Electrophoretic Orientation of DNA detected by Linear Dichroism Spectroscopy

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The observation is reported of a partial, but significant, alignment of DNA during electrophoretic migration in an aqueous polyacrylamide gel; orientation of elongated macromolecules due to electrophoretic motion has been postulated before but never directly measured.

Polyacrylamide gel electrophoresis (P.A.G.E.), which has become the standard method for analysis of DNA, separates DNA fragments of different size owing mainly to a sieving effect. It is well known that above a certain limit increasing field strength no longer favours but instead counteracts the separation. This has tentatively been ascribed to an increase in the alignment of rigid DNA molecules with the strong electric field;[1] alternatively, orientation or distortion of the polyacrylamide chains by the electric field might aid the passage of the DNA molecules through the gel matrix.[2] Extension of flexible coils along the electric field has also been proposed in connection with gel electrophoresis on large DNA.[3] Although a preferred orientation of elongated particles with the longest dimension parallel to the direction of motion should be an expected hydrodynamic fact, no successful experiments verifying this effect in electrophoresis have as far as we know been reported.

We are engaged in an investigation of the behaviour of DNA in gels and have applied linear dichroism (L.D.) as a tool for studying alignment effects of the DNA as well as of the gel structure. L.D. is today readily measured with the phase-modulation technique[4—7] and, as was demonstrated in the earliest experiments, it could also sensitively monitor orientation of only slightly oriented samples, such as dilute solutions of small guest molecules in anisotropic polymer hosts.[8] The linear dichroism, at a given wavelength, is defined by equation (1),[4] with $A_{\parallel}$ and $A_{\perp}$ denoting the absorbances with the electric vector of plane polarized light directed, respectively, parallel and perpendicular to a given external reference axis (in our case the electrophoretic field direction). The absorption process of polarized light is on a molecular level determined by a vectorial property known as the transition dipole moment, in that maximum absorption is achieved when the electric field of the light is parallel to the transition moment. In absence of any anisotropic force field, thermal motion in solution will randomize the orientation of the molecules (and transition moments) so that the sample is isotropic on a macroscopic level and $A_{\parallel} = A_{\perp}$ and L.D. = 0. Conversely, macroscopic electric and magnetic fields, and also 'hydrodynamic fields' and steric deformations, can produce macroscopically aligned samples.[4,6] The orientation of a molecule in such a sample can be characterized by the ratio L.D./A_{iso}, where A_{iso} is the absorbance of the isotropic sample, when the wavelength of light is chosen to correspond to a suitable transition moment in the molecule (L.D./A_{iso} = 3, for example, generally corresponds to perfect alignment[4]). For a qualitative purpose it is sufficient to note that L.D. > 0 implies a non-random orientational distribution.

$$\text{L.D.}(\lambda) = A(\lambda)_{\parallel} - A(\lambda)_{\perp} \quad (1)$$

Figure 1 reports L.D. and A spectra upon steady-state electrophoresis with and without DNA in polyacrylamide at moderate fields (technical details are given in the caption). Electrophoresis on a pure gel without DNA results in a slowly growing negative L.D. signal, when followed at a certain wavelength, and the featureless L.D. spectrum has the characteristic inverse wavelength dependence of Rayleigh

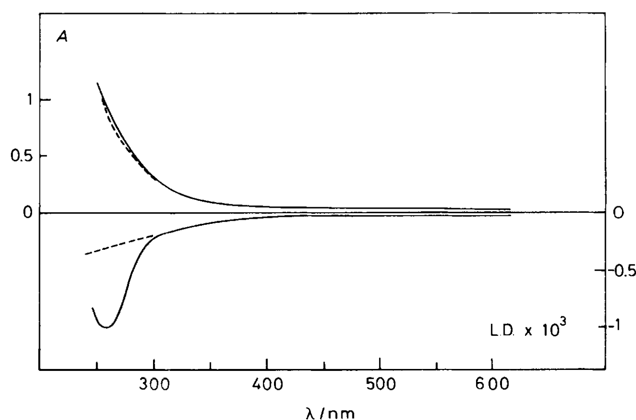

Figure 1. Linear dichroism (L.D.) and absorbance (A) of an electrophoresis gel (0.3 cm thick) containing DNA at a field strength of 20 V cm^{-1}. The L.D. baseline corresponds to zero field strength; optical pathlength of L.D. and A, 0.3 cm. DNA concentration *ca.* 0.05 mM phosphate, dashed curve corresponds to steady-state spectrum without DNA. Electrophoresis conditions: 5% (T) polyacrylamide, 5 mM NaH_2PO_4, 5 mM Tris, 0.12 mM ethylene diaminetetra-acetic acid, pH 7.5, sonicated calfthymus DNA (Sigma type I).

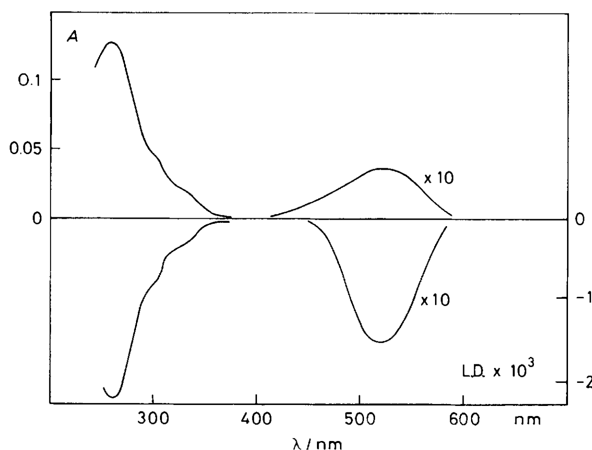

Figure 2. L.D. of DNA (*ca.* 0.025 mM) in an electrophoresis gel containing ethidium bromide (EB) (*ca.* 0.002 mM). The background due to light scattering has been subtracted in L.D.; the absorbance spectrum (A) corresponds to free buffer solution containing DNA (0.025 mM) and EB (0.002 mM). The absorption band around 500 nm of EB is broader than the L.D. band owing to the presence of both free and DNA-associated ligand (abs. max. at 470 and 520 nm, respectively, for free and bound EB). Experimental conditions as in Figure 1.

