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Pulsed-field acceleration: The electrophoretic behavior of large spherical particles in agarose gels

In agarose gel electrophoresis, periodically inverting or interrupting the applied field may greatly accelerate the migration of polystyrene microspheres, in a manner varying with pulse times, and the observed zones are made sharper. The particles concerned are just large enough that under constant field they appear not to enter the gel at all or to migrate very slowly: and merely lowering the applied field may also enhance their electrophoretic migration, though to a lesser extent than with field pulsing. These effects may be accounted for by gel mesh flexibility which, varying with the nature of the migrating species, may either help or hinder migration.

1 Introduction

Pulsed- or varying-field gel electrophoresis (PFGE) [1] is used to separate DNA of more than 20 kilobases, the main effect being that the migration velocity is slowed progressively more the longer the molecule. Thinking that the supporting medium may play a more important role in PFGE than is commonly envisaged, experiments were undertaken, *inter alia*, using agarose gel electrophoresis of spherical polystyrene particles. Under appropriate conditions, a large increase in migration velocity can be obtained by periodically inverting or disconnecting the applied field-apparently opposite of what is seen with DNA. Polystyrene microspheres had previously been used to determine apparent pore sizes in agarose and similar media [2, 3], either as size standards [4] or for mechanistic studies [5, 6].

2 Materials and methods

2.1 Polystyrene microspheres

The particles were "Polybead" polystyrene microspheres (Polysciences, Warrington, PA, USA) of 59 ± 5.9, 112 ± 2.3, 210 ± 6.0, 247 ± 1.4 and 380 ± 3.6 nm (diameter ± standard deviation; data from supplier). They were provided as 2.5% suspensions in water and were diluted appropriately to give a final suspension varying from 0.025 to 0.875% of the particles in 5% w/v sucrose, 0.3% w/v sodium dodecyl sulfate (SDS), and 25 mmol/L sodium phosphate, pH 7.5. SDS was used with the objectives of preventing aggregation and approaching a uniform surface charge density, though no such assumption is required in interpreting the results. The microspheres from batches used in these experiments were of uniform dimensions, qualitatively, as seen under the electron microscope, except those of 210 nm nominal diameter which contained a minority population of smaller size, and gave rise to 2 zones on electrophoresis. (Results described below relate to the major zone – full-sized particles). The microspheres of 112 nm diameter migrated slightly faster than those of 59 nm: this was presumably due to a difference in inherent surface charge, but does not affect the conclusions.

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Abbreviations: PFGE, pulsed-field gel electrophoresis; **SDS,** sodium dodecyl sulfate

2.2 Electrophoresis

Agarose was "High Strength Analytical Grade" from Bio-Rad (Richmond, CA, USA). In the results reported, no correction is made for electroendosmosis. The gel was 12.8 cm long, in the electrophoresis direction, × 14.0 (wide) × 0.56 cm and was arranged horizontally in a home-made tank, totally submerged in 2 L of the buffer of Section 2.1. Origin slots were 0.15 cm in the electrophoresis direction, × 1.00 (wide) × 0.4 cm, arranged 3.4 cm from one end of the gel. The applied field and pulses were generated by Bio-Rad Pulsewave 760, with programming block. The voltage gradient was measured with a vacuum tube voltmeter, between two platinum electrodes applied to the surface of the gel. Experiments were conducted at room temperature; the observed temperature in the buffer tank was 25 ± 4° C.

3 Results

Observed mobility is defined as migration distance ÷ (time × field strength), and is always calculated from the algebraic time-average of the field in the forward (anode) direction. No variation of observed mobility with elapsed time could be detected. The observed mobility of 59 nm particles was apparently unaffected by field variation under any conditions tested, which permitted us to work in terms of relative mobility (defined as observed mobility ÷ observed mobility of 59 nm particles measured simultaneously on the same gel) and thus eliminate experimentally several sources of variability. However, the observed mobility of 59 nm particles under constant field was diminished in a manner dependent on agarose concentration, giving a linear Ferguson plot (Fig. 1) and a retardation coefficient of 0.163. Using these results, the main body of results can be re-expressed, if desired, in terms of absolute velocities, but we prefer not to do so.

