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Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution

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Abstract

This review describes the electrophoresis of curved and normal DNA molecules in agarose gels, polyacrylamide gels and in free solution. These studies were undertaken to clarify why curved DNA molecules migrate anomalously slowly in polyacrylamide gels but not in agarose gels. Two milestone papers are cited, in which Ferguson plots were used to estimate the effective pore size of agarose and polyacrylamide gels. Subsequent studies on the effect of the electric field on agarose and polyacrylamide gel matrices, DNA interactions with the two gel matrices, and the effect of curvature on the free solution mobility of DNA are also described. The combined results suggest that the anomalously slow mobilities observed for curved DNA molecules in polyacrylamide gels are due primarily to preferential interactions of curved DNAs with the polyacrylamide gel matrix; the restrictive pore size of the matrix is of lesser importance. In free solution, DNA mobilities increase with increasing molecular mass until leveling off at a plateau value of $(3.17 \pm 0.01) \times 10^{-4}$ cm^2/Vs in 40 mM Tris-acetate-EDTA buffer at 20°C. Curved DNA molecules migrate anomalously slowly in free solution as well as in polyacrylamide gels, explaining why the Ferguson plots of curved and normal DNAs containing the same number of base pairs extrapolate to different mobilities at zero gel concentration.

Keywords

agarose gels; capillary electrophoresis; DNA; free solution mobility; polyacrylamide gels

1 Historical overview

The study of DNA electrophoresis began in 1964, when three groups of investigators [1-5] measured the mobility in free solution using moving boundary methods. They found that the mobility was independent of size for DNA molecules larger than ~400 base pairs (bp) [5], and varied with ionic strength [3,5] and the identity and valence of the cation in the background electrolyte [2,3]. At about the same time, inspired by the separation of proteins in synthetic gel matrices [6-10], other investigators began to use similar matrices to separate RNA [11-19] and DNA molecules [15,20-24] by molecular mass. The separation matrices included agar [11,18,20,21], agarose [22,23], polyacrylamide [13,14,19,24-28] and composite agarose-acrylamide [16,17] gels. As electrophoretic methods were improved by the purification of agarose [29-31] and the use of slab gels instead of tube gels [25,32], and as the discovery of restriction enzymes allowed the preparation of monodisperse DNA fragments of known size [33,34], it became apparent that the separation of DNA fragments by molecular mass depended on the gel matrix in which the separation was carried out

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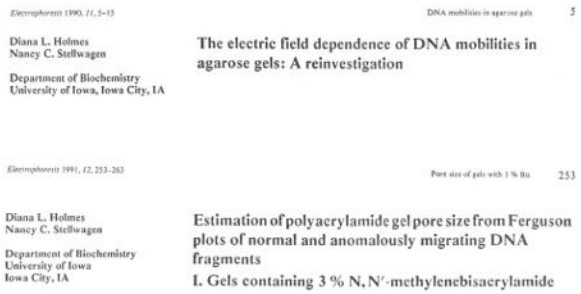
[33,35]. Electron microscopy experiments [36] indicated that the mobilities observed in agarose gels accurately reflected DNA molecular mass, while the mobilities observed in polyacrylamide gels did not. More detailed studies [37,38], carried out with restriction fragments ranging from 40 to 4000 base pairs (bp) in size, showed that DNA mobilities decreased monotonically with increasing molecular mass in agarose gels, but that ~25% of the same fragments migrated anomalously slowly in polyacrylamide gels.

Many investigators then turned their attention to the physical properties of DNA that could be responsible for the anomalously slow mobilities observed for certain restriction fragments in polyacrylamide gels. It was soon discovered that the largest mobility anomalies were observed for DNA molecules containing A-tracts, runs of 4 – 6 contiguous adenine residues repeated about every 10 base pairs, in phase with the helix repeat [39-41]. Other studies, using a variety of techniques [42-48], showed that DNA molecules that migrated anomalously slowly in polyacrylamide gels had helix backbones that were curved, not straight. Since curved DNA molecules have shorter end-to-end lengths and larger cross-sectional areas than linear molecules containing the same number of base pairs, curved DNAs would require larger pores to migrate through the gel matrix, behaving electrophoretically as though they were larger than their true sizes [40,47].

An equally important question is why curved, A-tract-containing DNA molecules migrate anomalously slowly in polyacrylamide gels, but not in agarose gels. The mobility differences in the two gel media are illustrated in Fig. 1, where the mobilities of monomers, dimers, trimers and higher multimers of normal (N) and anomalous (A) 167-bp DNA fragments are compared. As shown in the left panel, normal (N) and anomalous (A) multimers of equal size migrate with equal mobilities in 2% agarose gels, as expected because of their equal molecular masses. However, as shown in the right panel, the anomalous multimers (A) migrate more slowly than their normal counterparts (N) in polyacrylamide gels containing 5.7% and 1.5%C. Similar results are observed for polyacrylamide gels with different compositions [50-52].