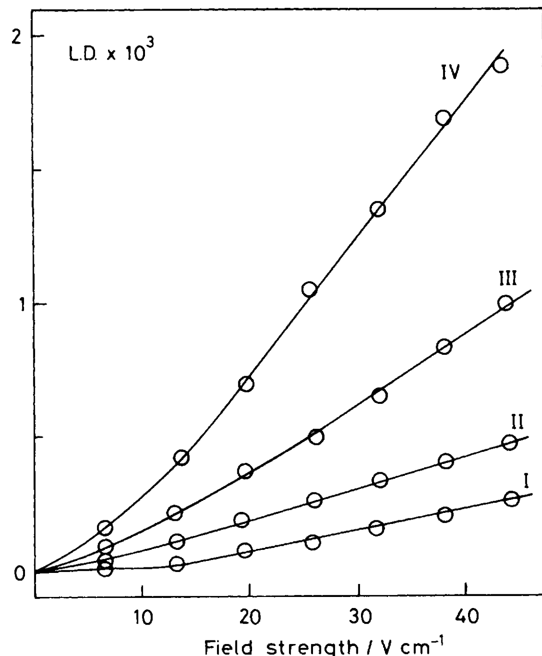

Figure 3. Orientation (L.D.) as a function of field strength. The curves represent the orientation at different positions along the gel. The sonicated DNA was first allowed to migrate to these positions at a low constant field strength (1 V cm^{-1}). The resulting DNA concentration is approximately uniform (*ca.* 0.1 mM phosphate), and the curves correspond to decreasing DNA length in the order IV—I (mobilities 115×10^{-6} $cm^2 V^{-1} s^{-1}$ for I, 80×10^{-6} for II, 45×10^{-6} for III, and 25×10^{-6} for IV).

scattering. This slow L.D. response (minutes) correlates with the stabilization of the electric current, indicating that the effect is due to a thermally induced anisotropic deformation of the gel. The relaxation of this L.D. signal, when the voltage is turned off, has a time constant of several hours. In order to confirm the orientation of DNA an intercalating dye ethidium bromide (EB) with an absorption band in the visible region was also used (Figure 2).

With DNA (or DNA including EB) in the gel the build-up as well as the decay of the L.D. takes less than 1 second, if measured at the wavelengths where the transition moments of DNA (or the DNA–E.B. adduct) are responsible for the light absorption (260 nm for DNA and 520 nm for EB). In fact, as seen in Figure 1, the spectrum is superimposed upon a pure gel electrophoresis L.D. spectrum. The two contributions are also easily separable because of their very different relaxation times.

The important conclusion from the results in Figures 1 and 2 is that DNA becomes oriented with its helix axis in the migration direction during P.A.G.E. The evidence for the DNA direction is the negative L.D. signals from both the 260 nm band of DNA and the 520 nm band of intercalated EB. The negative sign of L.D. comes from the fact that the transition moments in the DNA bases (for B form DNA) and the intercalated EB are directed more or less perpendicular to the helix axis, so that $A_{\perp} > A_{\parallel}$. Similar shapes of L.D. and A_{iso} for the DNA 260 nm band indicate that the oriented DNA remains in a B type of conformation in the gel.[9]

We find further that, within the investigated intervals, the orientation increases, apparently linearly, with the electric field strength and with the length of the DNA fragments (Figure 3). The orientation is far from perfect (L.D./A_{iso} = 10^{-2}—10^{-1} depending on DNA size, field strength, *etc.*), but a quantitative discussion of the different factors governing the orientation will have to await a full paper concerned with these measurements on well characterized DNA and oligonucleotides.

A short discussion of the orientation mechanism is warranted here, however. The orientation is only partial; however, it is still several orders of magnitude greater than can be obtained from electric dipole orientation (Kerr effect). The absence of any fast pure-gel orientations seems also to eliminate the possibility that the DNA orientation is an indirect effect of gel orientation (such effects are in fact known for mechanically deformed gels[10]). The electro-osmotic flow is low in polyacrylamide and its velocity gradients are confined to the electrolyte close to the matrix walls and should therefore only have a marginal effect on the DNA orientation. Check experiments with flow orientation in a Coutte cell of the DNA sample also indicate that flow gradients can be excluded as an orientation origin.

Finally, the orientation can be due to what we shall call steric and frictional interactions between the DNA molecules and the gel structure as the fragments migrate through the matrix (*e.g.* analogous to curly hairs passing through a comb). This will be our working hypothesis in the following work which will concentrate on the dependence of orientation, both regarding steady-state magnitude and transient behaviour, on DNA chain length, on electric field as a time dependent vectorial quantity, and on the properties of various kinds of electrophoresis gels.

We also point out three potential implications of electrophoretic orientation of DNA. First, studies of orientation may provide valuable information on the dynamics of biopolymers. Secondly, electrophoresis as a separation and analytical technique can probably be substantially improved by modifying the method guided by this new type of knowledge. Thirdly, electrophoretic orientation in gels, which could be made quite u.v.-transparent, could be used as a complement to other orientation methods for studies of DNA structure and interactions.

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