Tethering of double-stranded DNA to agarose gels for studies of conformation dynamics during electrophoresis

Björn Åkerman

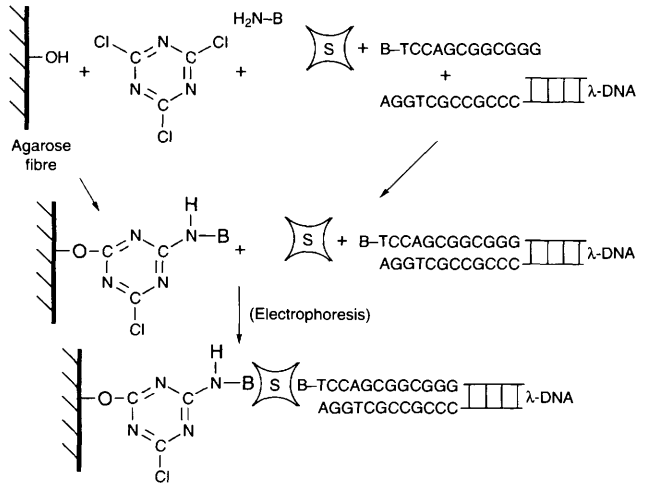
Department of Physical Chemistry, Chalmers University of Technology, Göteborg S-41296, Sweden

λ-DNA molecules are tethered specifically by one end to the fibres of an agarose gel so that the stretching and recoiling which occurs in a cyclic fashion during electrophoresis of free molecules can be studied as individual processes.

The electrophoretic migration of long DNA through gels is a highly dynamic process. Even in a constant field there is an ongoing cyclic conversion between extended and compact conformations,[1] as molecules first become stretched in U-conformations hooked around gel fibres and later recoil as they slide off the hook. In pulsed fields, separation of mega-base pair DNA has been achieved by creating an interplay between the pulsing and these stretching and recoiling motions of the DNA.[2] Direct observation of individual molecules by fluorescence microscopy has provided considerable insight into the migration and separation processes,[2–4] but spectroscopic methods to study the DNA motion are also necessary for obtaining quantitative data, especially since the spatial and temporal resolution of the microscope is not high enough to probe important aspects of the stretching and recoiling.[4] However, the spectroscopic responses usually contain contributions from both modes of motion since at any instant the molecules of the studied ensemble will be distributed over the phases of the migration cycle. Such averaging hampers the study of the stretching and recoiling. Here we demonstrate that by tethering the DNA to the gel (*via* an avidin–biotin bridge,[5] Scheme 1), an ensemble of molecules can be prepared which perform these important elements of the migration (or close mimics thereof) in a concerted manner. This will allow spectroscopic studies of them as separate processes.

Briefly, λ-DNA molecules (48 502 base pairs) were avidinated specifically at one end by first ligating a biotinylated oligomer complementary to one of the two different single-strand overhangs at the opposite ends of λ-DNA,[6] and then incubating agarose plugs containing the biotinylated DNA in 1 mol dm^{-3} NaCl containing a 100-fold excess (over DNA ends) of streptavidin, which diffuses into the gel plugs and binds to the DNA–biotin. Biotin–ethylenediamine (Molecular Probes) was activated with 2,4,6-trichloro-1,3,5-triazine in aqueous solution at 0 °C, and immediately incubated with an aqueous suspension of pieces of standard agarose gel (1%) at 20 °C in order to activate the second Cl-group.[7] The concentration of biotin covalently attached to the gel was about 0.5 μmol dm^{-3} according to absorption measurements on immobilised fluorescein–avidin, which also showed that the biotin remains covalently linked to the gel fibers in agarose blocks formed out of remelted biotinylated gel-pieces diluted with unmodified gel. At the biotin concentrations used here, the modification did not affect the gel structure, as monitored by gel turbidity and electrophoretic mobility of native DNA.