Several obvious questions therefore arise. Do agarose and polyacrylamide gels, on average, have different pore sizes and are these differences responsible for the anomalously slow mobilities observed in polyacrylamide gels? Does the electric field affect agarose and polyacrylamide gels differently, and can these differences explain the results? Do DNA molecules interact with agarose and polyacrylamide gels during electrophoresis and, if so, do curved and normal DNA molecules interact with the two matrices differently? To answer these questions, we have undertaken detailed studies of DNA electrophoresis in agarose and polyacrylamide gels. Two of these studies [51,52], which were designed to measure apparent gel pore size, are being cited in this issue of *Electrophoresis* as milestone papers:



Electrophoresis 1990, *11*, 5–15 DNA mobilities in agarose gels 5

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The electric field dependence of DNA mobilities in agarose gels: A reinvestigation

Electrophoresis 1991, *12*, 253–262 Pore size of gels with 3 % Bis 253

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Estimation of polyacrylamide gel pore size from Ferguson plots of normal and anomalously migrating DNA fragments
I. Gels containing 3 % N, N′-methylenebisacrylamide

These papers have been cited 59 times and 54 times, respectively, through 2008. The results obtained in the milestone papers and related studies, along with subsequent work on the effect of the electric field on agarose and polyacrylamide gels and interactions of the two gel

matrices with curved and normal DNA molecules, are described in the next three sections of this review. The following section describes the mobility of DNA in free solution. A brief summary concludes the paper.

2 Apparent gel pore size

2.1 Agarose gels

Agarose is an alternating copolymer of 1,3-linked β-D-galactose and 1,4-linked 3,6-anhydro-α-L-galactose, infrequently substituted with carboxylate, pyruvate and/or sulfate residues [29-33]. Agarose molecules in solution have a random coil structure at high temperatures [33-35]. Upon cooling, the agarose chains form helical fiber bundles held together by noncovalent hydrogen bonds; gelation occurs at still lower temperatures when the fiber bundles become linked together in "junction zones" by the formation of additional hydrogen bonds [35-40]. Strand partner exchange occurs in the junction zones by hydrogen bond rearrangements [32,33,38].

The effective pore size of agarose gels can be estimated from Ferguson plots (log mobility vs. gel concentration [65]) of DNA molecules of different sizes. Assuming a Gaussian distribution of pore sizes, the median pore radius of the gel in which the mobility of a given DNA molecule is reduced to one-half its mobility at zero gel concentration is equal to the radius of gyration of that DNA [51,66-68]. Ferguson plots, such as those illustrated in Fig. 2, were measured for normal DNA molecules ranging in size from 0.5 to 12.2 kilobase pairs in the first milestone paper cited above. The median pore radius of a 1% agarose gel was found to be ~100 nm [51], similar to the pore sizes obtained in other electrophoretic studies [67-71]. However, the estimated gel pore radius depends somewhat on the method used to determine it. Agarose gel pore radii estimated from lattice models of DNA gel electrophoresis [67,72] tend to be ~2-fold smaller than those determined by Ferguson plot methods, while the gel pore radii measured by NMR [73] or atomic force microscopy (AFM) [74] are ~2-fold larger. One could argue that the gel pore radii determined by NMR or AFM methods are more accurate, since the values determined by electrophoretic methods represent the subset of pores that are accessible to the migrating DNA molecules. However, the average size of the electrophoretically accessible pores in a given gel matrix is probably more relevant for interpreting gel electrophoresis experiments.

2.2 Polyacrylamide gels

Polyacrylamide gels are chemically crosslinked gels formed by the reaction of acrylamide with a bifunctional crosslinking agent such as N,N′-methylenebisacrylamide (Bis). The composition of the gel is given by %T, the total (w/v) concentration of acrylamide plus crosslinker, and %C, the (w/w) percentage of crosslinker included in %T. Polyacrylamide gels are polydisperse in structure, because Bis polymerizes with itself more rapidly than with acrylamide [74-81]. For this reason, polyacrylamide gels consist of highly crosslinked, Bis-rich nodules linked together by sparsely crosslinked, relatively acrylamide-rich fibers [75,80,81].

Because of the structural heterogeneity of the polyacrylamide gel matrix, the effective pore size determined by electrophoretic methods depends on the size of the analyte. If proteins are used as the analytes, the apparent pore size corresponds to the pores in the Bis-rich nodules [82]. Larger analytes, such as DNA, do not "see" the small pores in the nodules and instead are retarded by migration through the acrylamide-rich fibers [82]. In the second milestone paper cited above [52], Ferguson plots were measured for DNA molecules ranging in size from 123 to 1600 bp in polyacrylamide gels containing 3.5% to 10.5%T and 3% Bis. Typical examples of the Ferguson plots obtained for multimers of 167-bp curved and normal DNA fragments are illustrated in Fig. 3. The Ferguson plots obtained for the

curved multimers are steeper in slope and extrapolate to lower mobilities at zero gel concentration than the Ferguson plots of their normal counterparts. Analysis of the Ferguson plots indicates that the effective pore radius ranges from 20 to 140 nm in gels containing 3.5% to 10.5%T and 0.5% to 10%C [52,83]. Similar gel pore radii have been observed by scanning and transmission electron microscopy [84-86]. However, NMR relaxation experiments [72] have suggested that the average pore radius is ~5-fold smaller, possibly because this technique measures an average of the nodule and gel fiber pore radii.

3 Effect of the electric field on agarose and polyacrylamide gels

The mobilities observed for DNA molecules in agarose gels are highly dependent on the electric field applied to the gel [51,87], most likely because the electric field disrupts the hydrogen bonds in the junctiones zones, allowing the gel fibers and fiber bundles to orient in the electric field [88,89]. The oriented gel fibers and fiber bundles are very large, ranging up to 22 μm in length [88]. Surprisingly, the gel fibers and fiber bundles orient in the perpendicular direction when the electric field is reversed in polarity [89]. The resulting “flip-flop” orientation and reorientation of agarose fibers and fiber bundles in reversing electric fields provides a mechanism for creating transient pores in the gel matrix, allowing very large DNA molecules to migrate through the gel during pulsed field gel electrophoresis [89,91].