Under a steady, unidirectional field of 1.8 V/cm, the 59 and 112 nm particles entered a 0.7% agarose gel and migrated normally, as distinct zones. The 210 nm particles entered, though they migrated very slowly. The 247 nm particles appeared not to enter, but did so at a lower field strength of 0.60 V/cm, a change which produced a higher relative mobility of 210 nm particles but had no detectable effect on the observed mobility of the 59 and 112 nm particles. Failure to enter the gel was not due to a peculiar structure of the surface layer of the gel, since (after entering the gel) either the 247 or the 210 nm particles could be arrested at any time by

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raising the field strength sufficiently, and then again resumed migration when the field strength was diminished to its previous value.

When periodical inversion of the field was introduced, there was a further, larger increase in observed mobility of the 210 and 247 nm particles. Figure 2 shows the results at a fixed absolute value of field strength (1.8 V/cm) and a fixed ratio of forward and reverse pulse duration, namely 2. This yields an average forward field of 0.60 V/cm (as for the observations with a steady, unidirectional field noted in the previous paragraph). Relative mobility increases dramatically as the pulse duration in each direction is diminished. A similar pattern of results is obtained if the field is not inverted, but merely interrupted (Fig. 3). Relative mobility increases as the absolute period of field interruption increases.

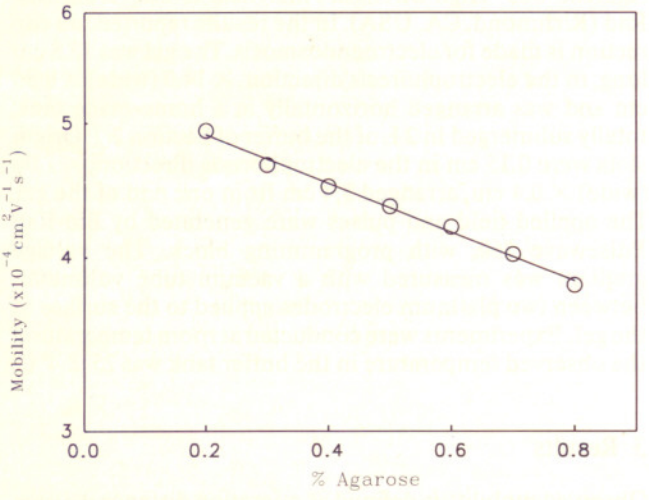

Figure 1. Observed mobility of 59 nm paticles: variation with agarose concentration.

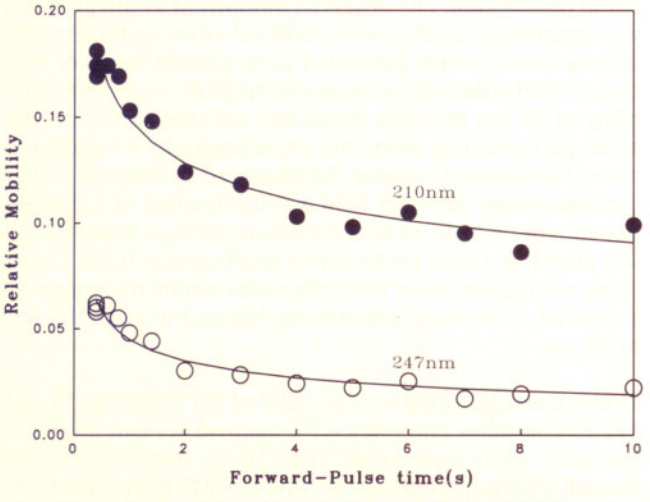

Figure 2. Relative mobility of 210 and 247 nm particles on field-inversion agarose gel electrophoresis in 0.7% agarose: variation with forward pulse time. Conditions, see Section 3. Relative mobility is mobility relative to 59 nm particles studied simultaneously on the same gel. Buffer, 25 mmol/L (sodium) phosphate, pH 7.5. Field strength, 1.8 V/cm. Ratio of forward/reverse pulse times = 2.0, so that net (average) forward field strength = 0.6 V/cm. ●, 210 nm particles, major (slower) band; ○, 247 nm particles. Particle concentration: 0.0625%.

