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Short Communication

DNA electrophoresis in uncross-linked polyacrylamide solution, studied by epifluorescence microscopy

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ABSTRACT

Electrophoresis of human DNA fragments (approximately $1 \cdot 10^5$ to $1 \cdot 10^7$ bases in size) was conducted in a solution of uncross-linked polyacrylamide contained in a horizontally mounted 1 mm diameter glass tube and monitored by epifluorescence microscopy. In presence of the polymer, molecular conformations described as a "trailing network" of DNA and a globular "head" were observed. The migration velocity varies between species differing in the size of the "head", and in the ratio between the size of the "head" and that of the trailing "network". By contrast, in pure buffer, λ phage DNA migrates in a globular form at a mobility consistent with known macroscopic data. When electrophoresis in the polymer solution of an agarose plug preparation of *Schizosaccharomyces pombe* DNA was carried out after melting at 70°C, a migrating DNA-agarose complex was observed. The complex was not fully dissociated by an agarose-hydrolyzing enzyme (Gelase).

INTRODUCTION

DNA electrophoresis in capillaries filled with polymer solutions providing size separation based on molecular sieving was pioneered by Heiger *et al.* [1]. Using uncross-linked polyacrylamide solutions, these authors separated DNA fragments up to 23 kilobase pairs (kb) in size. Schwartz *et al.* [2] have separated DNA in this size range by electrophoresis in 0.5% methyl-hydroxypropyl-cellulose. Recently, Boček and Chrambach [3] have demonstrated size separations of DNA up to 12 kb by capillary electrophoresis in solutions of agarose above its gelling temperature. The application of polymer-coated capillaries of narrow diameter to polystyrene separations [4] may also be considered in this context.

Electrophoresis in agarose gel slabs with continuous monitoring by epifluorescence microscopy [5–10] has been applied to the study of individual DNA molecules larger than 50 kb in size. This technique has yielded data concerning the relation between DNA conformation and mobility operative in electrophoretic size separations of DNA [11,12].

The work reported here combines the capillary technique of electrophoresis in polymer solution with the capacity of microscopic monitoring for relating DNA conformation with mobility.

MATERIALS AND METHODS

DNA

Human genomic DNA [MCF7 breast cancer cells (ATCC, Rockville, MD, USA)] were cultured in the Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum. They were harvested and washed with 100 m*M* NaCl, 50 m*M*

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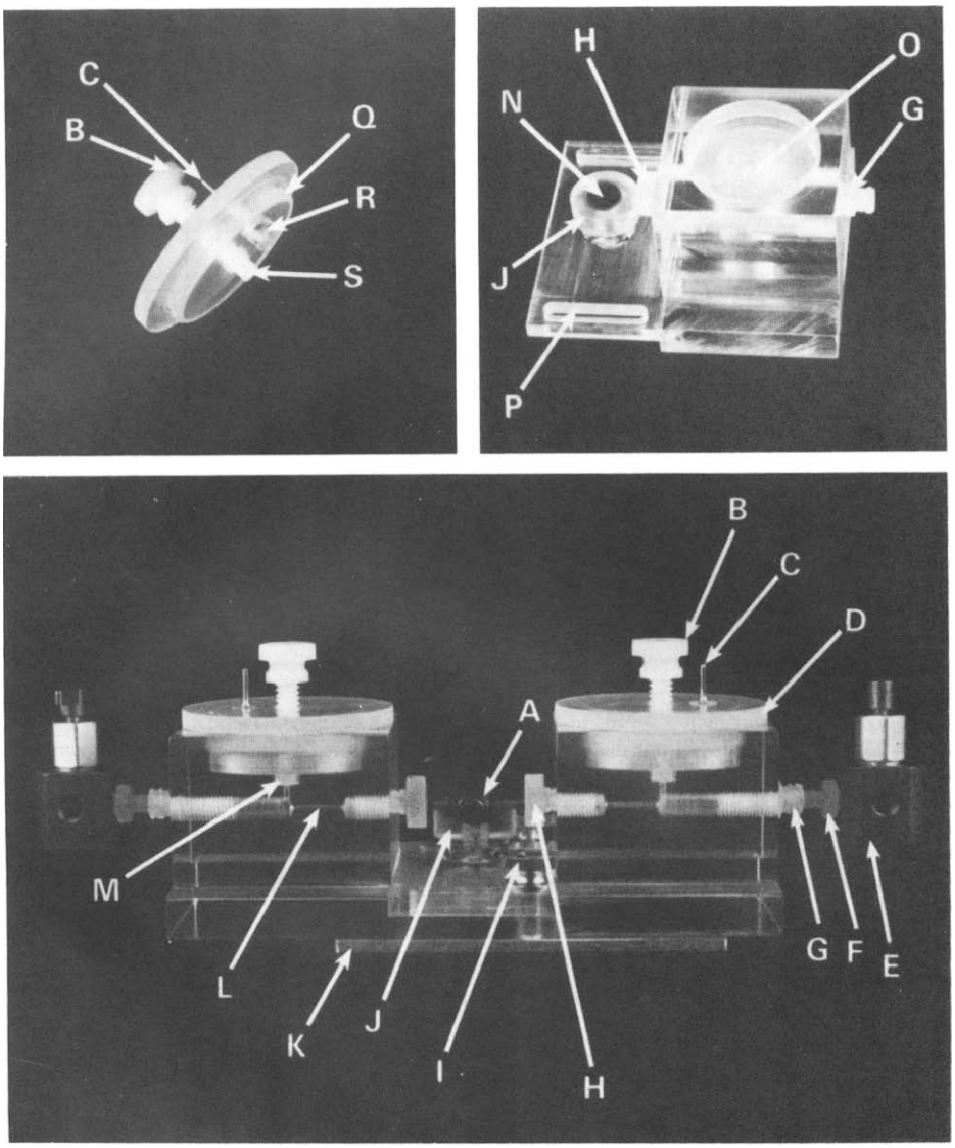