The orientation of the agarose gel fibers and fiber bundles also affects electrophoresis in unidirectional electric fields. If an agarose gel is pre-electrophoresed in a direction perpendicular to the eventual direction of electrophoresis, linear DNA molecules travel in lanes skewed toward the side of the gel, as though the pre-electrophoresis had created pores or channels in that direction [93]. The lanes gradually straighten out and become aligned in the parallel direction as electrophoresis is continued, presumably because the gel fibers and fiber bundles gradually become oriented in the new field direction. Lanes skewed toward the side of the gel are not observed if a gel is pre-electrophoresed in the direction in which electrophoresis will be carried out, or if a gel oriented in the perpendicular direction is allowed to stand for 24 hours before use [93]. Hence, the oriented agarose gel fibers and fiber bundles become randomized upon standing, presumably by rearrangement of the hydrogen bonds in the junction zones. DNA mobilities in such randomized gels are identical to those observed in gels that have not been subjected to pre-electrophoresis [93].

Polyacrylamide gels, which are chemically crosslinked, are distorted somewhat by the electric field, but significant orientation of the gel fibers does not occur [89]. For this reason, DNA mobilities observed in polyacrylamide gels are essentially independent of the electric field strength used for electrophoresis [38,50].

4 DNA interactions with agarose and polyacrylamide gel matrices

4.1 Agarose gels

According to the Ogston-Rodbard-Chrambach theory of gel electrophoresis [94,95], the mobility of a polyelectrolyte in a gel matrix is determined by the fractional volume of the gel that is accessible to the migrating macromolecules. Hence, Ferguson plots are expected to extrapolate to the free solution mobility of an analyte at zero gel concentration. For DNA, the Ferguson plots should extrapolate to a common intercept at zero gel concentration, because the free solution mobilities of DNA molecules larger than ~400 bp are independent of molecular mass [5,96]. However, as shown for agarose gels in Fig. 2, the intercepts at zero gel concentration decrease monotonically with increasing DNA size. Hence, DNA molecules migrating in agarose gels appear to be retarded by a molecular mass-dependent mechanism that occurs in addition to sieving. This molecular mass-dependent effect is most

likely the transient interaction of the DNA molecules with the agarose gel fibers during electrophoresis. Such an interaction is not surprising, since DNA is a highly negatively charged polyelectrolyte and agarose molecules are known to bind anions [97,98]. Other studies have also indicated that DNA molecules interact with the agarose gel matrix during electrophoresis [review: 99]. If the mobilities observed at zero agarose gel concentration are extrapolated linearly to zero DNA molecular mass, the resulting mobility is $(3.0 \pm 0.1) \times 10^{-4}$ cm^2/Vs in 40 mM Tris-acetate-EDTA buffer [100], very close to the mobility observed in free solution in the same background electrolyte [96].

4.2 Polyacrylamide gels

The Ferguson plots observed for DNA molecules in polyacrylamide gels extrapolate to very different mobilities at zero gel concentration, as shown in Fig. 3 [52,82,101]. If the mobilities observed at zero gel concentration are extrapolated linearly to zero DNA molecular mass, the free solution mobility of DNA is calculated to be $(3.1 \pm 0.1) \times 10^{-4}$ cm^2/Vs in 40 mM Tris-acetate-EDTA buffer, equal within experimental error to the value obtained from extrapolation of the Ferguson plots observed in agarose gels [100]. Hence, DNA molecules electrophoresed in polyacrylamide gels are also retarded by a molecular mass-dependent mechanism that occurs in addition to sieving, most likely transient interactions of the migrating DNA molecules with the gel matrix [52,83,100,103]. Curved DNA molecules appear to be more retarded by this mechanism than normal DNAs containing the same number of base pairs, because the Ferguson plots of curved DNAs have steeper slopes and extrapolate to somewhat lower mobilities at zero gel concentration (compare the solid and dashed lines in Fig. 3).

The relative importance of sieving and gel matrix interactions to the anomalously slow mobilities observed for curved DNA molecules in polyacrylamide gels can be evaluated by measuring the mobilities of curved and normal DNAs in gels in which the pore size is varied by changing %T at constant %C (the usual method of changing gel pore size) and by changing %C at constant %T. If sieving effects are the primary factor contributing to the anomalously slow mobilities, the mobility anomalies should be independent of the method used to vary the gel pore size. However, if preferential interactions of curved DNA molecules with the polyacrylamide gel fibers are responsible for the anomalously slow mobilities, the mobility anomalies should correlate with the acrylamide concentration in the gel, not the apparent gel pore size. When the polyacrylamide gel pore size is decreased by increasing %T at constant %C, the anomalously slow mobilities of the curved DNA molecules increase with decreasing gel pore radius [52,101,103], as though sieving effects were responsible for the mobility anomalies [39,104]. However, when the gel pore size is varied by changing %C at constant %T, the anomalously slow mobilities are *independent* of gel pore radius as long as the apparent gel pore radius is larger than the DNA radius of gyration [103]. If the DNA radius of gyration is larger than the apparent gel pore radius, the anomalously slow mobilities of the curved DNAs decrease with increasing gel pore radius until the gel pore radius becomes equal to the DNA radius of gyration, after which the mobility anomalies become constant and independent of gel pore size. Hence, the interaction of curved DNA molecules with the acrylamide-rich gel fibers appears to be the primary cause of the anomalously slow mobilities observed for curved DNAs in polyacrylamide gels; sieving effects are of secondary importance.

