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HIGH-SENSITIVITY LINEAR DICHROISM AS A TOOL FOR EQUILIBRIUM ANALYSIS IN BIOCHEMISTRY. STABILITY CONSTANT OF DNA–ETHIDIUMBROMIDE COMPLEX

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A stoichiometrical application of a sensitive method for linear dichroism (LD) detection is suggested for biochemical purposes. The complex formation between a binding site on a polynucleotide and a ligand may be studied with high precision if the following conditions are fulfilled: (1) The polymer can be given a fixed degree of orientation. (2) The site has a specific orientation with respect to the orientation axis of the polymer (e.g., intercalation). (3) The ligand has an anisotropic optical absorption property.

The method was applied to studying the complex between DNA and ethidiumbromide, which was detected by LD with precision of $\pm 0.5 \times 10^{-7}$ M in a 4×10^{-4} M DNA solution, i.e., 0.1% occupation of the total site concentration can be detected. The complexation could be explained by a single type of site ($n = 0.14 \pm 0.01$ sites per nucleotide residue) and a stability constant $K_1 = (2.5 \pm 1) \times 10^5$ M^{-1} at 0.2 M ionic strength.

From the specific LD an average angle 60° was concluded between the helix axis and the long axis of the ethidiumbromide molecule.

This value formally contradicts the Watson–Crick model or the intercalation model but may be explained by extension and deformation effects on the xhain by the flow.

1. Introduction

Among ordinary methods for equilibrium analysis commonly used in complex chemistry [1] only few seen applicable when studying the complexation between macromolecules and small ligands. The more sensitive methods are based upon a change in some optical spectroscopic intensity (UV absorption, circular dichroism, fluorescence, etc.) associated with the formation of the complex [2]. The specific change (M^{-1}) is generally small in comparison with the total intensity due to products and reactants, often it is less than 5%. The ideal case is of course an intensity signal that is proportional to the concentration of some of the participating species in the reaction. Such a signal is the induced linear dichroism (LD) to be observed at the complexation between an oriented polynucleotide and an optically anisotropic dye molecule, here demonstrated by DNA and ethidiumbromide (EB), respectively.

The present study is a consequence of an available very sensitive linear dichroism detection technique [3] recently found to be convenient at determining the content of DNA in helial conformation [4].

2. Basic theory

The radiant energy ("intensity") I_0 incident upon a sample may be attenuated by absorbance according to eq. (1), where I is the transmitted energy,

$$A = \log_{10}(I_0/I) . \qquad (1)$$

We define the linear dichroism, $LD = A_{\parallel} - A_{\perp}$, in eq. (2),

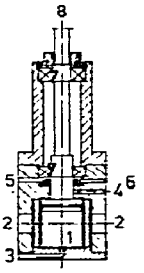

Fig. 1. Orientation cell: (1) Inner quartz cylinder. (2) Light path. (3–4) Inlet – outlet. (5–6) Drain (connected to water suction pump) preventing contamination between bearing grease and sample solution. (7) Ball-bearings. (8) Rotor axis, driving equipment and tachometer.

$$A_{\parallel} = \log_{10}(I_{\parallel 0}/I_{\parallel}), \qquad A_{\perp} = \log_{10}(I_{\perp 0}/I_{\perp}), \tag{2}$$

$$A_u = \log_{10}(I_{\parallel 0} + I_{\perp 0})/(I_{\parallel} + I_{\perp}), \tag{3}$$

where $\parallel$ and $\perp$ denotes that the electric vector of the incident light oscillates parallel and perpendicular, respectively, to a laboratory-fixed axis Z (Z perpendicular to the rotation axis of the orientation cell in fig. 1). The absorbance, A_u, observed with unpolarized light on the same (oriented) sample is defined in eq. (3).

Let a polynucleotide be introduced in a solution into the cylindrical cell space, fig. 1. If the molecule has a rigidly prolate shape a certain degree of orientation with the prolate long-axis trying to align parallel to the direction Z, may be accomplished by the shear field produced by rotating the inner cylinder. The orientation will depend on the viscosity of the fluid. In the present treatment it is required that the orientation is constant throughout a titration, i.e., it must either by checked so that the viscosity is not perturbed or a shear field be used where the orientation is perfect.