Tethering of the DNA to the gel (Scheme 1) was evaluated by migrating avidinated DNA from the gel plugs (where some DNA was trapped non-specifically) through inserts of biotinylated gel placed in a frame gel of unmodified agarose (Fig. 1). A narrow zone of immobilised DNA is seen at the top of the insert (lane 3), but a fraction of the DNA has migrated through the insert and forms a zone at the position expected for λ-DNA under present electrophoresis conditions. (During continued electrophoresis the zone in the insert did not move, whereas the lower zone migrated). No trapped DNA is observed in the insert if the gel is not biotinylated (lane 1), or if the DNA is not avidinated (lane 2), which shows that the immobilisation in lane



Scheme 1 Proposed structure of the biotin (B)/streptavidin (s)-link between λ-DNA and gel fibre. Each streptavidin can bind four biotin molecules.[5]

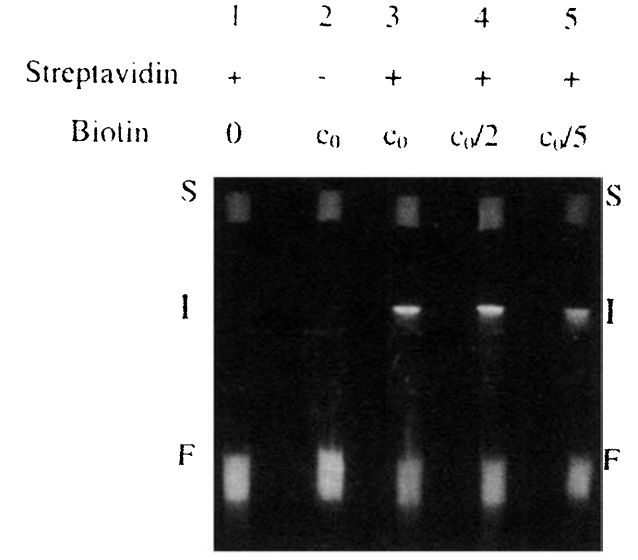


Fig. 1 Frame gel containing sample plugs (S) with avidinated DNA, and inserts (I) of biotinylated gel with biotin-concentrations as indicated. The length and position of the inserts, as indicated by the symbols I, were the same in all lanes. Approximate biotin concentration c_0 in lane 3 is 0.05 μmol dm^{-3}. All gels are 1% agarose in 0.5 × TBE (50 mmol dm^{-3} Tris, 50 mmol dm^{-3} borate, 1 mmol dm^{-3} EDTA). After electrophoresis at 3 V cm^{-1} for 7 h, the gel was stained with ethidium-bromide, to show DNA trapped in biotin-inserts and free-moving (F) DNA.

3 is due neither to a perturbed gel structure caused by the biotinylation, nor to non-specific interactions between avidinated DNA and the gel. It is also seen that in lane 3 the lower part of the insert contains no DNA, *i.e.* the free-moving DNA did not escape due to lack of biotin sites, and the integrated ethidium-bromide fluorescence in the two zones showed that the avidination yield therefore was about 45%. The ability to remelt the biotinylated gel was exploited to investigate the effect of dilution of the biotin sites in the gel. Lane 5 shows a more extended zone of lower concentration of trapped DNA, in agreement with more dispersed biotin-sites compared to lane 3. A similar but less accentuated trend is observed in lane 4, in agreement with its intermediate biotin concentration. A detailed tethering protocol and studies of the quantitative aspects of the tethering will be presented elsewhere.

The specific trapping of the avidinated DNA strongly indicates that the immobilised DNA is tethered with one end to the gel. Further evidence is provided by linear dichroism (LD) spectroscopy studies[1] of the orientation of the trapped DNA by an electric field (Fig. 2), measured in the same type of insert as in lane 3 of Fig. 1, similarly placed in a non-modified frame-gel in a spectroscopic electrophoresis cell.[8] The sign of the LD shows that upon application of a constant field the trapped DNA becomes preferentially oriented in the field direction, as is the case for migrating DNA.[1] However, the degree of orientation of the trapped DNA [Fig. 2(*a*), upper trace] is about three times higher and the build-up is slower and does not have the overshoot observed for free DNA (lower trace) of the same size at the same field. These observations are all in agreement with the immobilised DNA being tethered at one end: the orientation is stronger because the anchoring is now permanent, instead of the transient anchoring of migrating DNA molecules which are stretched because of the cyclic hooking and unhooking around gel fibres.[4] The orientation builds up more slowly because only one end can move in response to the field and stretch the molecule, whereas free DNA is stretched by both ends moving the molecule into a U-shape. Finally, no overshoot is observed, in agreement with the demonstration that the oscillatory LD signal for free DNA reflects the cyclic migration,[4] a mode of motion not available to tethered DNA.