Fig. 1. Horizontal tube electrophoresis apparatus, allowing for the detection of macromolecules by microscopy. A = point of detection for oil immersion microscope objective (oil drop surrounding electrophoresis tube was shown); B = screw regulating the size of the connection between buffer chamber and electrophoresis tube; C = male pin (gold) connector to electrode; D = buffer chamber lid with air-outlet pinhole; E = four-post 90°C Hamilton HV valve; F = male Luer connector; G = female Luer connector; H = perforated screw serving as an electrophoresis tube (I.D. range 1.1 to 0.05 mm) guide; the screw makes contact with a silicone rubber "doughnut" seal; I = lock screw regulating the distance of the buffer chambers from one another; J = support plate of adjustable height for the electrophoresis tube and immersion oil drop; K = base plate (90 mm length) allowing the calipers on the microscope stage to hold the apparatus; L = channel connection between electrophoresis tube and buffer chamber; the length of the electrophoresis tube between the two channel connections and supported at A was 100 mm; M = seat for B; N = light trap blackening below the capillary at the top of J); O = buffer chamber; P = slot allowing distance of the buffer chambers along the base plate; Q = threads on D regulating the electrode position and sealing the buffer chamber; R = platinum electrode; S = sealing flat bottom of B.

Tris–HCl, 3 m*M* $MgCl_2$, pH 8, then in the same buffer containing 0.2% Triton *X*-100. The washed nuclei were incubated in phosphate-buffered saline (PBS), 0.01 *M* disodium ethylenediaminetetraacetate (Na_2, EDTA), 50 μg/ml proteinase K, 1.5 *M* lithium acetate, 0.2% sodium dodecyl sulfate (SDS) at 37°C for 24 h. DNA was extracted 3 times with phenol–chloroform and dialyzed against 0.25 m*M* Na_2EDTA (Fig. 5) or 50 m*M* Tris–Hcl, 10 m*M* Na_2EDTA, 10 m*M* NaCl (Fig. 3). The procedure was a modification of that given by McGhee *et al.* [13].

Yeast chromosomal DNA (*Schizosaccharomyces pombe*) was obtained encased in an agarose plug (melting point 65°C) from Bio-Rad Labs (Richmond, CA, USA). The plug was heated to 70°C for 15–20 min prior to loading and, in the cases indicated, cooled to 40°C and incubated with Gelase (0.5 U/ml, Epicentre Technologies [14]) for 1 h.

Lambda phage DNA was obtained from Gibco BRL (Gaithersburg, MD, USA).

Polyacrylamide

Uncross-linked polyacrylamide was obtained from Polysciences (Warrington, PA, USA) as a 1% (w/v) aqueous solution, weight-average molecular weight $5.5 \cdot 10^6$, intrinsic viscosity 10.835 $(dl/mol)^{1/3}$. The solution was diluted with TBE buffer (89 m*M* Tris, 89 m*M* boric acid, 2 m*M* Na_2EDTA) to yield 0.9% (w/v) polyacrylamide in 0.5 × TBE buffer, pH 8.3. This will be referred to as "PA buffer".

Electrophoresis apparatus

Horizontal glass tube apparatus suitable for microscopic monitoring of electrophoresis at room temperature is depicted in Fig. 1. A central point of detection on the tube connects to the oil immersion microscope objective via an oil drop. The microscopic setup was similar to the one described previously [8]. Applicable tube diameters range from the 1 mm used in this study to 50 μm (not reported here).

