ELSEVIER

Biophysical Chemistry 58 (1996) 151–155

Biophysical Chemistry

Dynamics of single-stranded DNA in polyacrylamide gels during pulsed field gel electrophoresis. A birefringence study

Jean Sturm *, Alain Pluen, Gilbert Weill

Institut Charles Sadron, CNRS and Université Louis Pasteur, 6 rue Boussingault F-67083 Strasbourg Cedex, France

Received 27 December 1994; revised 16 February 1995; accepted 23 February 1995

Abstract

The study of the orientation of single-stranded DNA in polyacrylamide gels in denaturing conditions has been undertaken by electric birefringence in order to determine the mechanism involved in the electrophoretic transport. The presence of an overshoot in the birefringence signal, when applying the electric field, and the study of the influences of the electric field and of the gel concentration on the dynamics show that a mechanism of reptation with elongation of the molecule occurs in polyacrylamide gels with low T values. Therefore it is suggested that the use of pulsed fields in sequencing electrophoresis is possible and can lead to a large increase of the length of the fragments that can be sequenced in one single run.

Keywords: Sequencing; Gel electrophoresis; Reptation; DNA separation; Polyacrylamide gels

1. Introduction

The use of pulsed fields in gel electrophoresis led to an enormous increase in the separability of double-stranded DNA in agarose according to their length for very long molecules, allowing separation up to several millions of base pairs [1]. Indeed, if gel electrophoresis with a continuous field is appropriate to separate DNA fragments up to 50 000 base pairs, it fails for longer fragments since the mobility at low electric field E varies according to $\mu = \mu_0 (1/N + \alpha E^2)$, where α is independent of N but depends on the gel mesh size a. The importance of the transient states of the molecules during the separation process is thus put in evidence and it is important to understand the molecular mechanisms and to determine the relevant times of orientation of DNA when the electric field is applied, changed or reversed. These have been studied by several groups: linear dichroism in the UV band was used by Jonsson et al. [2], fluorescence detected linear dichroism of intercalated ethidium bromide by Holzwarth et al. [3] and linear birefringence by Sturm and Weill [4]. Several features characterise this mechanism: (i) the degree of steady state orientation as a function of the field E depends on the number of base pairs N, in contradiction with the initial biased reptation theory [5], (ii) the decay of the orientation signal is highly non exponential, and (iii) an overshoot in the orientation occurs when the electric field is turned on. This behaviour shows that, in addition to the alignment of the molecule in the direction of the electric field by

* Corresponding author.

SSDI 0301-4622(95)00094-1

biased reptation, there exists the possibility for the molecule to elongate its primitive path. This means that, if the length of the DNA subchain contained in one pore is initially longer than the pore size and if the whole DNA chain initially occupies several pores, the number of occupied pores increases when an electric field is applied [6,7]. It implies the double inequality

$$P < a < L \qquad (1)$$

where P is the persistence length of the DNA molecule, a is the pore size of the gel and L is the contour length of the DNA molecule.

The transposition of this mechanism to conditions used for separating single-stranded DNA instead of double-stranded DNA is of particular interest for the sequencing techniques. Continuous field techniques are still the only ones used up to the present time for sequencing. Determining experimental conditions where a mechanism of reptation with elongation is occurring with single-stranded DNA, i.e., taking advantage of the knowledge of the internal deformation dynamics in order to build appropriate pulse trains according to DNA length and gel mesh size, might enable higher resolution in sequencing gels for the bands corresponding to the long fragments and thus displace the limit of separability with length to higher values. The present study shows that such conditions can be obtained.

