNA chain can also deform the network. The actual elastic-energy barriers that the DNA chain encounters in tight gels are probably much smaller than those predicted assuming rigid fibres; hence the low effective value of B.

6. ANOTHER VIEW

In a recent paper Calladine *et al.* (1991) have proposed a very different approach to the theory of gel electrophoresis, based on an extensive set of new experiments with a variety of gels, including polyacrylamide as well as unusually high concentrations of agarose.

From their experiments they make $\log -\log p$ lots of velocity, v, against doublestranded DNA length, N, at various gel concentrations of agarose, 'Nu-Sieve' agarose, and PA, and at fields from 0.5 to 8 V/cm. At the short-length side, down to N = 10 bp, all plots have same shape, with velocity, v, approaching a limit, f, at short lengths. This limiting velocity at small N is proportional to field and practically independent of gel concentration; it is reasonably interpreted as the free-solution velocity of the DNA, which is known to be independent of N(Olivera et al. 1964). By translating the log-log curves along the axes they can superpose those of different voltage gradients and gel concentrations; they call the resulting curve the 'master curve'. On the high-N side individual plots break away from the master curve and level off to a high-field v plateau that depends on voltage and gel concentration and is practically independent of DNA length. The short-length, master curve region is called 'region 1' and the other, the plateau, 'region 2'. (It is stated that preliminary data show single-stranded DNA conforming to the same picture.) Region 1 corresponds to the unique curve at the left side of our Fig. 1; region 2 to the multiplicity of high-voltage curves at the right side.

The master curve of region 1 is characterized by the value of $N_{0.5}$, which is the value of N at which v equals f/2. The master curve for all gels is empirically represented in terms of the reciprocal of a unique third-degree polynomial,

$$v/f = 1/(1 + N' + 0.01 N'^3),$$
 (24)

where $N' = N/N_{0.5}$. N' ranges from a few tenths up to more than 30. Different gels, which can give quite different mobilities, are thus distinguished in region 1 only by their values of $N_{0.5}$. This master curve is notable in that it has a 1/N'dependence like Southern's (1979) data (Fig. 4) over a limited domain but then steepens to approximately $1/N'^3$. The steepening itself is apparently a new discovery. The steepening occurs at low fields, ≤ 1.0 V/cm, and at high gel concentrations, $w \geq 0.015$ with agarose and $w \geq 0.08$ with PA, where w is the gel concentration in mass/volume. A problem is how to reconcile this steepening with Southern's very convincing 1/N plots in the region w = 0.004 to 0.028 agarose at about 1.0 V/cm (see Fig. 4). In the data of Calladine *et al.* (1991) at lower gel concentrations and higher fields the break-away to region 2 occurs before the steepening, and a I/N' fit to the data looks reasonable; this may help reconciliation with Southern's data.

Calladine *et al.* (1991) interpret the master curve by considerations of the Ogston type involving the probabilities of the DNA being in contact with either one, or a pair, or a triple of randomly spaced obstacles; they have to introduce three empirical parameters corresponding to the amount the velocity of the DNA is lowered while in these contacts.

Region 2, the plateau, begins at a transition velocity, v_T , which is close to but higher than the high-N limiting velocity. (It is actually defined as the velocity at which the experimental curve crosses the master curve translated to the right by log (2).) For agarose itself they find that the relative velocity v_T/f varies as $(E/w)^{1.5}$; for Nu-Sieve agarose and PA there is such a slight breakaway effect that it is hard to identify v_T .

Calladine *et al.* (1991) have a very original theory for region 2, which we paraphrase as follows. Suppose that the gel is made up of cubic cells with edge length, *a*. If we represent the gel fibres as rods much longer than *a* lying along the edges of the cubic cells, then by computing the volume of a cell we find that *a* varies as $w^{-\frac{1}{2}}$. The DNA is supposed to move freely across a cell with velocity *f* until the leading end encounters the cell wall at time a/f, at which point the DNA starts to pile up on the cell wall. It piles up until the pile exerts enough force to either rupture or bend the gel, and then it breaks through the cell wall. The amount in the pile at breakthrough is *l*, and the time to accumulate it is l/f. To get the net velocity, v_T , we add the times of free movement and of piling up and get $a/v_T = a/f + l/f$, which gives

$$\frac{v_T}{f} = \frac{I}{I + l/a}.$$
(25)