Procedure of electrophoresis monitored by microscopy

Tubes (1 mm I.D.) coated internally with linear polyacrylamide [15] were filled with PA buffer, uncoated tubes were filled with 0.5 × TBE buffer, as specified in the figure legends. A solution of human DNA or melted DNA–agarose plug (70°C) was injected by Hamilton syringe (25 μl) into the tube through port G (Fig. 1). Electrophoresis was conducted at 2 V/cm. Fluorescent labeling with 4′,6-diamidino-2-phenylindole (DAPI), microscopic detection, acquisition of the image and measurements were carried out as previously described [8]. Images shown in the figures were photographed as displayed on the computer monitor at high contrast.

RESULTS

Microscopic data on fluorophore-labelled DNA during and prior to electrophoresis in 0.5 × TBE buffer in presence of uncross-linked polyacrylamide

λ Phage DNA, fluorophore labeled by DAPI, when placed into a solution of 0.5 × TBE buffer (in the absence of uncross-linked polyacrylamide) appears spherical under microscopic observation (Fig. 2A). It maintains its spherical appearance and exhibits a constant average velocity during electrophoretic migration (Fig. 2B), consistent with the previous macroscopic findings and the notion of a common charge density for DNA of all sizes [16]. During migration, the globular λ-DNA molecules were not displaced linearly but at variable angles to the orientation of the electrodes. The migrating molecules appear to move into and out of the confocal plane of the microscope in time. Such a mode of migration appears consistent with the previously noted the three-dimensional migration behavior of individual DNA molecules during electrophoresis in agarose gels [11].

By contrast, when electrophoresis of human DNA was carried out in PA buffer, microscopy reveals three types of conformational features, designated descriptively as (a) a globular "head"; (b) an apparent DNA boundary emanating from the "head" and comigrating at 69.5°C (standard error, S.E. = 0.5, $n = 3$) to the direction of electrophoresis; and (c) a comigrating trailing and widely spatially spread DNA trailing "network" (Fig. 3). The area ratio between "head" and "network" varies among the various migrating DNA molecules observed microscopically (Fig. 3A, B). Such DNA trailing "networks", observed in the presence of polyacrylamide, are not observed during electrophoresis either in TBE buffer [17] or in agarose gels [11]. Large