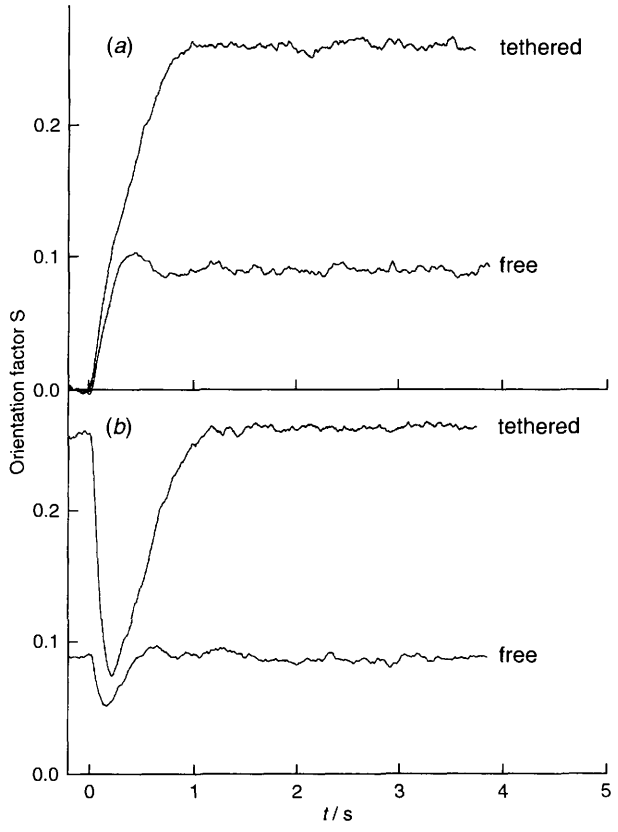


Fig. 2 LD response of tethered and free λ-DNA in 1% gel to a field of 15 V cm^{-1}. LD is presented in terms of the orientation factor S, which varies between 0 and 1 for random and perfect helix orientation, respectively.[8] DNA-phosphate concentration was 60 μmol dm^{-3} for tethered and 81 μmol dm^{-3} for free DNA, and each LD-trace is the average of 9–16 measurements. (*a*) Response to a constant field applied (at time zero) to the samples in equilibrium. (*b*) Response to an inverted field applied (at time zero) to the samples in steady-state orientation.

Fig. 2(*b*) shows that for both tethered and free DNA the LD response to an inverted field exhibits a minimum. For migrating DNA the minimum is mainly due to a field-driven collapse of both arms of molecules that happened to be in a U-conformation at the time of field reversal.[9] Also in this case a slower response is therefore expected for tethered DNA, because reorientation can only occur by motion of one end, and then from a more extended state. Notably, the minimum is considerably deeper for the trapped DNA, indicating that the tethering leads to a more concerted response of the ensemble of molecules, as anticipated. Free molecules will reorient from a wider distribution of conformations, and the spectroscopic averaging results in a smeared-out LD-response and therefore in a shallower minimum than for tethered molecules. These observations show that tethered DNA can be used to study stretching and other field-induced motions of DNA in electrophoresis gels, as separate processes.

This work was supported by the Magn. Bergwall foundation.

References

1 B. Norden, C. Elvingsson, M. Jonsson and B. Åkerman, *Q. Rev. Biophys.*, 1991, **24**, 103.
2 S. B. Smith, P. K. Aldridge and J. B. Callis, *Science*, 1989, **243**, 203.
3 T. Kotaka, S. Adachi and T. Shikata, *Electrophoresis*, 1993, **14**, 313.
4 A. Larsson and B. Åkerman, *Maromolecules*, 1995, **28**, 4441.
5 M. Wilchek and E. A. Bayer, *Methods Enzymol*, 1990, **184**, 5.
6 J. Sambrock, E. F. Fritsch and T. Maniatis, *Molecular Cloning*, Cold Spring Harbor Laboratory Press, 1989.
7 C. R. Lowe and J. C. Pearson, *Methods Enzymol.*, 1984, **10**, 97.
8 M. Jonsson, B. Åkerman and B. Norden, *Biopolymers*, 1988, **27**, 381.
9 K. J. Platt and G. Holzwarth, *Phys. Rev. A., Gen. Phys., Ser 3*, 1989, **40**, 7292.

Received, 14th September 1995; Com. 5/06092C