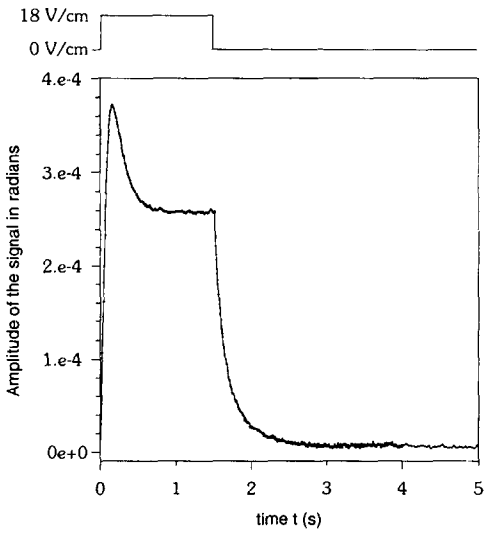

Fig. 1. Rise of the birefringence when an electric pulse of 18 V/cm is applied (left part), and decay of the birefringence at the cut-off of the electric field (right part), for a pKS single-stranded DNA ($N = 2961$ bases), 250 μg/ml, in an acrylamide gel, $T = 6\%$, $C = 5\%$, 6mm thick, 1×TBE buffer and 8M urea denaturing conditions and 25°C.

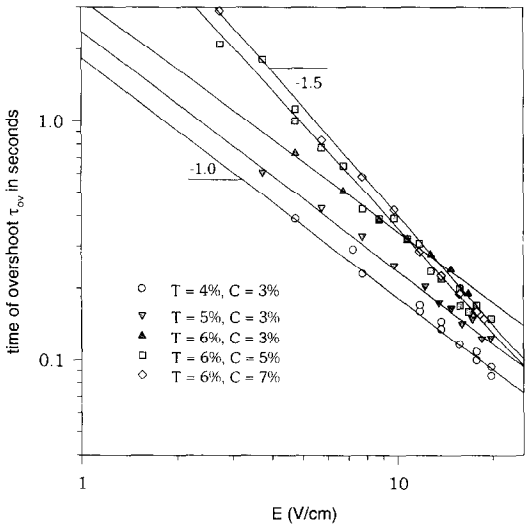

Fig. 2. Variation of the overshoot time τ_{OV} as a function of the electric field E, for a pKS DNA in polyacrylamide gels of different concentrations.

2. Material and methods

Our fully automated set-up has been described in details elsewhere [6]. A horizontal electrophoretic cell with a bottom suprasil window to minimize stray birefringence is crossed by a vertical He–Ne laser beam for birefringence measurements. Two stepping motors allow the displacement of the cell to bring the DNA spot in the laser beam. They also permit a scanning of the gel, using the birefringence induced by one single electric pulse to detect the presence of DNA in the laser beam and thus to locate the DNA bands and to measure the electrophoretic mobility.

Single-stranded DNA: pKS DNA $N = 2961$ base pairs was used: the plasmid DNA was linearised by digestion with the restriction enzyme EcoRI. Denaturation was undertaken by heating the solution to 100°C during 5 min in the presence of 8 M urea. Polyacrylamide gels were prepared by mixing acryl-

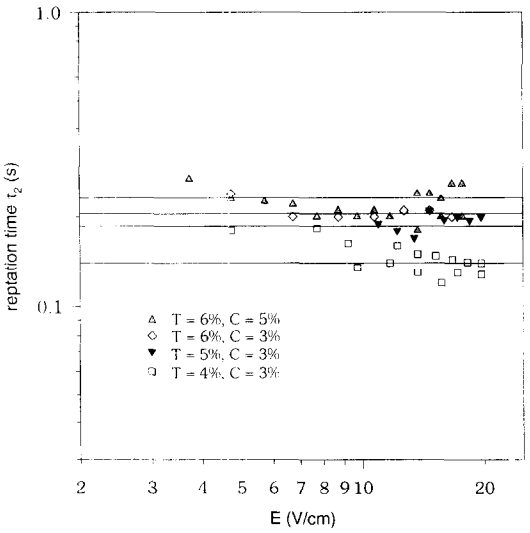

Fig. 3. Variation of the reptation time, τ_2, as a function of the electric field applied before, for a pKS DNA in polyacrylamide gels of different concentrations.