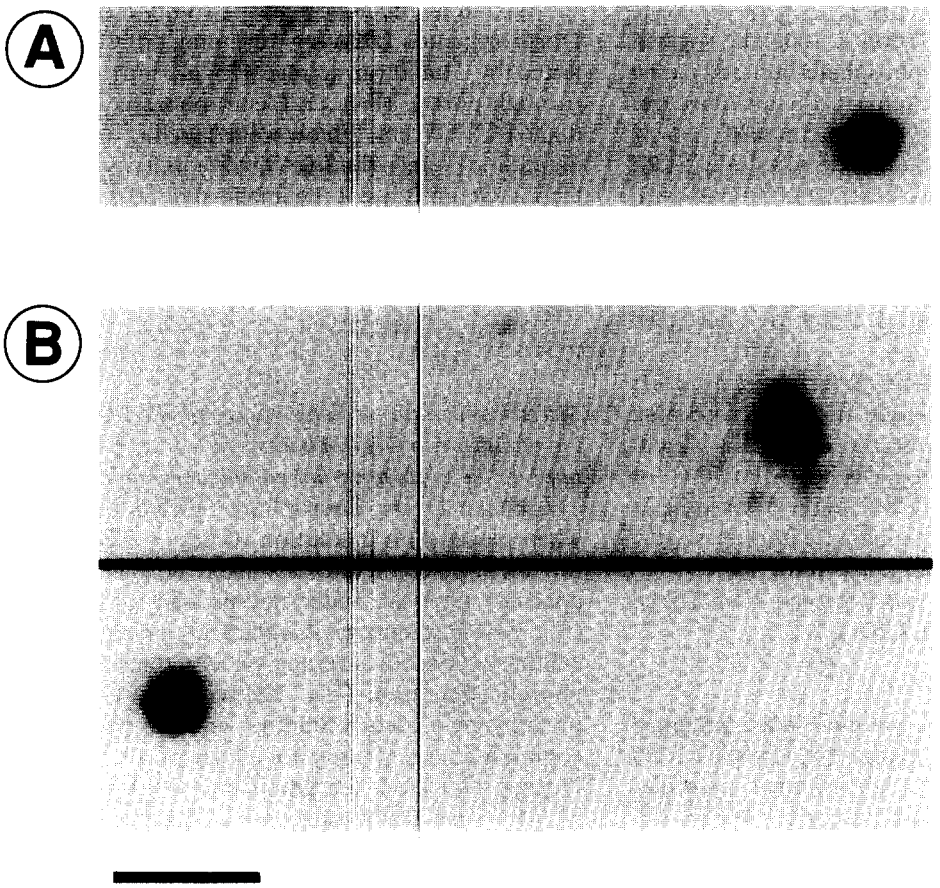

Fig. 2. Epifluorescence videomicrograph of λ-DNA in 0.5 × TBE buffer, uncoated soda glass tube, 1 mm I.D., and at room temperature. (A) Prior to electrophoresis; molecule was positioned at the tube wall and not undergoing Brownian motion; (B) during electrophoresis (from right to left), 1.8 V/cm (minimal field strength for distancing the molecule from the wall), 6 μm/s migration rate. The black line in panel B separates the videomicrograph taken at 0 time (top of the line) from that taken after 4 s of migration (below the line). Fluorescent area 3.0 μm^2. Bar = 5 μm. Anode was on the left.

DNA in buffer is deformed rather than perfectly spherical under the conditions used.

Different migration velocities for human DNA fragments exhibiting condensed "heads" of differing fluorescent areas

When the migration rates of the "head" portions of human DNA exhibiting different fluorescent areas, interpreted as a measure of DNA size (Fig. 2 in ref. 18), were compared, differences in migration rates by a factor of more than two were observed, as well as the absence of a correlation between size and migration rate (Fig. 4).

Microscopic data on fluorophore labeled DNA contained in a solution of 0.25 mM Na_2EDTA and 0.9% uncrosslinked polyacrylamide

When the human DNA configuration was spread by placing the molecule into a milieu of low ionic strength (0.25 m*M* Na_2EDTA), electrophoresis results in progressive deceleration of migration and ultimate arrest. The arrested DNA molecule can exhibit, in addition to a globular "head", a trailing DNA network (Fig. 5A). [The strands in the 2-dimensional projection of the microscope appear linked at a 30° (S.E. = 1.5, $n = 20$) angle, with a node density of 25 per 1000 μm^2]. Arrest can also be ac-