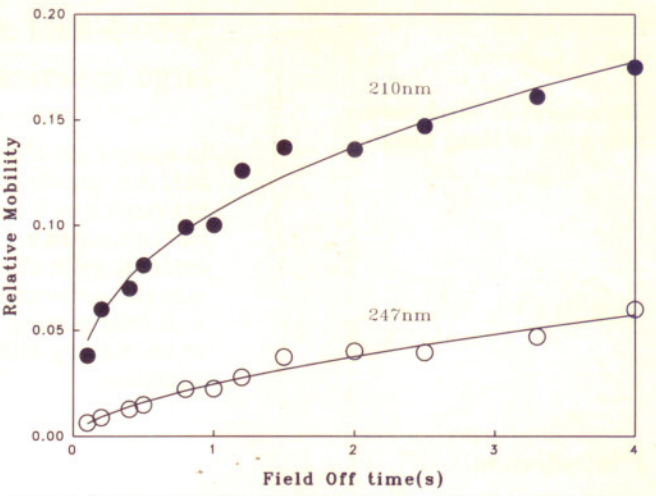

Figure 3. Relative mobility of 210 and 247 nm particles on interrupted-field agarose gel electrophoresis in 0.7% agarose: variation with null-field time. Definitions, symbols and conditions – see text and legend to Fig. 2. The forward pulse time (1.8 V/cm) was fixed at 2.0 s.

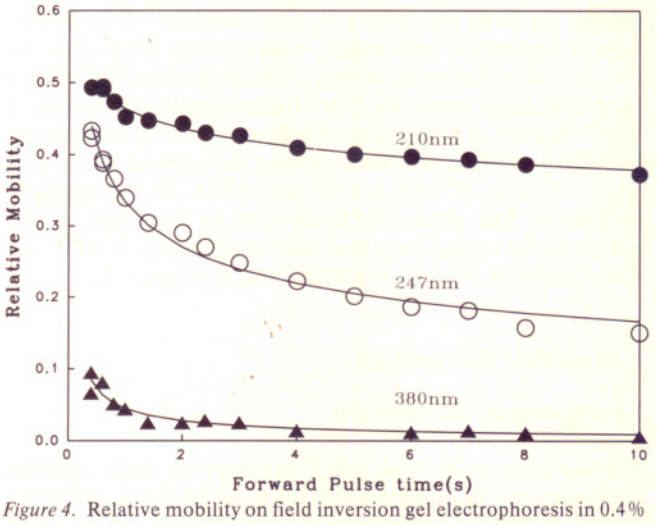

Figure 4. Relative mobility on field inversion gel electrophoresis in 0.4% agarose: variation with forward pulse time. ▲, 380 nm. Particle concentration: 0.025%. Other definitions, symbols and conditions, see text and legend to Fig. 2.

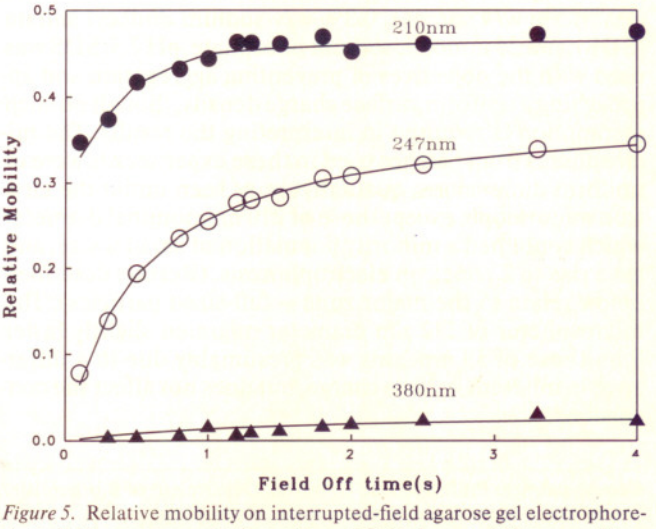

Figure 5. Relative mobility on interrupted-field agarose gel electrophoresis in 0.4% agarose: variation with null-field time. ▲, 380 nm particles. Other definitions, symbols and conditions, see text and legends to Figs. 2 and 4.