If we define a site, S, of the polynucleotide so that its occupation by a ligand, L, yields a defined orientation of L with respect to the orientation axis of the polynucleotide and the latter has the fixed average orientation mentioned above, eq. (4), can be shown to hold.

$$\mathrm{LD} = \Delta\epsilon\, d\, [\mathrm{SL}] . \tag{4}$$

Here, d (cm) denotes the path length, $\Delta\epsilon$ ($M^{-1}cm^{-1}$) is a linear dichroism coefficient specific for the complex SL. The requirement of a defined orientation may be formulated so that the probability of getting a certain orientation of SL should be independent of the degree of occupation of the total site concentration C_S.

If several different types of sites S(i) are occurring, LD may be composed by a sum $\Sigma\Delta\epsilon_i d\,[\mathrm{S}(i)\mathrm{L}]$ instead, or, eq. (5), when written in terms of stability constants and free ligand concentration according to the equilibria defined in eq. (6).

$$\mathrm{LD} = [\mathrm{L}]\, \Sigma K_i \Delta\epsilon_i d [\mathrm{S}(i)] , \tag{5}$$

$$\mathrm{S}(i) + \mathrm{L} \overset{K_i}{\rightleftharpoons} \mathrm{S}(i)\mathrm{L} ,$$

$$K_i = [\mathrm{S}(i)\mathrm{L}]/[\mathrm{S}(i)]\,[\mathrm{L}] . \tag{6}$$

A site density, n, may be defined according to eq. (7) where C_N stands for the concentration of the nucleotide unit and n_i is the number of sites S(i) per unit.

$$C_{S(i)} = n_i C_N . \tag{7}$$

3. Experimental study

In the case of DNA and EB there is reason to believe that at high ionic strength, only one strong complex is formed (by an intercalation site S(1)) [5] and that any further complexation (S(2)) is due to weak ($K_2 \ll K_1$) species with less well defined structure ($|\Delta\epsilon_2| \ll |\Delta\epsilon_1|$). Hence eq. (5) turns over into eq. (4).

Further, LD is observed to approach a saturation value at high EB concentrations, implying that the occupation of the site ensemble is complete, i.e., $C_S = [\mathrm{SL}]$. By this the situation simplifies considerably and a ligand number, $\bar{n}_1$, [1] for the S(1)L complexation is directly obtained according to eq. (8).

$$\bar{n}_1 = [\mathrm{S}(1)\mathrm{L}]/C_{S(1)} = \mathrm{LD}/\mathrm{LD}_{max} ,$$

$$\bar{n}_2 = [\mathrm{S}(2)\mathrm{L}]/C_{S(2)} , \qquad 0 \leqslant \bar{n}_1, \bar{n}_2 \leqslant 1 . \tag{8}$$

K_1 is now estimated from eq. (9), straightforwardly derived from eqs. (6)–(8) and the conditions $C_{S(i)} = [\mathrm{S}(i)] + [\mathrm{S}(i)\mathrm{L}]$ and $C_L = [\mathrm{L}] + \Sigma_i[\mathrm{S}(i)\mathrm{L}]$.

$$1/(1-\bar{n}_1) = K_1 C_L/\bar{n}_1 - K_1 n_1 C_N - K_1 \bar{n}_2 C_{S(2)}/\bar{n}_1 . \tag{9}$$

By plotting $1/(1-\bar{n}_1)$ versus $C_L/\bar{n}_1$ a straight line should thus be obtained when the last term of eq. (9) can be neglected, i.e., in the concentration range where [S(2)L] is negligible, with K_1 as the slope and $n_1 C_N$ as the intercept at $1/(1-\bar{n}_1) = 0$.

Calf-thymus DNA, Sigma type I, was used without further purification in a buffer solution of 0.1 M NaH_2PO_4, 0.1 M NaCl, 10^{-3} M Na_2 versenate (adjusted to pH 7.1 with HCl). Ion strength with respect to cations was 0.2 M.

Ethidium bromide (Aldrich) was used in a stock solution 2.14×10^{-3} M in water without any buffer. The concentration was determined by ϵ (480 nm) = 5600 M^{-1} cm^{-1} [6]. This solution was added in amounts of 10–20 μl to a titration bulk of 4.3 ml of the DNA (10^{-4} M)-buffer solution. The DNA concentration was determined by UV absorption, using ϵ (260 nm) = 6600 M^{-1} cm^{-1} [7].

