Vol. 179, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1991 Pages 482-486

Electrophoretic separation of S. Pombe chromosomes in polyacrylamide solutions using a constant field

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Received July 17, 1991

Previous electrophoretic separations of megabase (Mb) sized DNA have been achieved in pulsed electric fields, using agarose gel as a matrix. The present study demonstrates separations of Mb sized DNA due to a retardation of migration in proportion to the concentration of uncrosslinked polyacrylamide of $5x10^6$ molecular weight, using a constant electric field. Potentially, the method should be applicable to large DNA in general, greatly reducing the instrumental complexity of such separations and rendering them compatible with capillary electrophoresis apparatus. © 1991 Academic Press, Inc.

To date, Mb sized DNA has been exclusively separated by electrophoresis in anticonvective agarose gels, using pulsed electric fields (1). Molecular sieving, defined in electrophoresis as a retardation of migration and resulting separation due to molecular size diffferences which is proportional to polymer (gel) concentration, has not been achieved previously with DNA in excess of about 50 kb, although it is the separation method par excellence for DNA fragments and other charged molecular species of lesser size (2). The present study replaces the relatively stationary polymer fiber of a gel in pulsed field gel (PFG) electrophoresis by one which is variably oriented in solution. At the same time, it replaces a variably oriented electric field of PFG electrophoresis by one which is constant.

0006-291X/91 $1.50
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MATERIALS AND METHODS

The electrophoretic method, applied to commercial grade S. Pombe DNA (BioRad), employs horizontal thermostated 1 mm diameter glass tube apparatus (3) and procedures of electrophoresis in polymer solutions [(4); Garner, M. M. and Chrambach, A., submitted to Nucleic Acids Res.] previously described. Polyacrylamide of $5x10^6$ molecular weight, a commercial 1% solution (Polysciences) in water, was diluted to contain 1xTBE buffer (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA). Dilutions were filled into the tubes [inner walls coated with 2% methylcellulose-4000 (Serva) and dried at 110°C] plugged at the distal end with 20% polyacrylamide gel in TBE buffer. The entire commercial agarose plug of S. Pombe DNA was submerged in 20-30 µl of TBE buffer, and DNA was fluorescent labeled with 2 µl of ethidium homodimer (0.3 mg/ml, Molecular Probes Inc.) at R.T. in the dark for 40 min. An aliquot (5 mg) of the agarose plug of S. Pombe DNA was inserted into the tube and positioned at the origin of the linear tube segment. Initiating the flow of thermostating fluid from the waterbath, the tube, submerged under buffer in the glass-covered horizontal apparatus, was heated to 70°C for 15-20 min to melt the agarose (M.P. 65°C) plug. The agarose solution was cooled to 40°C and digested by injecting 5 µl Gelase (0.5 U/µl, Epicentre Technologies) into the melted agarose droplet. Digestion time was minimally 1 h. The waterbath was then reset to 25°C. Electrophoresis was conducted at 60 V (regulated) across the tubes (6.0 V/cm) for approximately 120 min. Gel tubes were photographed using the GelPrint (Biophotonics) system and UV-illumination as described (4). Migration distances were measured on the photographs and mobility ($cm^2/s/V$) was calculated for each band.

RESULTS AND DISCUSSION

S. pombe DNA separates into 4 components (Fig.1). Their mobilities are indistinguishable whether the agarose plug is digested with Gelase (left panel) or not (right panel). All components exhibit mobilities at several concentrations of uncrosslinked polyacrylamide which yield a linear plot of log(mobility) vs polymer concentration (Ferguson plot) (Fig.2). The slopes (K_R, a measure of size) and intercepts with the mobility axis (Y_o, a measure of charge) (5), as well as evidence for linearity (correlation coefficients) are listed in Table 1. Three of the components exhibit an indistinguishable Y_o value, suggesting the same free mobility (related to surface net charge density) (5) and are therefore likely to be DNA (6), i.e. chromosomes I, II and III. That conclusion is also supported by the resemblance of their pattern (Fig.1) with the gel patterns of S. Pombe chromosomes on PFG electrophoresis (7). The fourth component with 5-fold lower free mobility may be a DNA-agarose interaction product.