In 0.4% agarose, similar effects were observed, but at a different range of relative migration velocities, and it also was possible to study migration of 380 nm particles (Figs. 4 and 5). Figure 6 shows that bands are sharper under a pulsed than under a constant field, and there is also a dramatic acceleration, especially of 247 and 380 nm particles, under a pulsed field in 0.4% agarose. Similar observations were made in 0.7% agarose for 210 and 247 nm particles. Experiments were conducted to determine whether the passage of such very large particles had damaged the gel. Upon reversing the direction of the net field and hence of migration, no change was found in observed mobility between the first direction and rerunning over the same track.

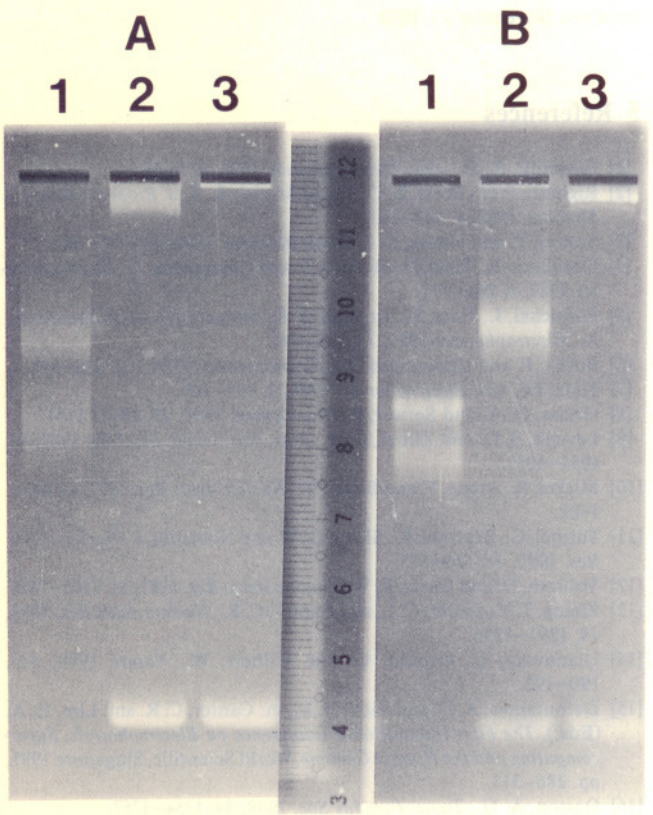

Figure 6. The effect of pulsed field on migration of particles in 0.4% w/v agarose gel. (A) Electric field, 1.80 V/cm, 2.7 h. (B) Electric field, 1.80 V/cm pulsed with 2.0 s forward and 1.0 reverse, 8.1 h. Lanes: (1) particles of nominal diameter, 210 nm: two zones appear, of which the upper, slower-migrating zone consists of particles of about 210 nm while the faster-migrating zone is contaminating particles of smaller size. (2) Mixture of 247 nm and 59 nm particles. (3) Mixture of 380 nm and 59 nm particles. (Concentration of 59 nm particles in both lanes: 0.875%.) Conditions otherwise, including final concentration of each type of particle used, as in legends to Figs. 2 and 4.

4 Discussion

Righetti *et al.* [2] reported entry to agarose gel under low-field conditions, of plastic microspheres which were apparently excluded at higher field strengths. Their results leave open, however, whether this was due to a special structural state of the gel at its surface. Gombocz *et al.* [4] note that, for their largest-sized particles, mobility was diminished at higher field strengths, but they give few details (see also [7]). Our experiments were completed before we heard of the work of Griess and Serwer [8]. These authors used larger particles than ours, in more dilute agarose gels, obtaining results qualitatively resembling those described above, in that particles trapped under an electric field could be released by field interruption or inversion and would then resume their net forward movement. The continuous, quantitative relationships of mobility to pulse duration, described in the present paper, have apparently not been recorded elsewhere.

Trapping or slowing have been reported for certain DNA molecules (if the field strength is too high) with relief of trapping, and band sharpening, at lower field strengths or if the field is modulated [9–13]. The trapping mechanism which underlies the phenomena described here, however, must be quite different. (The case of DNA-protein complexes [14, 15] may be more nearly similar to the effects we have studied.) A plausible explanation of these results must acknowledge that larger particles become trapped in the polymer network as a consequence of their movement induced by electrophoresis, and that the trapping is released by reversing or interrupting the field. We suggest that the particle is thus given another chance to penetrate the gel network in another place when the original field is restored, and this cycle of trapping and release is repeated again and again during the experiment. The observed sharpening of particle bands can be rationalized by suggesting that during an experiment at constant field, and provided the field is not too large, detrapping may occur spontaneously by thermal motion. (Griess and Serwer [8] saw this under the microscope.) But spontaneous detrapping is a stochastic process and trapping events may be long-lived with possibly an exponential decay of the "trapped" condition. Under pulsed-field, however, the trapping event is terminated by an externally applied force, and this occurs simultaneously for all particles which remain trapped at the moment of field reversal.