amide (ultra pure, ICN) and N,N′-methylene-bisacrylamide (ultra pure, ICN) in a 1 × TBE (0.01 M Tris borate–EDTA buffer) containing 8 M urea (ultra pure, ICN). The solution was degassed by nitrogen bubbling, then 0.8 μl of TEMED per ml of acrylamide solution, and 0.8 μl of ammonium persulfate (from a stock solution of 25%) per ml of acrylamide solution were added. A layer of 6 mm thickness is spread in a circular mold from which it can be transferred to the electrophoretic cell. Gelation was completed 90 min after mixing. The DNA was introduced in the gel at such concentrations that the concentration in a band is always much smaller than the overlap concentration (which is 24.9 mg/ml in that case, by assuming a persistence length of 50 Å for the single-stranded DNA), by filling a well of about 2 × 3 mm, which has been prepared in the gel mother layer during gelification. Optical measurements were carried out after electrophoretic displacement into the gel. Voltages from 0–200 V can be applied to two platinum wire electrodes at a distance of 10 cm, allowing measurements in the range 0–20 V/cm, without inaccuracies arising from electrode polarisation.

All experiments were carried out in a 0.01 M Tris borate–EDTA buffer, pH 8.3 (1 × TBE), and 8 M urea.

3. Results and discussion

All the results presented here are to be compared with the similar results for double-stranded DNA presented in Refs. [6] and [7]. Fig. 1 shows a typical birefringence signal of pKS single-stranded DNA when an electric field E is applied and cut off once the steady state value is reached. This figure has to be compared to the similar signals for double-stranded DNA shown in Fig. 2 of Ref. [6].

3.1. Rise of the signal

An overshoot is present as for double-stranded DNA, with a relative amplitude which is larger with single-stranded DNA. In addition, one can notice the absence of the undershoot in the case of single-stranded DNA. Fig. 2 shows the variation of the time at the overshoot with the electric field. It shows an $E^{-1.5}$ dependence which shifts to an E^{-1} dependence when the acrylamide concentration is low ($T < 6\%$) or, for $T = 6\%$, if the relative bisacrylamide concentration is low ($C = 3\%$); such a behaviour is obviously linked to the average mesh size of the gel. For high values of T (8%), the overshoot does not exist anymore.

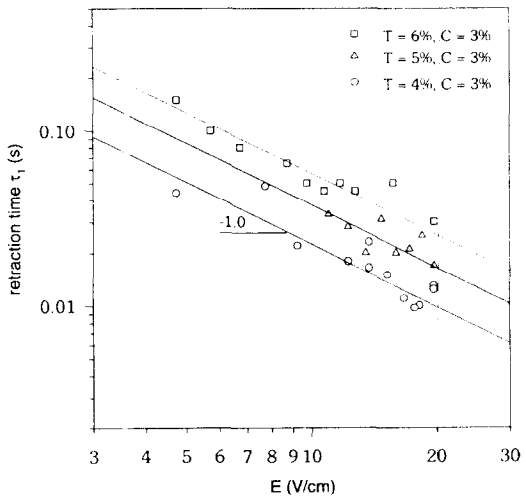

Fig. 4. Variation of the retraction time, τ_1, as a function of the electric field applied before, for a pKS DNA in polyacrylamide gels of different concentrations.

3.2. Decay of the signal

The relaxation process after cutting off the electric field looks similar in both experiments (Fig. 1). Moreover the present results fit with the analytical expression of the birefringence signal proposed in Ref. [6] to account for the convolution of retraction of the tube (τ_1) and reptation (τ_2):

$$\Delta n(t) = \exp(-t/\tau_2)\left[A_2 + A_1 \exp\left(-\frac{t+t_0}{\tau_1}\right)^{\alpha}\right]$$

One obtains always $t_0 = 0$ and $\alpha = 1$ in the present study.

Fig. 3 shows that, as expected, the reptation time τ_2 does not depend on the field that induces the orientation of the molecule. On the contrary, Fig. 4 shows that the retraction time τ_1 is proportional to E^{-1}, a behaviour which is identical to the one obtained with double-stranded DNA in agarose gels [6] (in Ref. [6], it has to be compared to the behaviour of δ^{-1}, the inverse of the slope at $t = 0$, which is proportional to τ_1 at $t = 0$).