Although chromosomes I, II and III are shown to be separated (Figs.1,2), they remain unidentified. However, the inverse

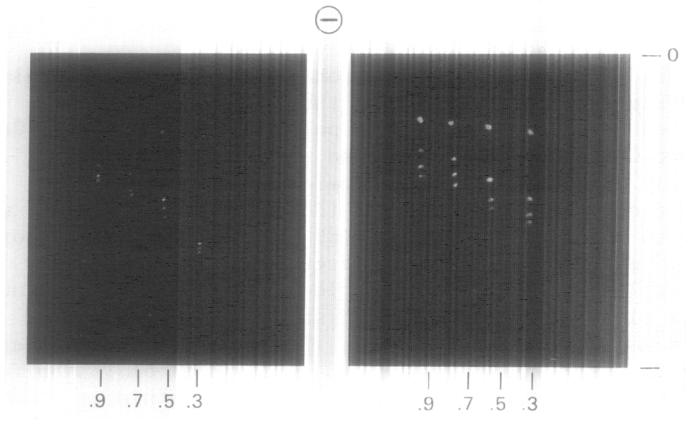

Fig.1. Separation pattern of S. Pombe DNA (BioRad) on electrophoresis in solutions of uncrosslinked polyacrylamide ($5x10^6$ molecular weight). The DNA is fluorescent labeled with ethidium homodimer. Electrophoresis is conducted on 5 mg aliquots of the S. Pombe DNA plug in horizontal 1 mm ID glass tubes coated with methylcellulose; 1xTBE buffer, 25°C. Origin is denoted by 0. Polyacrylamide plug at the end of the tube is marked. Left panel: Gelase digestion of the agarose plug in situ. Right panel: No digestion.

relation between molecular size (radius) and retardation (K_R) in uncrosslinked polyacrylamide of $5x10^6$ molecular weight of polystyrene sulfate particles (3) suggests the same inverse relationship for the DNA particles (Fig.3). Accordingly, the

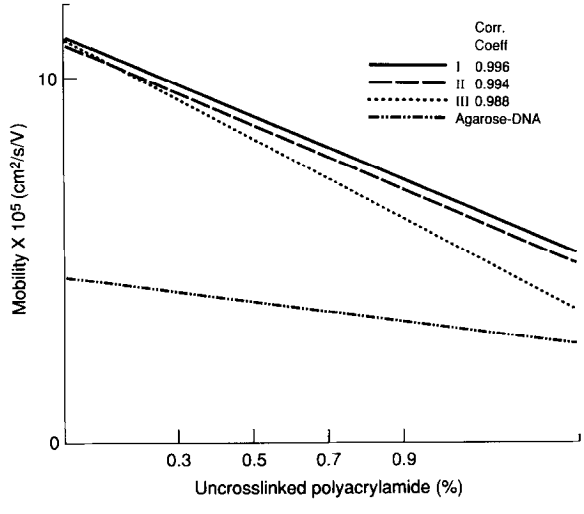

Fig.2. Ferguson plot of S. Pombe DNA components in solutions of uncrosslinked polyacrylamide. Conditions as in Fig.1, left panel. Values of slopes (K_R), intercepts on the ordinate (Y_o) and correlation coefficients are listed in Table 1.

Table 1. Ferguson Plots (8) of S. Pombe DNA in uncrosslinked polyacrylamide ($5x10^6$ molecular weight)

	R (8) (μ)	R_G** (9)* (μ)	Mb	K_R	σ_{K_R}	Y_o	σ_{Y_o}	Poly-acrylamide Range (%)	Corr. Coeff.**
Ag.-DNA (Gelase)				0.14	0.01	2.8	0.07	0.3-0.9	0.994
III	0.115	7.625	3.5	0.43	0.02	12.2	0.68		0.996
(Gelase)									
II	0.126	8.741	4.6	0.44	0.03	11.7	0.88		0.994
(Gelase)									
I	0.135	9.731	5.7	0.53	0.05	12.0	1.57		0.988
(Gelase)									
Ag.-DNA				0.17	0.02	3.7	0.10	0.3-0.9	0.995*
III	0.115	7.625	3.5	0.38	0.07	10.9	1.21		0.983
II	0.126	8.741	4.6	0.43	0.02	11.5	0.36		0.998
I	0.135	9.731	5.7	0.56	0.05	12.4	1.03		0.995