5 DNA mobility in free solution

5.1 Dependence of free solution mobility on DNA molecular mass

The free solution mobility of DNA is often assumed to be independent of molecular mass, because the linear charge density is essentially constant. The early moving boundary

electrophoresis measurements of DNA solutions [5], using relatively polydisperse DNA samples, were consistent with this assumption. However, more accurate mobility measurements carried out by capillary electrophoresis, using monodisperse DNA samples, have shown that this assumption is not correct [96,105]. The mobilities of small DNA molecules increase with increasing molecular mass, as shown by the open circles in Fig. 4, before leveling off and reaching a constant plateau value for DNAs larger than ~400 bp. Since theoretical studies have suggested that the mobilities of charged rods should increase with increasing molecular mass [106,107], the results in Fig. 4 suggest that small DNA molecules are rigid and rod-like in solution. DNAs larger than ~400 bp begin to adopt a random coil conformation and migrate as free-draining coils [5,96,105,108,109]; the mobility is then dependent only on the effective charge per unit mass. The plateau mobility observed for DNA molecules ≥400 bp in size in 40 mM Tris-acetate-EDTA buffer is $(3.17 \pm 0.01) \times 10^{-4}$ cm^2/Vs [110], essentially equal to the mobilities calculated by the double extrapolation of the Ferguson plots obtained in agarose and polyacrylamide gels to zero gel concentration and zero DNA molecular mass. Hence, the double extrapolation procedure leads to reasonably accurate values of the free solution mobility of DNA.

5.2 Dependence of free solution mobility on DNA curvature

The free solution mobilities of curved DNA molecules (as well as the mobilities observed in polyacrylamide gels) depend on the extent of curvature of the helix backbone [110]. This effect is illustrated by the closed circles in Fig. 4, which correspond to 199-bp DNA fragments containing 1 – 5 A-tracts (from top to bottom) located in a “curvature module” [111] in the center of each fragment. The fragment with a single A-tract in the curvature module has the conformation of normal DNA [111] and exhibits a mobility that falls on the same smooth curve describing the mobilities of other normal DNA fragments. However, fragments containing 2 – 5 A-tracts in the curvature module, which become increasingly more curved as the number of A-tracts increases [111,112], migrate increasingly more slowly in free solution. The anomalously slow mobilities observed for curved DNAs in free solution are due in part to the dependence of the frictional factor on molecular shape [112] and in part to the preferential binding of monovalent cations by DNA A-tracts, which decreases the effective net charge [112,113].

6 Concluding remarks

The milestone papers cited in this review were the first of a series of papers designed to better understand why curved DNA molecules migrate anomalously slowly in polyacrylamide gels, but not in agarose gels. The milestone papers showed that the restrictive pore size of the polyacrylamide gel matrix is not the primary cause of the mobility anomalies, since gels with equivalent apparent pore sizes can be cast in both gel matrices. The milestone papers also suggested that DNA molecules interact with agarose and polyacrylamide gels during electrophoresis, since the Ferguson plots do not extrapolate to a common intercept at zero gel concentration. The free solution mobility of DNA can be recovered if the mobilities observed at zero gel concentration are also extrapolated to zero DNA molecular mass.

Subsequent work, also described here, showed that the anomalously slow mobilities observed for curved DNA molecules in polyacrylamide gels are correlated with the acrylamide concentration in the gel matrix, not the gel pore size, because the mobility anomalies are observed even in gels in which the effective gel pore radius is larger than the DNA radius of gyration. Hence, curved DNA molecules appear to have a greater affinity for the acrylamide-rich gel fibers than normal DNAs containing the same number of base pairs. Mobility differences between curved and normal DNA molecules containing the same number of base pairs are not observed in agarose gels, most likely because the agarose gel

fibers are oriented by the electric field, creating transient pores in the gel matrix. Since the orienting fibers and fiber bundles are very large, the transient pores are insensitive to small differences in DNA conformation.

The free solution mobility of DNA increases with increasing molecular mass until leveling off at a constant plateau value for fragments larger than ~400 bp. Curved DNA molecules that migrate anomalously slowly in polyacrylamide gels also migrate anomalously slowly in free solution, most likely because the curved DNAs usually contain multiple A-tracts and A-tracts are known to bind monovalent cations [114]. Monovalent cation binding by DNA A-tracts would decrease the effective charge of curved DNAs, decreasing the observed mobility [110,112,113]. However, a detailed discussion of cation binding by DNA A-tracts is another story for another day.