At break the bending moment of a beam is proportional to force times the span, so if we assume that the gel fibre is a beam, the moment is *Ela*, giving

$$l/a = k/(Ea^2), \tag{26}$$

where k is a material-dependent constant. This gives the relative velocity, v_T/f , varying as E at low fields where l/a is large compared to unity and becoming constant, 'saturating', at high fields where l/a becomes small. If the gel fibre is considered to bend instead of break, we get a similar result but with a^3 or a^4 instead of a^2 , depending on whether the displacement of the fibre necessary to pass the DNA is assumed to be proportional to a^0 or a^1 . Since a is assumed to vary as $w^{-\frac{1}{2}}$, these translate into $f/v_T - I = l/a$ varying as w/E in the 'break' case and as $w^{\frac{3}{2}}/E$ or w^2/E in the 'bend' case. As noted above, empirically v_T/f varies as $(E/w)^{1.5}$, which lies between the above extremes if we assume that f/v_T is large compared to unity, as it usually is. In any case by this model in region 2 the velocity at a given field is independent of chain length.

Actually, in Hervet & Bean's (1987) data at low fields (Fig. 2) the mobility goes as a constant plus an E^2 term. In any physically reasonable theory the mobility must depend only on even powers of the field at low fields, since the mobility must not reverse sign when the field is reversed. The E^1 region theoretically predicted or the $E^{1\cdot 5}$ empirically seen must correspond to the inflexion region in Hervet & Bean's data where saturation is starting to appear at higher fields. (A dependence on the first power of E is also seen in Stellwagen's (1985b) data over a limited range of E.) Presumably, we have to say that this region-2 model of Calladine *et al.* (1991) applies only when the field is above a certain threshold, and their region-1 model takes over below this threshold.

An important new result of this work is the discovery that one master curve fits the data on three very different kinds of gels in region I, and that this curve includes a region of steep dependence on the length of the DNA, approaching N^{-3} . A new theoretical departure is the attribution of the levelling of mobility at high fields and chain lengths exclusively to the DNA breaking through the gel rather than to chain orientation, as has usually been done hitherto (Section 3). (The idea of piling up and breaking through also has an important part in the lakes-straits model discussed in Section 4, and that model also leads to a levelling of the mobility at high fields and chain lengths, although it is not clear whether the levelling is due only to the mechanism of piling up and breaking through.) A problem in the Calladine *et al.* (1991) model is the lack of a quantitative theory of the transition between regions I and 2; that is, at what DNA size, gel concentration, and electric field does the DNA case to slide around gel fibres and start to bend or break them?

7. MISCELLANEOUS

In all of this the problem of gel structure is a fundamental concern. The fact that the measured pore sizes of PA and of agarose are so different though the volume concentrations are within a factor of ten of each other brings up the question of whether both gels can be represented as random arrays of fibres. For PA with low degree of cross-linking the fibres should be simple acrylic-polymer chains; from the chemical structure we get a length to mass ratio of 284 Daltons per nanometre, which gives λ , the length of fibres per cubic centimetre in a gel of concentration w, as $2 \cdot 1 \times 10^{14} w$ centimetres. By the Ogston method (Rodbard & Chrambach, 1970; Giddings *et al.* 1968; Lumpkin *et al.* 1985) we can then calculate the mean pore size, $\langle a \rangle$, for a gel of 8% concentration:

$$\langle a \rangle = 1/(2\lambda^{\frac{1}{2}}) = 1.2 \text{ nm}.$$

The measured values compiled by Chrambach & Rodbard (1971) range from 1.5 to 2.3 nm for lightly cross-linked gels, so the theoretical model is not bad. At 15-25% cross-linker the pore sizes range up to 3.5 nm, reflecting the great inhomogeneity of highly cross-linked dilute polymers (Zimm *et al.* 1958).

On the other hand, a similar calculation for agarose, assuming that the agarose fibres are bundles of twelve helices, as suggested by small-angle X-ray scattering (Djabourov *et al.* 1989) gives a value of approximately 14 nm for a 1 % gel, in wild discordance with the measured values of 100 nm or more. Nevertheless, Waki *et al.* (1982) found that the agarose fibres are distributed approximately randomly,

depending somewhat on the salt concentration, with a spacing of the order of 100 nm. Attwood *et al.* (1988) found similar spacing in their electron micrographs. Righetti *et al.* (1981), Serwer & Allen (1983), and Griess *et al.* (1989) found similar numbers using charged spherical particles of known size as electrophoretic probes. Griess *et al.* (1989) analysed their results using Ogston theory and Ferguson plots (mobility against gel concentration). Agarose gels are precipitation gels (Leone *et al.* 1987), which perhaps accounts for their coarse structure. West (1987*a*, *c*, 1988) has discussed the geometrical problems of gel structure.

