

Solid Friction and Polymer Relaxation in Gel Electrophoresis

In a recent report (1), S. Burlatsky and J. Deutch propose a theoretical investigation of the influence of friction between a polyelectrolyte and a gel on the electrophoretic mobility of the polyelectrolyte. This question has received little attention in the past (2). The model proposed by Burlatsky and Deutch, based on solid friction, is simple and amenable to analytical solution. As they point out (1, p. 1782), this model leads to "significantly different behavior than [that] predicted by conventional theory." However, carefully comparing the new predictions (1) with available experimental data reveals contradictions.

First, there is not one, but two well-separated ranges of size within which flexible polyelectrolytes that are larger than the pores of a gel present mobilities, μ , that decay with their increasing size, L , faster than the dependence, L^{-1} , predicted by the biased reptation model (BRM) (3). The first range corresponds to moderate polyelectrolyte sizes [typically 2 to 20 kbp for duplex DNA in agarose (4), 1000 to 10,000 monomers for polystyrene sulfonate (5), and 200 to 1000 bases for single-strand DNA in acrylamide (6)] such as considered by Burlatsky and Deutch [reference 34 in (1)]. The second range corresponds to much longer molecules (typically above 1 Mb for duplex DNA in agarose), which have been reported to be immobilized by the gel (7, 8). We shall examine these two regimes separately to determine whether either can be adequately described by the proposed model.

The first mentioned deviation from the BRM prediction is rather moderate, it happens only in particular conditions (low electric fields or high gel concentration), and it is immediately followed by a saturation of μ/L to a value independent of L , known as the "compression band." This saturation, which may be accompanied by subtle band inversion effects (9), is the most spectacular and practically important feature of μ , and it is well accounted for by recent versions of the BRM (10). Burlatsky and Deutch predict a monotonic decrease of the apparent exponent of μ/L , which is consistent with an exponent of less than -1 , as has been transiently observed (4, 5), but not with the subsequent increase in the exponent of μ/L displayed by the same data sets (and well documented elsewhere). Another contradiction is that the freezing of chain configurations predicted in (1) is

field-independent, whereas experiments reveal a strong field dependence of the mobility in this range of polyelectrolyte size (4–6). Considering the mechanistic aspects of the model, we are concerned by the application of solid friction, which implies permanent contact, to macromolecules that may retain a strong Brownian character.

The typical size of Brownian fluctuations is most easily evaluated for duplex DNA, the physical properties of which have been studied at length. In the buffers used for gel electrophoresis, duplex DNA bears an effective charge of at most 0.1 electron per base pair (11). Let us consider a duplex DNA of, say, 10 kb in an electric field $E = 100$ V/m [that is, conditions similar to those in which Calladine *et al.* (4) report large deviations from L^{-1} mobility dependence]. Following the model of Burlatsky and Deutch, we assume that the chain adopts the conformation most favorable for solid friction to manifest: This corresponds to a "U-shape" conformation with one free arm (of at most 5 kb in the present case) stretched in the field, pulling on an entangled and unoriented "central section." With the parameters mentioned above, the maximum value of the tension (obtained at the top of the free arm) is on the order of $F = qE \approx 7.5 \times 10^{-15}$ Newton, where q is the electric charge of the arm and E is the electric field. At room temperature, the typical Brownian excursion under such tension, d , is on the order of $d \approx kT/F \approx 500$ nm, which is larger than the pore size of the gel. In practice, this means that even the parts of the chain submitted to the highest tension spend most of their time wiggling all around their surrounding pore and collide with the gel only from time to time. This does not rule out specific friction between the DNA and the gel, but to our understanding, it is incompatible with solid friction as proposed by Burlatsky and Deutch (1). For these reasons, the interpretation initially proposed by both Arvanitidou and Hoagland (5) and Mayer *et al.* (6), which is consistent with earlier theoretical work of Muthukumar and Baumgartner (12), seems correct: The divergence from BRM predictions (4–6) results from entropic trapping in larger pores and not from solid friction.