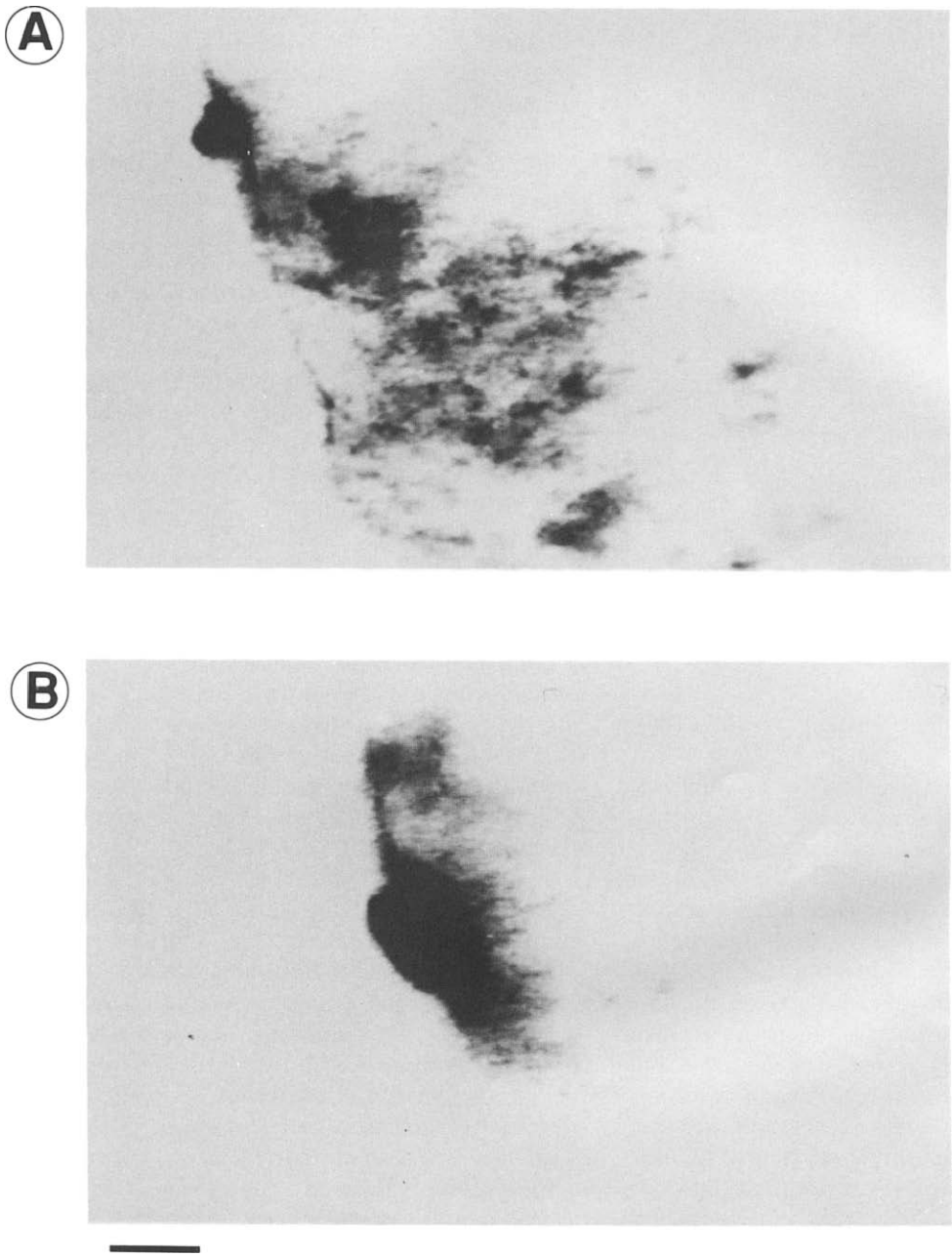

Fig. 3. Epifluorescence videomicrograph of human genomic DNA fragments in 0.5 × TBE buffer containing 0.9% uncross-linked polyacrylamide with a weight-average molecular weight of $5.5 \cdot 10^6$. Tube internally coated with uncross-linked polyacrylamide [15]; field strength 2 V/cm; temperature 24°C. (A) Representative DNA molecule with small "head", large trailing "network", 2.3 μm/s, 45 μm^2. (B) Representative DNA molecule with large "head", small trailing "network", migration rate 3.25 μm/s, 117 μm^2 fluorescent area. Bar = 10 μm. Anode was on the left.

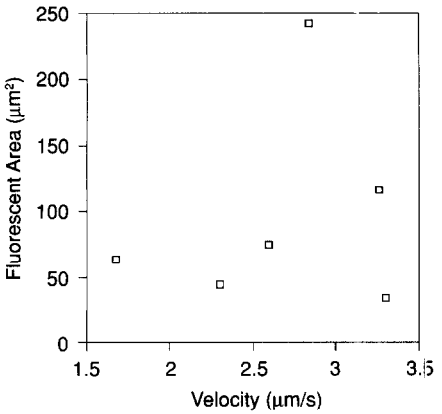

Fig. 4. Differences in migration rate between human genomic DNA fragments during electrophoresis in 0.5 × TBE buffer containing 0.9% uncross-linked polyacrylamide with a weight-average molecular weight of $5.5 \cdot 10^6$. Conditions as in Fig. 3. The migration rate of the DNA depicted in Fig. 3 was plotted in Fig. 4 in addition to four other data points.

companied by nodulated structures connected by branches (Fig. 5B).

An electrophoretically migrating agarose-DNA complex

Fluorophore labeled yeast chromosomal (*S. pombe*) DNA, loaded as an agarose plug [19] melted at 70°C, when monitored by epifluorescence microscopy (Fig. 6A) during electrophoresis in PA-buffer was seen to comigrate with a cluster of agarose molecules which were detected by light microscopy (Fig. 6B). The area occupied by fluorescent DNA (Fig. 6A) is smaller than that of the DNA–agarose complex viewed by light microscopy because DNA is contained within the agarose. The evidence that the comigrating material in Fig. 6B was agarose derives from a comparison of its micrograph with that of an unmelted agarose sample gel plug (Fig. 6C). The DNA-agarose complex was not dissociated by the action of agarase at 40°C under conditions under which free agarose was hydrolyzed.

DISCUSSION

We cannot as yet account in physical terms for the observed "head", emanating boundary and trailing "network" of DNA electrophoresed in a solution of polyacrylamide. It appears likely, however, that an originally globular DNA molecule —as it exists in buffer— through an interaction with the surrounding polyacrylamide was stretched so as to give rise to an open trailing network of DNA strands. The observed angles between DNA strands of the arrested "network" (Fig. 5B) may possibly relate to the intramolecular angles between DNA strands previously measured during agarose gel electrophoresis [11,12]. The observed DNA boundary emanating from the "head" may be due to the progressive resistance of the polymer network, that through its interaction with the traversing DNA gives rise to a steady state between the electrophoretic pull and the polymer induced drag. Since neither the trailing DNA "network" nor the boundary emanating from the "head" is observed during electrophoresis in either TBE buffer or agarose gel, it appears highly likely that the conformational interactions are due to interaction with linear polyacrylamide in solution.