LD was measured by employing a converted Legrand–Grosjean circular dichroism spectrometer according to a recent design [8–10]. The measuring cell was a Couette device, construction described in fig. 1. The recording was done 5 min after adding the EB solution, at which time it could be shown that equilibrium was reached (constancy in LD during at least 2 hours).

LD was measured at 330 nm at which wavelength the LD contribution from DNA is zero. The shear gradient, G, was kept at a value ensuring perfect orientation of the DNA molecules (3000 s^{-1}) [11]. LD was evaluated as the difference between the baseline at zero gradient (in practice the baseline was identically zero) and the recorded LD deflection at G = 3000 s^{-1}.

The sensitivity of the instrument corresponded to 1.250 absorbance units for a pen deflection over the whole chart. The circular dichroism instrument is calibrated in ellipticity units (°). The LD (absorbance units) was obtained according to eq. (10), where ϑ is the ellipticity, without using any optical or electronic corrections [10].

$$LD = \vartheta/33.0 . \qquad (10)$$

4. Results

The first important observation is that an LD signal appears upon addition of ethidium bromide to a sheared DNA solution, at the wavelengths characteristic for the $\pi \rightarrow \pi^*$ spectrum of EB. Fig. 2 shows the $\Delta\epsilon$ spectrum of the SL complex. The spectral shift of EB upon binding from 480 nm to 520 nm is clearly visible in the LD spectrum. (With only DNA, LD = 0 in this wavelength range).

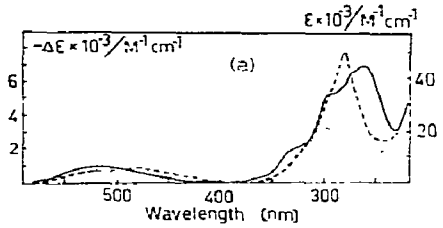

Fig. 2. (a) Linear dichroism spectrum of DNA – EB complex at G = 3000 s^{-1} (——), absorption spectrum of DNA – EB complex (·····), absorption spectrum of EB (– – – – –).

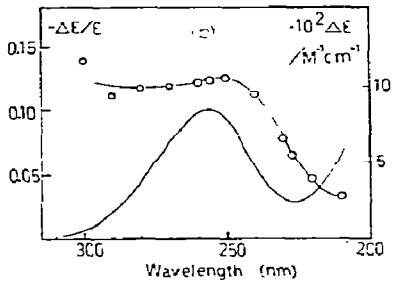

Fig. 2. (b) LD of DNA (calf-thymus, 0.3 mg/ml) at G = 3000 s^{-1} in the UV region, 0 LD/A.

The signal thus detected by the new linear dichroism method is an extremely sensitive measure of [SL]: $\Delta\epsilon$ estimated by means of eq. (4) is $\Delta\epsilon = LD/d\,[SL] = -11.36 \times 10^{-3}/0.100 \times 55.4 \times 10^{-6} = -2051$ M^{-1} cm^{-1} at 330 nm, where we have used in advance knowledge of [SL] for this purpose of demonstrating the sensitivity. LD can be measured with a resolution sensitivity of $\pm 10^{-5}$ absorbance units and hence [SL] can be measured $\pm 0.5 \times 10^{-7}$ M, that is, within 0.1& of the total site concentration in the case of 4×10^{-4}M DNA.

Fig. 3 shows some titration curves at different C_N and the corresponding plots according to eq. (9) are found in fig. 4, together with the estimated K_1 and n_1 values. Further two evaluation methods for K_1 were tried (figs, 6,7, see section 5).

On the basis of observed specific LD due to the DNA–EB complex and the intrinsic chromophores of DNA (fig. 5) we have discussed the possible structure of the complex (vide infra).

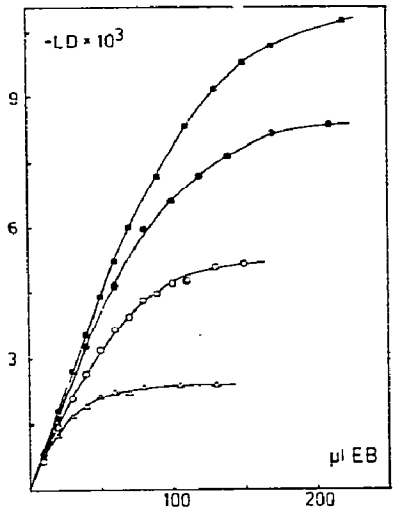

Fig. 3. Titration curves (LD versus added amount of 2.14 × 10^{-3} M EB) for different DNA concentrations: (△) 1.03 × 10^{-4} M, (○) 2.26 × 10^{-4} M, (●) 3.20 × 10^{-4} M, (■) 4.26 × 10^{-4} M. Ionic strength 0.2 M (except for △ = 0.1 M).