* Calculated (10) for 0.005 M Na^+.
** Weighted regression.

mechanism of their "size separation" appears not to be molecular sieving (implying a proportionality between K_R or its square root and molecular size), but rather a mechanism of exclusion

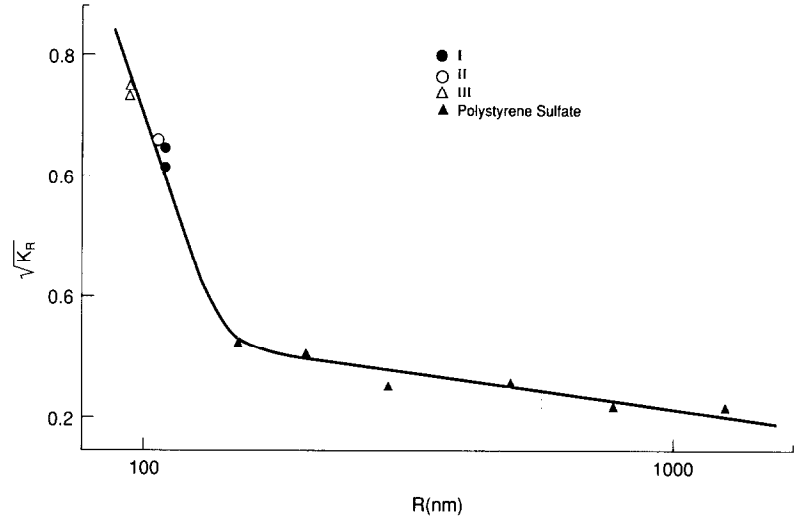

Fig.3. Relation of K_R of S. Pombe DNA components and of standard sized polystyrene sulfate particles to their molecular size. Sizes are given in terms of geometric mean radii of equivalent spheres [R (5), equ.10 of (2)]. Radii of gyration (R_G) are also given to indicate the dependence of size assignments on the underlying models. Data on polystyrene particles from (3).

from the polymer fiber network similar to that known as a gel permeation mechanism in chromatography. Corresponding data on polystyrene sulfate particles obtained with different polymers varying in size and degree of interaction (3) show that a fiber displacement mechanism is unlikely since the inverse relation between particle size and retardation is not exhibited when the molecular weight of the polymer is substantially decreased or when its fiber interactions are diminished.

The demonstration of Mb DNA separations in a constant field, presumably by size-dependent exclusion from a polymer network in solution, poses many new questions to be answered by experiment: i) The identity of the separated yeast chromosomes; ii) the dependence of the quality of separation on the size of the polymer and the degree of fiber interaction in solution; iii) the interdependence between DNA size and polymer size required for separation in solution; iv) the adaptability of the procedure to capillary electrophoresis apparatus; v) the adaptation of the procedure for preparative purposes.

REFERENCES

1. Gemmill, R. M. (1991) Advances in Electrophoresis (Chrambach, A., Dunn, M. J. and Radola, B. J., Eds.) Vol.4, VCH, Weinheim, Germany.

2. Stellwagen, N. C. (1987) Advances in Electrophoresis (Chrambach, A., Dunn, M. J. and Radola, B. J., Eds.) Vol.1, VCH, Weinheim, Germany.

3. Guszczynski, T., Garner, M. M., Deml, M. and Chrambach, A. (1991) Appl. and Theor. Electrophoresis, in press.

4. Bocek, P. and Chrambach, A. (1991) Electrophoresis, in press.

5. Rodbard, D. and Chrambach, A. (1970) Proc. natl. Acad. Sci. USA 65, 970-977.

6. Olivera, B. M. Baine, P. and Davidson, N. (1964) Biopolymers 2, 245-257.

7. Smith, C. L., Matsumoto, T., Niwa, O., Klco, S., Fan, J.-B., Mitsuhiro, Y. and Cantor, C. R. (1987) Nucleic Acids Res. 15, 4481-4489.

8. Rodbard, D. and Chrambach, A. (1970) Anal. Biochem. 40, 95-134.

9. Benoit, H. and Doty, P. J. (1953) Phys. Chem. 57, 958-963.

10. Campbell, A. M. and Jolly, D. J. (1973) Biochem. J. 133, 209-226.