Although physico-chemical evidence exists which suggests a DNA–agarose interaction [20], the migrating agarose–DNA complex has not been previously documented. We infer its existence from viewing the migrating species by light and fluorescent microscopy in alternating fashion. It appears that the migrating agarose complexed to DNA is similar in appearance to the agarose gel plug containing *S. pombe* DNA (Fig. 6C). This suggests that the agarose within the DNA complex was not in solution, and would explain why the DNA in the migrating complex remains localized (Fig. 6A), and why during electrophoresis the embedded DNA was not displaced to the front of the complex. It is also evident from Fig. 6A that a concentration of DNA present in the commercial agarose plug was capable of propelling the entire agarose–DNA complex. The notion that complexed agarose was not in a solubilized state was also supported by the fact that the complex when subjected to agarase (Gelase [14]) in a heat-solubilized bulk phase fails to dissociate or digest its agarose gel moiety. One corollary of these findings was that a previously reported separation of DNA species from a yeast chromosomal (*S. pombe*) agarose plug [21] refers to DNA-agarose complexes, not to DNA. These complexes also appeared white and were resistant to Gelase treatment. It is not as yet known whether any DNA–agarose association is maintained under the conditions of agarose gel electrophoresis.

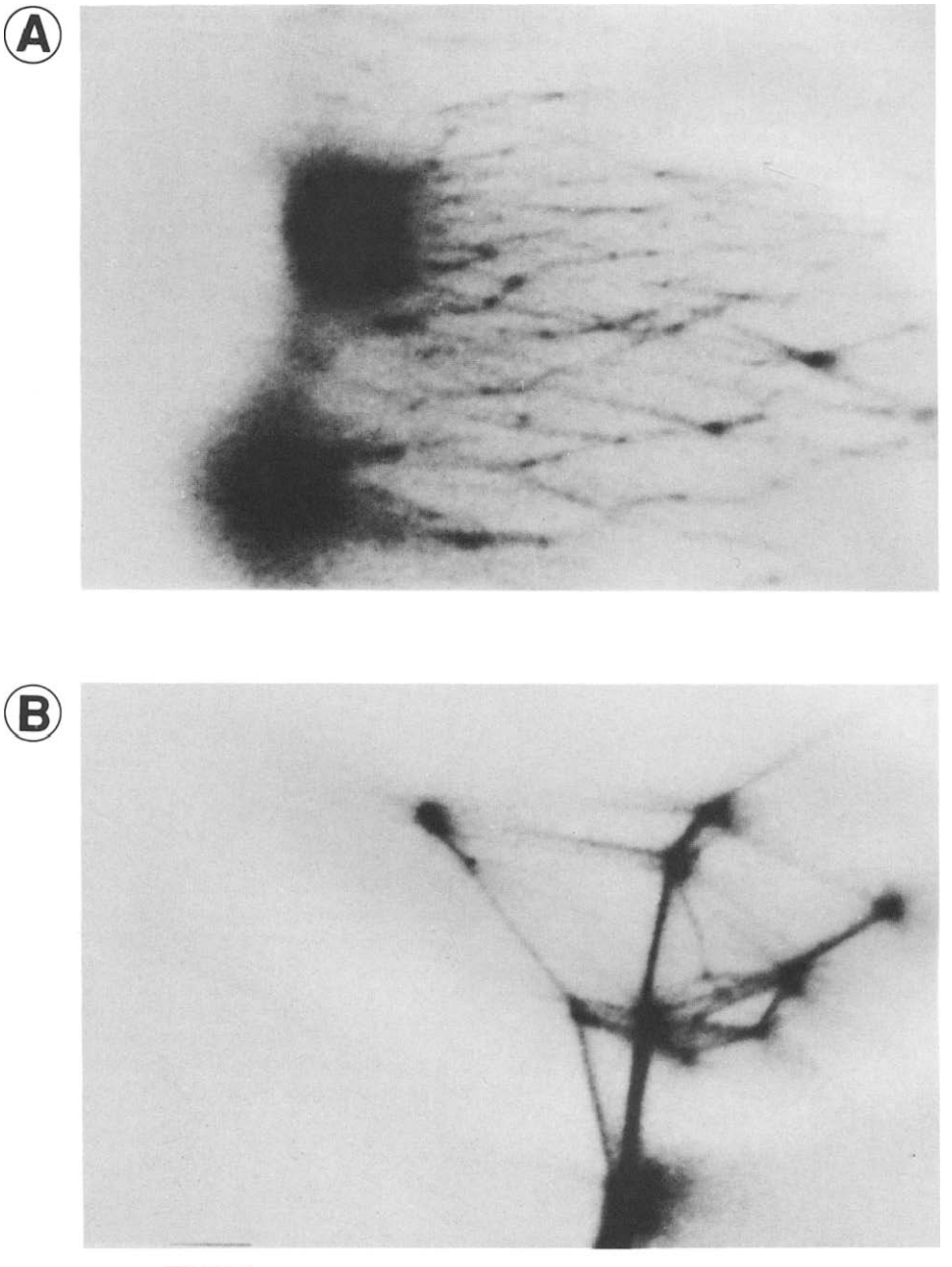

Fig. 5. Two forms of human genomic DNA fragments arrested during electrophoresis in 0.25 m*M* Na_2EDTA containing 0.9% uncross-linked polyacrylamide. The arrested state after 30 s of electrophoresis was depicted. (A) "Heads" with trailing networks; (B) DNA nodes linking the branches of a "network". Bar = 10 μm.