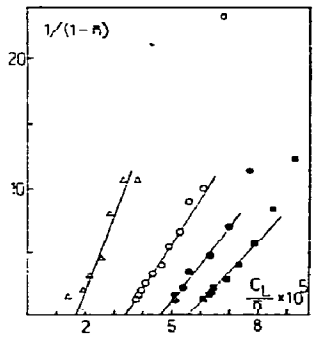

Fig. 4. K_1 and n_1 from the titrations visualized in fig. 3, with the same symbols, by means of eq. (9): (△) $K_1 = 5.2 \times 10^5$ M^{-1}, $n_1 = 0.16$; (○) $K_1 = 3.5 \times 10^5$ M^{-1}, $n_1 = 0.15$; (●) $K_1 = 2.9 \times 10^5$ M^{-1}, $n_1 = 0.14$; (■) $K_1 = 2.4 \times 10^5$ M^{-1}, $n_1 = 0.13$.

A lot of work on LD of biopolymers has been reported [11,17] and the construction of our Couette cell is very similar to that developed by Shimadzu Ltd. and Wada [11].

In contrast to Wada, who obtained a non-zero baseline LD for water, we can establish LD = (0 ± 5) × 10^{-5} over the whole wavelength range for water at G = 4000 s^{-1} as well as for DNA solutions at rest.

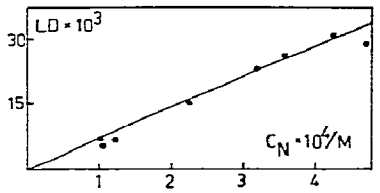

Fig. 5. LD due to the DNA purine and pyrimidine chromophores (260 nm) versus DNA concentration at G = 3000 s^{-1}.

We thus conclude that the technical design of the Couette cell is satisfactory (mechanically and optically) and well suited for its combination with the high-sensitivity LD spectrometer.

The present method may also be applied in studies directed towards the hydrodynamic properties of flow-oriented molecules. In fig. 8, LD/A at various gradients is visualized. It is of interest to approach the range of zero flow if it is suspected that the flow may perturb the structure or the hydrodynamics be anomalous in some way (see section 5).

5. Discussion

Besides the use above of LD for the equilibrium analysis we may note that the sign (negative) of the dichroism gives an important qualitative information about the structure of the DNA – ethidium bromide complex. The excited transitions responsible for the observed absorption are expected to be polarized within the molecular plane, which has recently been confirmed by traditional methods on EB in stretched PVA films [12]. The negative LD thus proves that the average orientation of the transition moment in the EB molecular plane lies beyond a "magic" angle of 54.7° (at which a zero LD should be recorded) with respect to the DNA helix axis [8]. In other words, the EB planes may lie more or less perpendicular to the helix axis, in agreement with the location expected in an intercalation model.

In fig. 5 we have found a linear dependence of the intrinsic DNA LD on the DNA concentration. Since mutual chain interactions at higher concentration should be expected to increase the orientation, this

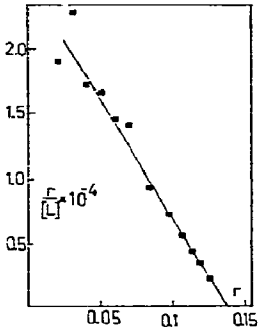

Fig. 6. Scatchard plot (referring to the titration series denoted (▪) in fig. 4) according to eq. (12). $K_1 = 1.8 \times 10^5\ M^{-1}$, $n = 0.14$.

behaviour is an indirect evidence for almost complete orientation. The $\Delta\epsilon/\epsilon$ ratio estimated from fig. 5 (260 nm) is −0.12. If the structure in solution had been identical with that revealed in crystal, i.e., with the purine and pyrimidine ring planes perpendicular to the helix axis, a value of −1.5 is expected.

From a study of the wavelength dependence of LD/A (fig. 2b) the decrease at 210 nm may be noted, supporting the suggestion by Wada about a positive component in this region. No such trend can however be detected above 290 nm which has been claimed to be the probable location of the $n \rightarrow \pi^*$ transition of the azanitrogens [17].