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References

1. Ross PD. Biopolymers. 1964; 2:9–14.
2. Ross PD, Scruggs RL. Biopolymers. 1964; 2:231–236.
3. Ross PD, Scruggs RL. Biopolymers. 1964; 2:79–89.
4. Costantino L, Liquori AM, Vitagliano V. Biopolymers. 1964; 2:1–8.
5. Olivera BM, Baine P, Davidson N. Biopolymers. 1964; 2:245–257.
6. Smithies O. Biochem J. 1955; 61:629–641. [PubMed: 13276348]
7. Raymond S, Nakamichi M. Anal Biochem. 1962; 3:23–30. [PubMed: 14490716]
8. Ornstein L. Ann N Y Acad Sci. 1964; 121:321–349. [PubMed: 14240533]
9. Tombs MP. Anal Biochem. 1965; 13:121–132.
10. Fawcett JS, Morris CJOR. Sep Sci. 1966; 1:9–26.
11. Bachvaroff R, McMaster PRB. Science. 1964; 143:1177–1179. [PubMed: 14108301]
12. Richards EG, Gratzer WB. Nature. 1964; 204:878–879. [PubMed: 14235710]
13. Richards EG, Coll JA, Gratzer WB. Anal Biochem. 1965; 12:452–471. [PubMed: 5859509]
14. Richards EG, Lecanidou R. Anal Biochem. 1971; 58:43–71. [PubMed: 4928341]
15. Bishop DHL, Claybrook JR, Spiegelman S. J Mol Biol. 1967; 26:373–387. [PubMed: 5339793]
16. Peacock AC, Dingman CW. Biochemistry. 1968; 7:668–674. [PubMed: 4868544]
17. Dahlberg AE, Dingman CW, Peacock AC. J Mol Biol. 1969; 41:139–147. [PubMed: 4979520]
18. Tsanev R, Staynov D, Kokileva L, Mladenova I. Anal Biochem. 1969; 30:66–85. [PubMed: 4892964]
19. Philippsen P, Zachau HG. Biochim Biophys Acta. 1972; 277:523–538. [PubMed: 4341777]
20. Thorne GV. Virology. 1966; 29:234–239. [PubMed: 4287545]
21. Thorne GV. J Mol Biol. 1967; 24:203–211. [PubMed: 4291530]
22. Aaij C, Borst P. Biochim Biophys Acta. 1972; 269:192–200. [PubMed: 5063906]
23. Helling RB, Goodman HM, Boyer HW. J Virology. 1974; 14:1235–1244. [PubMed: 4372397]
24. Maniatis T, Jeffrey A, van deSande H. Biochemistry. 1975; 14:3787–3794. [PubMed: 1174504]
25. Studier FW. J Mol Biol. 1973; 79:237–248. [PubMed: 4760132]
26. Jeppesen PGN. Anal Biochem. 1974; 58:195–207. [PubMed: 4363434]
27. Flint DH, Harrinigton RE. Biochemistry. 1972; 11:4858–4864. [PubMed: 4655257]
28. Elson E, Jovin TM. Anal Biochem. 1969; 27:193–204. [PubMed: 5767178]
29. Hickson TGL, Polson A. Biochim Biophys Acta. 1968; 165:43–58. [PubMed: 5672843]

30. Duckworth M, Yaphe W. Carbohydr Res. 1971; 16:189–197.
31. Duckworth M, Yaphe W. Carbohydr Res. 1971; 16:435–445.
32. McDonell MW, Simon MN, Studier FW. J Mol Biol. 1977; 110:119–146. [PubMed: 845942]
33. Roberts RJ. Crit Rev Biochem. 1976; 4:123–164. [PubMed: 795607]
34. Modrich P. Q Rev Biophys. 1979; 12:315–369. [PubMed: 232555]
35. Zeiger RS, Salomon R, Dingman CW, Peacock AC. Nature New Biol. 1972; 238:65–69. [PubMed: 4625705]
36. Thomas M, Davis RW. J Mol Biol. 1975; 91:315–328. [PubMed: 1102702]
37. Stellwagen NC. Biochemistry. 1983; 22:6180–6185. [PubMed: 6318809]
38. Stellwagen NC. Biochemistry. 1983; 22:6186–6193. [PubMed: 6318810]
39. Wu HM, Crothers DM. Nature. 1984; 308:509–513. [PubMed: 6323997]
40. Koo HS, Wu HM, Crothers DM. Nature. 1986; 320:501–506. [PubMed: 3960133]
41. Hagerman PJ. Proc Natl Acad Sci USA. 1984; 81:4632–4636. [PubMed: 6087336]
42. Hagerman PJ. Biochemistry. 1985; 24:7033–7037. [PubMed: 4084556]
43. Levene SD, Wu HM, Crothers DM. Biochemistry. 1986; 25:3988–3995. [PubMed: 3017412]
44. Stellwagen NC. Biopolymers. 1991; 31:1651–1667. [PubMed: 1814510]
45. Muzard G, Théveny, Révet B. EMBO. 1990; 9:1289–1298.
46. Griffith J, Bleyman M, Rauch CA, Kitchen PA, Englund PT. Cell. 1986; 46:717–724. [PubMed: 3742596]
47. Macdonald D, Herbert K, Zhang X, Polgruto T, Lu P. J Mol Biol. 2001; 306:1081–1098. [PubMed: 11237619]
48. Barbič A, Zimmer DP, Crothers DM. Proc Natl Acad Sci USA. 2003; 100:2369–2373. [PubMed: 12586860]
49. Stellwagen NC. Electrophoresis. 2003; 24:3467–3475. [PubMed: 14595693]
50. Stellwagen A, Stellwagen NC. Biopolymers. 1990; 30:309–324. [PubMed: 2177663]
51. Holmes DL, Stellwagen NC. Electrophoresis. 1990; 11:5–15. [PubMed: 2318191]
52. Holmes DL, Stellwagen NC. Electrophoresis. 1991; 12:253–263. [PubMed: 2070781]
53. Rees, DA.; Morris, ER.; Thom, D.; Madden, JK. The Polysaccharides. Aspinall, GO., editor. Vol. 1. Academic Press; New York: 1982. p. 196-290.
54. Serwer P. Electrophoresis. 1983; 4:375–382.
55. Kirkpatrick, FH. Electrophoresis of Large DNA Molecules: Theory and Applications, Current Communications in Molecular Biology. Lai, E.; Birren, BW., editors. Vol. 1. Cold Spring Harbor Laboratory Press; Plainview, NY: 1990. p. 9-22.
56. Dea ICM, McKinnon AA, Rees DA. J Mol Biol. 1972; 68:153–172. [PubMed: 5050360]
57. Arnott S, Fulmer A, Scott WE, Dea ICM, Moorhouse R, Rees DA. J Mol Biol. 1974; 90:269–284. [PubMed: 4453017]
58. Norton IT, Goodall DM, Austen KRJ, Morris ER, Rees DA. Biopolymers. 1986; 25:1009–1029.
59. San Biagio PL, Madonia F, Newman J, Palma MU. Biopolymers. 1986; 25:2255–2269.
60. Pines E, Prins W. Macromolecules. 1973; 6:888–895.
61. Feke FT, Prins W. Macromolecules. 1974; 7:527–530.
62. Leone M, Sciortino F, Migliore M, Fornili SL, Vittorelli MB. Biopolymers. 1987; 16:743–761.
63. Emanuele A, DiStefano L, Giacomazza D, Trapanese M, Palma-Vittorelli MB, Palma MU. Biopolymers. 1991; 31:859–868.
64. Bulone D, San Biagio PL. Chem Phys Lett. 1991; 179:339–343.
65. Ferguson KA. Metabolism. 1964; 13:985–1002. [PubMed: 14228777]
66. Slater GW, Guo HL. Electrophoresis. 1996; 17:1407–1415. [PubMed: 8905255]
67. Slater GW, Rousseau J, Noolandi J, Turmel C, Lalande M. Biopolymers. 1988; 27:509–524. [PubMed: 3359012]
68. Stellwagen NC, Holmes DL. Electrophoresis. 1990; 11:649–652. [PubMed: 2289466]
69. Stellwagen NC. Electrophoresis. 1992; 13:601–603. [PubMed: 1333950]