There were limitations regarding the interpretation of the migration velocity (mobility) in Fig. 4. (i) The ordinate in this figure refers to the DNA "head", neglecting the presence and relative size contribution of the trailing network, while the abscissa refers to the velocity of both —the "head" with its comigrating trailing network. Therefore, the measured velocities may equally pertain to a large "head" with small "network" as to a small "head" and a large "network". (ii) The polymer used in this study, polyacrylamide with a weight-average molecular weight of $5.5 \cdot 10^6$, when compared to methyl-hydroxypropyl-cellulose, agarose and polyvinyl alcohol solutions, has been found to be relatively ineffective in retarding the migration of model polystyrene sulfates of diameters comparable to large DNA [22]. It does not seem surprising, therefore, that no dependence of mobility on size could be demonstrated in Fig. 4. Nonetheless, the data indicate that the free mobility of DNA, equal

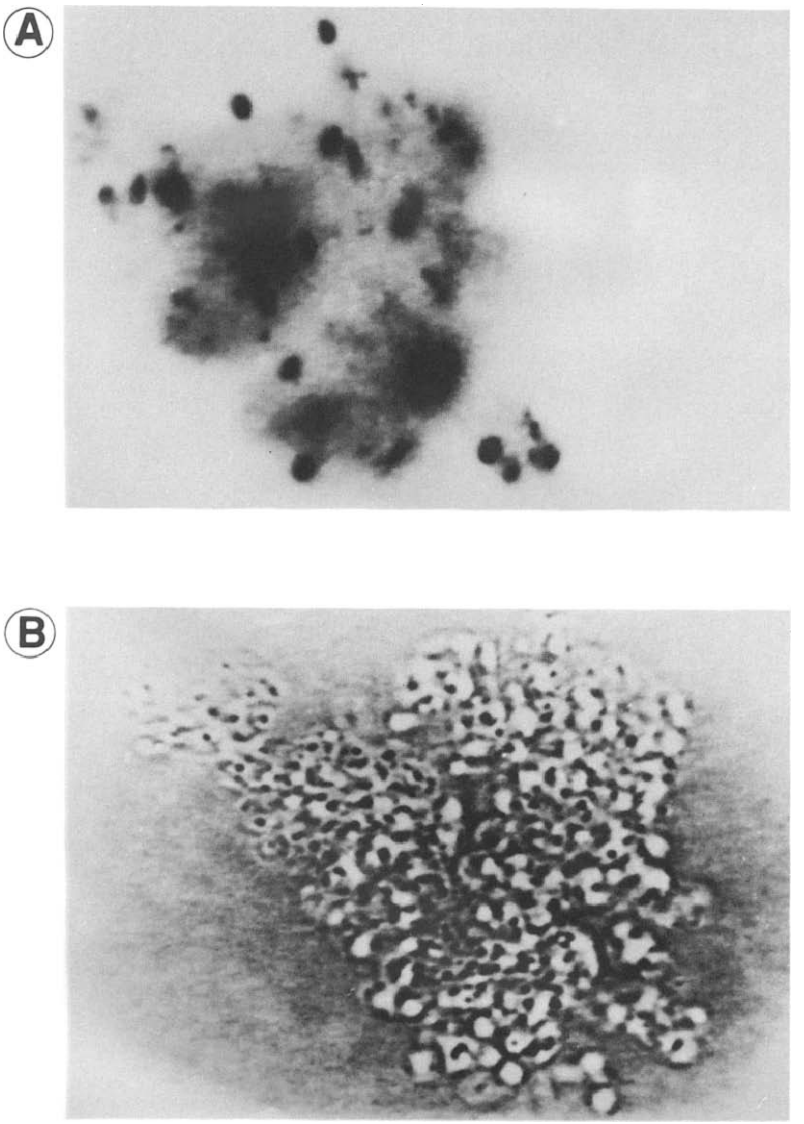

Fig. 6.