In the second regime, the abrupt decrease of mobility with size has been attributed to molecular trapping (7, 8). The mechanism by which such "freezing of

conformations" occurs has not yet received sufficient experimental and theoretical attention. The work of Burlatsky and Deutch is worth detailed consideration in this context because, for such long molecules, enormous tensions can build up and solid friction is more likely to be relevant. Unfortunately, available data in this regime also seem to contradict the predictions of Burlatsky and Deutch. First, all data agree with a strong field-strength dependence of the critical size above which trapping occurs (7, 8). In contrast, Burlatsky and Deutch predict a threshold for "freezing" of conformations independent of field. Second, they "expect the effect of solid friction on mobility to be more pronounced in pulsed, alternating electric field electrophoresis. . ." (1, p. 1783). According to their model, changing the direction of the field should favor U-shaped conformations with large values of L_2 , that is, a freezing of conformations. This prediction is also contradicted by experiments. We observed (9) that the threshold for trapping is larger (that is, the average mobility for a given DNA size is larger) for orthogonal field alternation at 90° than for a constant field. Corroborative experimental studies of the trapping of very large DNA are still scarce, but (on a more qualitative ground) it is well acknowledged among practitioners of pulsed fields that contour-clamped homogeneous electric field or orthogonal-field-alternation gel electrophoresis are able to pull into a gel very large chromosomes that would not enter it under constant-field conditions. Considering the rather striking irreversibility of DNA trapping in agarose gels, we proposed (8) a different interpretation, based on the tightening of topological "knots." The potential barrier involved in this approach may be seen as some kind of "solid friction," but it leads to conclusions different from, or opposite to, those proposed by Burlatsky and Deutch (1). (In particular, a DNA that is mobilized in a low field may be irreversibly arrested in a stronger one.)

In spite of these problems, we hope that the report by Burlatsky and Deutch and the present comment will renew the interest of theoreticians and experimentalists in the following question: Why is it necessary to decrease the field so dramatically in order to perform electrophoresis with large DNA, and (as a consequence) why can we not mobilize DNA larger than 6 or 10 Mb in a gel? At present, this question of considerable practical importance is answered neither by BRM, nor by the solid friction model proposed in the report (1).

Note added in proof: As mentioned by one reviewer of this comment, the inverse dependence of μ in relation to solvent

viscosity (13) also argues against solid friction.

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Response: Viovy and Duke do not offer quantitative comparisons between our or other model predictions, or experiments that would permit firm conclusions to be drawn. The solid friction model still seems the best starting point for explaining freezing of conformations, including field dependence. The treatment we offered in our report (1) was necessarily brief and did not include, for example, the electric field dependence of the factor α through the factor ϵ , which would take into account the likelihood of a segment interacting with gel points.

When the quantitative implications of electric field dependence in the solid friction model are fully worked out, we believe that many of the experimental observations noted by Viovy and Duke will be explained. For example, our unpublished analysis shows that, for large fields and long chains, there is a finite chain length mobility limit that is approached with an inverse electric field dependence. For smaller fields and

large chains, the mobility limit is inversely proportional to the field. Qualitative reasoning based on the solid friction model also explains why alternating fields are better able than constant fields to pull a large ionophore into a gel.

Only the solid friction model has the simple qualitative feature that relates conformational freezing to polymer length and thus provides the best basis for answering the question of DNA immobilization.

Our treatment did not include intersegment forces [note 29 in (1)] or thermal fluctuation [note 31 in (1)]. However, we do not agree with Viovy and Duke that Brownian motion might lead to less contact on average between entangled chain segments and the gel and hence to a smaller effect of solid friction. Thermal fluctuations will lead to more frequent contacts as well as to interruptions and thus should not change fundamentally the solid friction effect described in our report (1).

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