70. Serwer P, Hayes SJ. Anal Biochem. 1986; 158:72–78. [PubMed: 3799974]
71. Slater GW, Treurniet JR. J Chromatogr A. 1997; 772:39–48.
72. Chui MM, Phillips RJ, McCarthy MJ. J Colloid Interface Sci. 1995; 174:336–344.
73. Maaloum M, Pernodet N, Tinland B. Electrophoresis. 1998; 19:1606–1610. [PubMed: 9719534]
74. Hecht AM, Duplessix R, Geissler E. Macromolecules. 1985; 18:2167–2173.
75. Baselga J, Hernández-Fuentes I, Piérola IF, Llorente MA. Macromolecules. 1987; 20:3060–3065.
76. Baselga J, Llorente MA, Nieto JL, Hernández-Fuentes I, Piérola IF. Eur Polym J. 1988; 24:161–165.
77. Baselga J, Llorente MA, Hernández-Fuentes I, Piérola IF. Eur Polym J. 1989; 25:477–480.
78. Righetti PG, Caglio S. Electrophoresis. 1993; 14:573–582. [PubMed: 8375347]
79. Weiss N, Van Vleit T, Silberberg A. J Polym Sci: Polym Phys Ed. 1979; 17:2220–2240.
80. Joosten JGH, McCarthy JL, Pusey PM. Macromolecules. 1991; 24:6690–6699.
81. Heuer DM, Saha S, Archer LA. Biopolymers. 2003; 70:471–481. [PubMed: 14648758]
82. Stellwagen NC. Electrophoresis. 1998; 19:1542–1547. [PubMed: 9719523]
83. Holmes DL, Stellwagen NC. Electrophoresis. 1991; 12:612–619. [PubMed: 1752240]
84. Rüchel R, Brager MC. Anal Biochem. 1975; 68:415–428. [PubMed: 1200345]
85. Rüchel R, Steere RL, Erbe EF. J Chromatogr. 1978; 166:563–575.
86. Hsu TP, Cohen C. Polymer. 1989; 174:336–344.
87. Stellwagen NC. Biopolymers. 1985; 24:2243–2255. [PubMed: 4092089]
88. Stellwagen J, Stellwagen NC. Biopolymers. 1994; 34:187–201. [PubMed: 8142588]
89. Stellwagen J, Stellwagen NC. Biopolymers. 1994; 34:1259–1273. [PubMed: 7948738]
90. Stellwagen J, Stellwagen NC. Electrophoresis. 1992; 13:595–600. [PubMed: 1459072]
91. Stellwagen NC, Stellwagen J. Electrophoresis. 1993; 14:355–368. [PubMed: 8500468]
92. Stellwagen J, Stellwagen NC. J Phys Chem. 1995; 99:4247–4251.
93. Holmes DL, Stellwagen NC. J Biomol Struct Dyn. 1989; 7:311–327. [PubMed: 2604908]
94. Ogston AG. Trans Faraday Soc. 1958; 54:1754–1757.
95. Rodbard D, Chrambach A. Proc Natl Acad Sci USA. 1970; 65:970–977. [PubMed: 4191703]
96. Stellwagen NC, Gelfi C, Righetti PG. Biopolymers. 1997; 42:687–703. [PubMed: 9358733]
97. Piculell L, Nilsson S. J Phys Chem. 1989; 93:5596–5601.
98. Piculell L, Nilsson S. J Phys Chem. 1989; 93:5602–5611.
99. Stellwagen NC, Stellwagen E. J Chromatogr A. 200910.1016/j.chroma.2008.11.090
100. Strutz K, Stellwagen NC. Electrophoresis. 1998; 19:635–642. [PubMed: 9629889]
101. Stellwagen NC. Electrophoresis. 2006; 27:1163–1168. [PubMed: 16440397]
102. Chiari M, D'Alesio, Consonni R, Righetti PG. Electrophoresis. 1995; 16:1337–1344. [PubMed: 8529594]
103. Stellwagen NC. Electrophoresis. 1997; 18:34–44. [PubMed: 9059818]
104. Marini JC, Effron PN, Goodman TC, Singleton CK, Wells RD, Wartell RM, Englund PT. J Biol Chem. 1984; 259:8974–8979. [PubMed: 6086618]
105. Stellwagen E, Lu YJ, Stellwagen NC. Biochemistry. 2003; 42:11745–11750. [PubMed: 14529285]
106. Völkel AR, Noolandi J. J Chem Phys. 1995; 102:5506–5511.
107. Mohanty U, Stellwagen NC. Biopolymers. 1999; 49:209–214.
108. Stellwagen E, Stellwagen NC. Electrophoresis. 2002; 23:2794–2803. [PubMed: 12210184]
109. Viovy JL. Rev Modern Phys. 2000; 72:813–872.
110. Stellwagen E, Lu YJ, Stellwagen NC. Nucleic Acids Res. 2005; 33:4425–4432. [PubMed: 16085753]
111. Lu YJ, Weers BD, Stellwagen NC. Biophys J. 2005; 88:1191–1206. [PubMed: 15556988]
112. Lu YJ, Stellwagen NC. Biophys J. 2008; 94:1710–1725.
113. Stellwagen NC, Magnúsdóttir S, Gelfi C, Righetti PG. J Mol Biol. 2001; 305:1025–1033. [PubMed: 11162111]