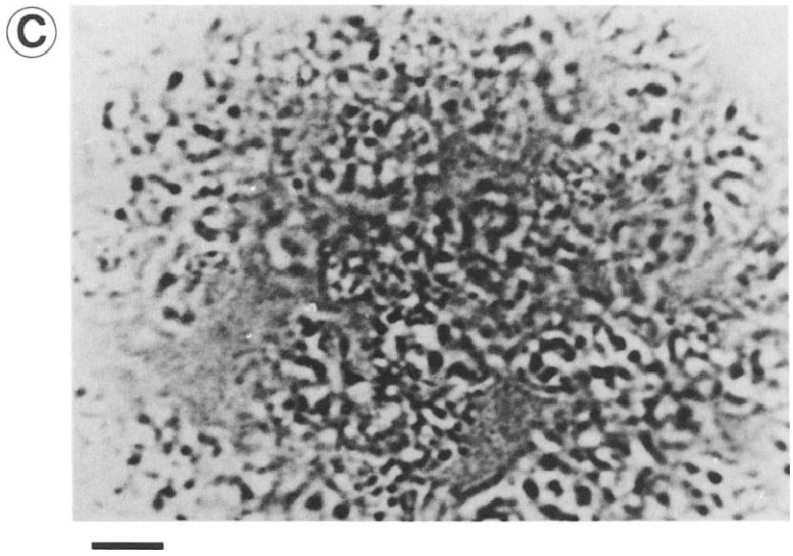

Fig. 6. Light and epifluorescence micrographs of agarose and agarose-DNA complex during electrophoresis in PA buffer. (A) Epifluorescence micrograph of yeast chromosomal DNA (*S. pombe*) loaded as an agarose gel plug (Bio-Rad) melted at 70°C; 2 s migration time; (B) light micrograph of A at 0 s; (C) light micrograph of agarose gel plug of yeast chromosomal DNA (*S. pombe*, Bio-Rad). Bar = 10 μm. Anode was on the left.

for all DNA sizes in the absence of polymer [16], was unequal in the polymer solution. This fact raises the possibility that under other conditions large DNA of different sizes may be separated by electrophoresis in a constant electric field using polymer solutions.

REFERENCES

1 D. N. Heiger, A. S. Cohen and B. L. Karger, *J. Chromatogr.*, 516 (1990) 33.
2 H. E. Schwartz, K. Ulfelder, F. J. Sunzeri, M. P. Busch and R. G. Brownlee, *J. Chromatogr.*, 559 (1991) 267.
3 P. Boček and A. Chrambach, *Electrophoresis*, in press.
4 B. B. VanOrman and G. L. McIntire, *J. MicroColumn Sep.*, 1 (1989) 289.
5 S. B. Smith, P. K. Aldridge and J. B. Callis, *Science (Washington, D.C.)*, 243 (1989) 203.
6 D. C. Schwartz and M. Koval, *Nature (London)*, 338 (1989) 520.
7 N. J. Rampino, *Doctoral Thesis*, University of California at Berkeley, Berkeley, CA, 1989.
8 N. J. Rampino and A. Chrambach, *Anal. Biochem.*, 194 (1991) 278.
9 S. B. Smith, in G. C. Salzman (Editor), *Proceedings of the International Society for Optical Engineering (SPIE), Los Angeles, CA, January 1990*, SPIE, Bellingham, WA, 1990, p. 90.
10 S. Guirreri, E. Rizzarelli, D. Beach and C. Bustamante, *Biochemistry*, 29 (1990) 3396.
11 N. J. Rampino, *Biopolymers*, 31 (1991) 1009.
12 N. J. Rampino and A. Chrambach, *Biopolymers*, 31 (1991) 1297.
13 J. D. McGhee, J. M. Nickol, G. Felsenfeld and D. C. Rau, *Nucl. Acids Res.*, 11 (1983) 4065.
14 *Gelase Worksheet*, Epicentre Technologies, Madison, WI, 1991.
15 S. Hjertén, *J. Chromatogr.*, 347 (1985) 191.
16 B. M. Olivera, P. Baine and N. Davidson, *Biopolymers*, 2 (1964) 245.
17 N. J. Rampino, unpublished results.
18 N. J. Rampino and P. G. Johnston, *Biochem. Biophys. Res. Commun.*, 179 (1991) 1344.
19 D. C. Schwartz and C. R. Cantor, *Cell*, 37 (1984) 67.
20 S. S. Smith, T. E. Gilroy and F. A. Ferrari, *Anal. Biochem.*, 128 (1983) 138.
21 T. Guszczynski and A. Chrambach, *Biochem. Biophys. Res. Commun.*, 179 (1991) 482.
22 A. Chrambach, P. Bocek, T. Guszczynski, M. M. Garner and M. Deml, in B. J. Radola (Editor), *Elektrophorese '91*, Technical University Munich Publ., Munich, 1991, p. 35.