The trapping observed here cannot be explained by entanglement arising from the elongated and flexible nature of the migrant particle, as is suspected in the case of DNA. We can start, perhaps, with an image of a massive object being pressed up against a flexible mesh by its own movement under the influence of an electric field. The mesh is deformed (compressed) so as to become more and more dense in the zone just ahead of the particle and so that more and more elements of the mesh are brought into play. Finally, the elastic resistance of a sufficient number of mesh elements brings the object to rest. There is a balance of forces. If the field is switched off, the immobilized object probably moves backwards a little by elastic recoil of the mesh, but, unless it has time to diffuse away from the potentially trapping local mesh configuration, may be trapped again when the field is restored. Thus relative mobility should increase with zero-field time (Figs. 3 and 5). But actual reversal of the field makes certain that the particle will migrate completely away, allowing the mesh to return to its original, unstressed configuration and the particle to try again in a new place. A long forward pulse is time wasted in the trapped condition, so that in this case relative mobility is inversely related to pulse time (Figs. 2 and 4).

Since the medium appears to be undamaged after passage of these large particles, and its properties unaltered, we

must conclude further that a particle above the apparent limiting pore size can always find its way through our meshwork, provided it is allowed to try repeatedly, and that such particles do not simply tear the mesh to pieces. It follows that the apparent pore size limit is illusory and there is not a regular mesh of fixed pore dimensions, or at least that such a mesh is interrupted by a proportion of much larger gaps, which also must be readily accessible. Indeed, such gaps must communicate with each other to the extent that large-dimension channels can be opened up throughout the gel without apparent damage – and must therefore have been present from the outset, at least potentially. This experimental conclusion can be accommodated by the Ogston model [16] of gel structure and subsequent developments [7], combined with acknowledging that gel mesh elements are also flexible in the sense of being deformable [15] and susceptible to thermal motion [17].

It is popular, however, to treat gels as having pores of a definite size or distribution of sizes [2, 3] and it is not clear whether such theories may not also fit our data [18] provided that due account is again taken of the anomalies which may result from deformability of mesh elements [15]. There is independent evidence of this deformability or mobility in the case of agarose [19]. Deformation of the gel mesh, under impact, should be considered in relation to the size, charge, momentum, and kinetic energy of the moving particles. Our experiments so far refer to very large particles compared, for instance, with protein molecules. Nevertheless, it is reasonable to forecast that similar pulsed-field acceleration effects will be observed with much smaller particles (or large molecules) and more readily deformed gel elements, notably polyacrylamide: but it is likely that this will be observed only with high-frequency field modulation. Conversely, Brassard *et al.* [20] found a slowing of migration of SDS-protein complexes under field-interruption regimes.

Current theories as to the mechanism of PFGE and of the trapping of DNA molecules on electrophoresis suppose that the flexible migrating molecule is entangled with a relatively rigid gel mesh. By constrast, we may view the trapping and release phenomena described here as due to the entanglement of a flexible gel mesh with a rigid and massive migrating particle – a complete inversion of the theoretical explanation, just as our experimental findings are the opposite of the dominant effect with PFGE of DNA. Though not usually considered in the theory used to date (but see [15]), flexibility of the gel mesh could no doubt be involved in the behavior observed with DNA and this is made the more probable by noting that the masses of the particles concerned are similar (200 nm sphere of density 1.0 g/cm^3; 4.2×10^{-15} g: 2 Mbp DNA; 2×10^{-15} g). Exemplary figures are also easily provided for mobility in free solution and the radius of gyration of DNA molecules, but the most important thing to know would be the force imposed on the gel mesh, per unit of cross-sectional area, by an arrested, charged particle in an electric field – and this is more difficult to estimate with any confidence.

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5 References

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