114. Egli M. Chem Biol. 2002; 9:177–186.

Abbreviations

bp base pairs

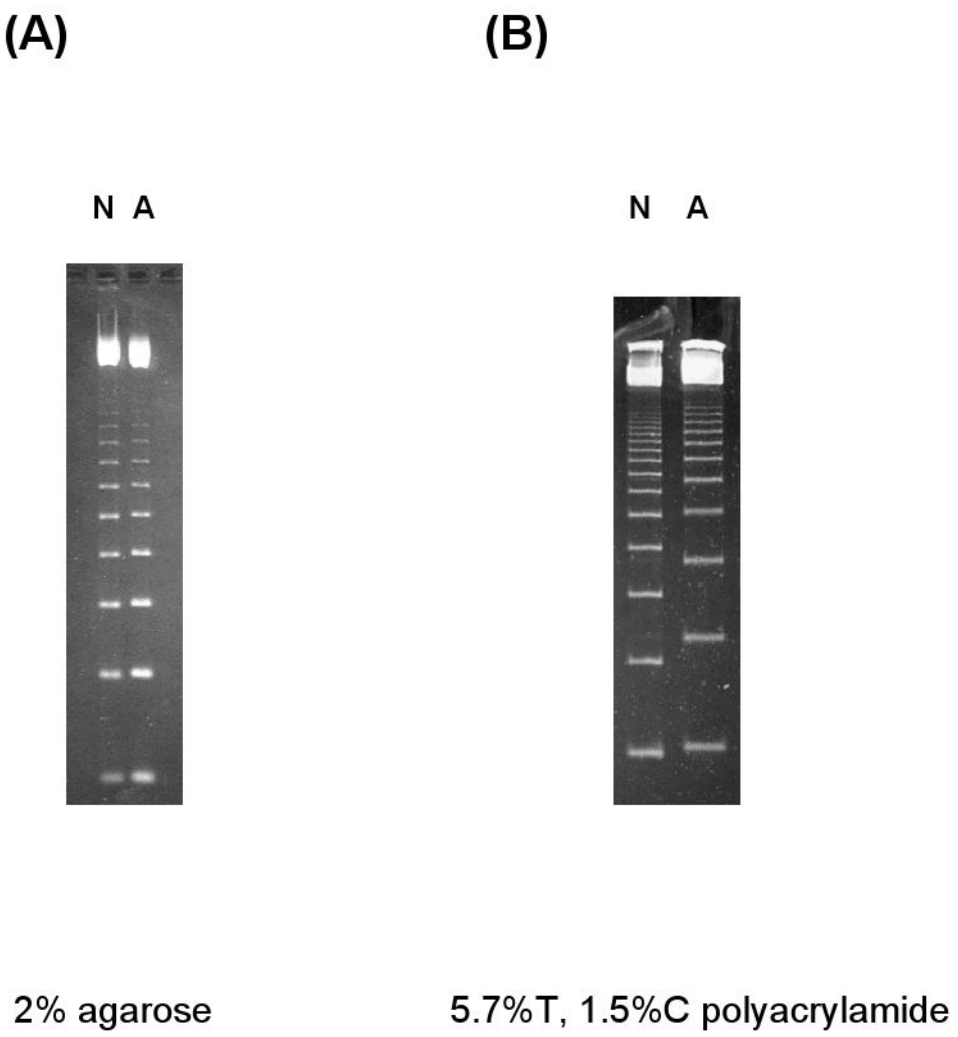

Figure 1.