So far we have discussed only linear DNA. Actually, circular molecules, both covalently closed circles which form supercoils and nicked circles which do not, usually migrate similarly to linear molecules though at somewhat different velocities. An exception is the behaviour of nicked circles larger than about 20 kb which migrate very slowly. This has been attributed to a 'hoop-and-stick effect' (Mickel *et al.* 1977) in which the open circle gets caught on a projecting gel fibre. Periodic reversal of the field eliminates the effect (Levene & Zimm, 1987), as might be expected.

A complication that has not received the attention that it probably deserves is the effect of randomness in the gel structure. It is known that diffusion in random media can be anomalous; for review see Havlin & Ben-Avraham (1987). Naghizadeh & Kovac (1986) made a computer simulation of random-flight chains diffusing with no applied field in a random array of rods and found a tendency for the chains to occupy selectively the larger spaces between the rods. Baumgärtner & Muthukumar (1987) made a similar simulation with a random array of cubic obstacles and compared the results to a simulation with a regular array of the same obstacles on a cubic lattice. They found very different results in the two cases. In the regular array the usual random-flight statistics for the radius of gyration were valid, and motion followed the reptation formula, diffusion constant proportional to the reciprocal of the chain length squared. (This corresponds to electrophoretic mobility varying as reciprocal length to the first power; mobility varies as length times the diffusion constant.) In the random array longer chains diffused much more slowly, diffusion constant varying approximately as the reciprocal cube of the length, and at the same time the chains became compressed so that their radius of gyration did not increase with chain length. Both phenomena were attributed to the chains becoming trapped in the larger spaces in the obstacle array.

By a scaling argument, reinforced by the simulation, Baumgärtner & Muthukumar concluded that these effects were functions of the universal variable, $N^{\frac{1}{2}}(1-p)$, N the number of Kuhn segments in the chain and p the fraction of space not occupied by the obstacles. Significant effects of the obstacles appeared when this variable was greater than unity. Putting in numbers, assuming that a Kuhn length is 0.4 kbp, and taking 0.01 as the concentration of the gel, we find that the variable reaches unity at a length of 4000 kbp. This is in fact near the upper limit of successful agarose-gel electrophoresis of DNA; molecules larger than the limit do not migrate through the gel.

The high exponent in the length dependence suggests a possible connection with the steep part of the master curve of Calladine.

Further simulations of random-obstacle fields have been reported by Muthukumar & Baumgärtner (1989 a, b) and by Honeycutt & Thirumalai (1990). The analytical theory has been developed further by Edwards & Muthukumar (1988) and Muthukumar (1989) using field-theoretic techniques, and by Honeycutt & Thirumalai (1989, 1990) using a simple mean-field approach. Only a preliminary study by simulation of the electrophoretic problem has been made (Melenkevitz & Muthukumar, 1990), but in this simulation, which had a strong electric field, the chains frequently broke out of the traps and moved in a way that was superficially similar to the other simulations described above.

8. CONCLUSION

If we now stop for an overview of the theory of the electrophoresis of DNA through gels, we see a decidedly mixed picture with some problems solved but some challenges unmet. The lack of a comprehensive and detailed theory for electrophoresis of molecules too small to be described by reptation is a major gap in our understanding. At the other extreme of molecular sizes, there is at present no explanation of why DNA molecules larger than about 10000 kb do not migrate through gels at all.

For molecules of intermediate size the theory is more satisfactory but still appears incomplete. The randomness of the gel structure may not have been satisfactorily incorporated. For some purposes it appears necessary also to consider the elastic properties of the gel. At low fields the by now 'classical' reptation theory has held sway, but is being challenged for molecules larger than a gel-dependent critical size by the random-obstacle results described in the last Section. At higher fields there seems to be general agreement that reptation theory is insufficient and more attention must be paid to the processes of trapping, breaking out of traps, and climbing over barriers.

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