All these results show that the mechanism of orientation controlling the electrophoretic transport of single-stranded DNA in acrylamide gels is the same as with double-stranded DNA in agarose gels. For concentrations of acrylamide, $T = 6\%$ and $C \geq 5\%$, we seem nevertheless to be close to the limit of the region where 'reptation with elongation' occurs. Indeed, with a concentration of acrylamide of $T = 8\%$, the overshoot when the electric field is applied disappears completely, showing that the DNA subchain in one pore is no more long enough to allow extension. On the opposite, for the low concentrations of acrylamide, the dependence of the overshoot time with E becomes the same as the one observed for double-stranded DNA (E^{-1}) and is then in agreement with the analytical model of Lim et al. [8].

The persistence length of single-stranded DNA is not yet known, but is reasonably expected to be around 50 Å for the buffer concentration used. On the other hand, according to Hsu and Cohen [9], the pore size of an acrylamide gel of $T = 5\%$ and $C = 2.5\%$ is about 90 Å. Taking into account that the pore size of the gel depends, according to Griess et al. [10], on $T^{-0.6}$, and that the increase of the concentration of cross-linking bisacrylamide will also decrease the pore size, one expects, according to Eq. (1), a change of regime in the electrophoretic transport when a becomes lower than P, and this shows the self-consistency of the results in the view of a reptation model with elongation.

In order to benefit by the enormous increase in separability of long DNA fragments which is made possible by the use of pulsed fields, the electrophoresis experiments have to be performed in conditions where the reptation with elongation regime is active. We have shown in this paper that such conditions are already fulfilled with relatively diluted polyacrylamide gels, and Turmel et al. [11] showed an electrophoregram where the feasibility of pulsed fields to increase the separability range in sequencing was already proved; nonetheless, to remain in the reptation with elongation regime, more dilute acrylamide gels are more likely to be adequate. Unfortunately, such gels are hard to handle. Therefore, we suggest that the use of new types of gels with larger pores should be of great interest. Such gels are now investigated in our laboratory. Pulsed field techniques proved to enhance greatly the possibilities of separation for double-stranded DNA in agarose; this work shows that one can work in the same electrophoretic regime with single-stranded DNA in acrylamide or new gels with larger pores. The optimization of the different parameters involved will therefore enable the use of pulsed field techniques in sequencing, and might give rise to a large improvement of the sequencing techniques.

Acknowledgements

We thank Prof. Soucier (Université Louis Pasteur, Strasbourg) for the kind supply of linearized pKS DNA. This work is supported by a grant of the Human Frontier Science Program (RG329/93). Alain Pluen's work on this project is part of the Biomed1 GENE-CT930024 programme of the European Community.

References

[1] D.C. Schwartz and C.R. Cantor, Cell, 37 (1984) 67.
[2] M. Jonsson, B. Åkerman and B. Norden, Biopolymers, 27 (1988) 381.

[3] G. Holzwarth, K.J. Platt, C.B. McKee, R.W. Whitcomb and G.D. Crater, Biopolymers, 28 (1989) 1043.
[4] J. Sturm and G. Weill, Phys. Rev. Lett., 62 (1989) 1484.
[5] G.W. Slater and J. Noolandi, Biopolymers, 25 (1986) 431.
[6] P. Mayer, J. Sturm and G. Weill, Biopolymers, 33 (1993) 1347.
[7] P. Mayer, J. Sturm and G. Weill, Biopolymers, 33 (1993) 1359.
[8] H.A. Lim, G.W. Slater and J. Noolandi, J. Chem. Phys., 92 (1990) 709.
[9] T.P. Hsu and C. Cohen, Polymers, 25 (1984) 1419.
[10] G.A. Griess, E.T. Moreno, R.A. Easom and Ph. Serwer, Biopolymers, 28 (1989) 1475.
[11] C.B. Turmel, E. Brassard and J. Noolandi, Electrophoresis, 13 (1992) 620.