Electrophoresis of normal and anomalous DNA fragments in: (A), 2.0% agarose gels; and (B), 5.7%T, 1.5%C polyacrylamide gels. Monomers of normal (N) and anomalous (A) DNA restriction fragments containing 167 bp were ligated (separately) to create multimers of various sizes. From bottom to top, successive bands in each lane of each gel correspond to monomers, dimers, trimers, 4-mers, 5-mers and higher multimers of the normal (N) and anomalous (A) DNAs. The effective pore radius of the agarose gel is estimated to be 51 nm [69]; the effective pore radius of the polyacrylamide gel is estimated to be 132 nm [83].

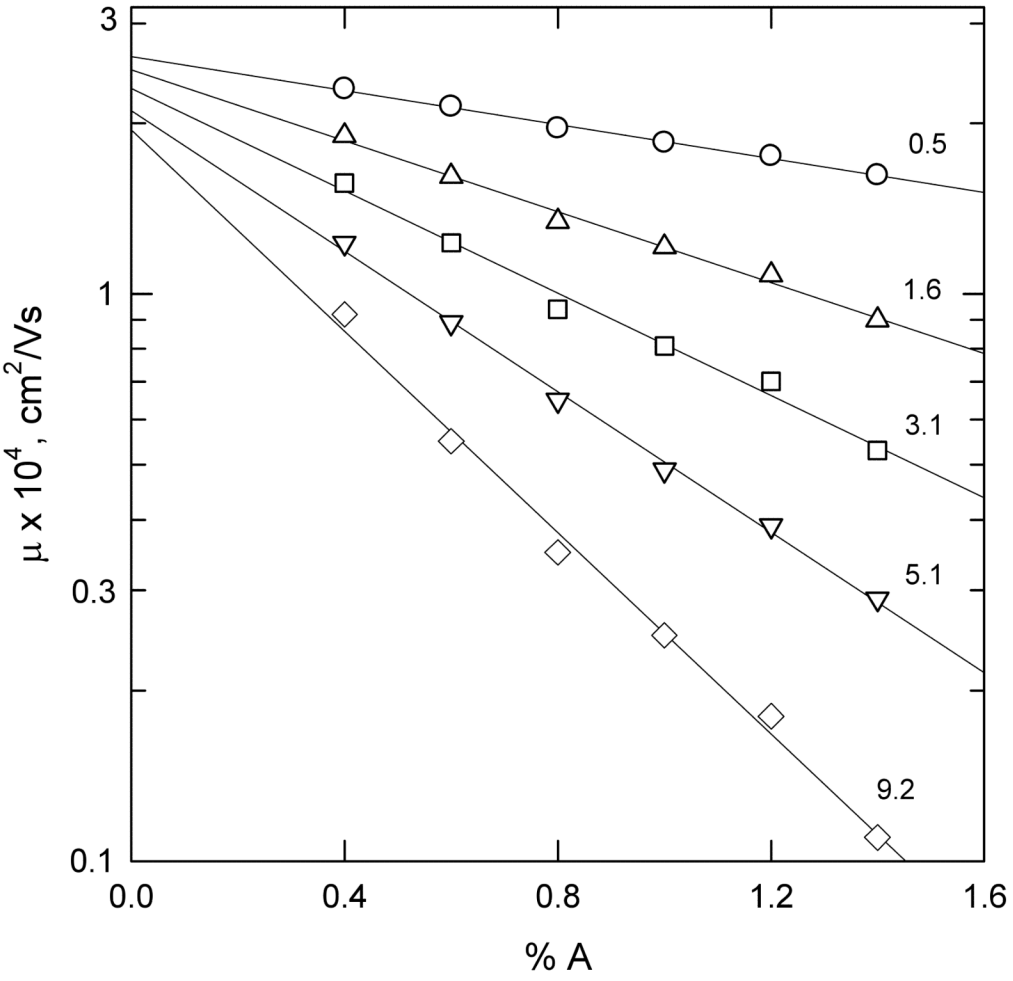

Figure 2.
Ferguson plots observed for normal DNA molecules in agarose gels. The logarithm of the mobility, extrapolated to zero electric field strength at each gel concentration, is plotted as a function of agarose concentration, %A. The lines were drawn by linear regression; the size of each DNA, in kilobase pairs, is given beside each line. Adapted from [51] with permission.

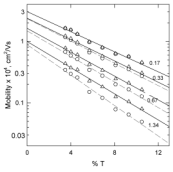

Figure 3.
Ferguson plots observed for normal (Δ) and anomalous (○) DNA fragments in polyacrylamide gels. The logarithm of the mobility is plotted as a function of polyacrylamide concentration, %T, in gels containing 3%C. The normal and anomalous fragments contained one, two, four or eight monomers, from top to bottom; the size of each pair of fragments, in kilobase pairs, is indicated beside each pair of lines. All lines were drawn by linear regression. Adapted from [101] with permission.

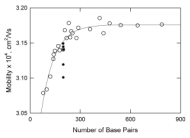

Figure 4.

Free solution mobility of normal (open circle) DNA fragments of various sizes in 40 mM Tris-acetate-EDTA buffer, plotted as a function of the number of base pairs in each fragment. The closed circles indicate the mobilities of curved 199-bp fragments containing 1 – 5 A-tracts in a curvature module located in the center of each fragment. Adapted from [110] with permission.