A further test of the LD data was obtained by applying a "Scatchard plot" [14], fig. 6, to one of the titration series:

$$r/[L] = n_1 K_1 - rK_1 , \qquad (11)$$

where

$$r = n_1 \times LD/LD_{max} = [S(1)L]/C_N \text{ and}$$

$$[L] = C_L - n_1 C_N \times LD/LD_{max} .$$

The value $n_1 = 0.13$ estimated from the intercept in fig. 4c was used. Then $K_1 = 1.8 \times 10^5\ M^{-1}$ was obtained and $n_1 = 0.14$.

Another method to evaluate stability constants has been described by Fronaeus [1]. This approach is somewhat different and it seems interesting to compare the result with those above, when it is applied to the same material. Fronaeus et al. have shown that $\bar{n}_1 = d \log X/d \log [L]$, where $X = 1 + K_1 [L]$. From

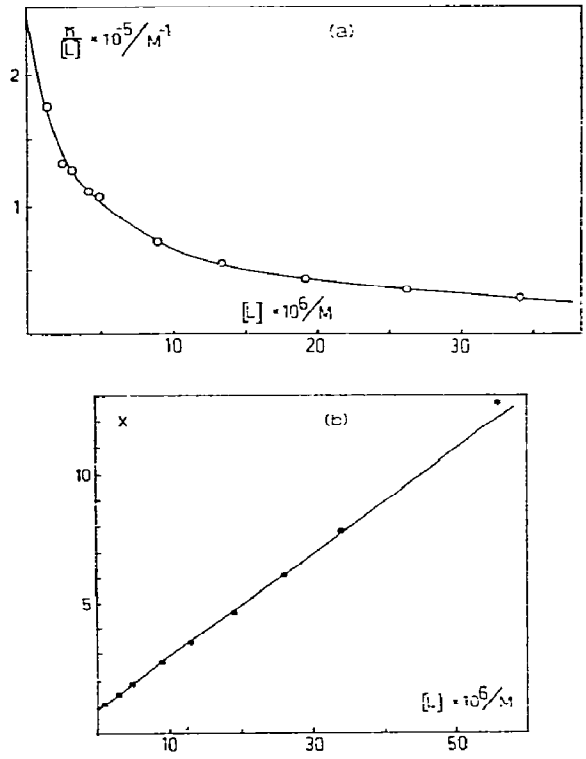

Fig. 7. (a) Plot of $\bar{n}_1/[L]$ versus $[L]$. (b) Plot of X versus $[L]$. $K_1 = (2.0 \pm 0.1) \times 10^5\ M^{-1}$.

this follows that $\int d \log X = \int (\bar{n}_1/[L]) d[L]$.

If $\bar{n}/[L]$ is plotted versus $[L]$ one obtains by a graphical integration a series of corresponding values of X and $[L]$. Fig. 7a shows $\bar{n}/[L]$ versus $[L]$, and fig. 7b shows X versus $[L]$. From the slope of the last plot a $K_1 = (2.0 \pm 0.1) \times 10^5\ M^{-1}$ is evaluated.

The obtained value of the complex constant agrees well with studies by other methods, i.e., by spectrophotometry, spectrofluorimetry and equilibrium dialysis [5,15,16]. The curvature in the plots according to eq. (9), in fig. 4, at high $\bar{n}_1$ values is probably not due to cooperativity between the binding sites, S(1), nor to any important formation of S(2)L complexes. The good linear correlation obtained over the whole titration range in the Scatchard plot and in the plot of X versus $[L]$ can be taken as an indication for this. Our initial assumption of a negligible [S(2)L] is thereby supported.

It may be noted that the linear curve shape in fig. 6

makes a fitting by a curve according to the exclusion model very difficult (see fig. 1, ref. [16]), our value of n is considerably lower than earlier values (0.20–0.25). Hence, it seems interesting to scrutinize the real importance of an exclusion effect by a careful LD study at high EB concentrations.

To be able to use the LD data in a more critical consideration of the structure of SL it is necessary to scrutinize on which factors $\Delta\epsilon$, or $\Delta\epsilon/\epsilon$, where ϵ is the specific absorption (M^{-1} cm^{-1}) at $G = 0$, may depend. Eq. (12) gives the expected dichroism as a function of an order function (α, β angles relating the molecule orientation to the laboratory system) and the angle, θ, between the rod axis and the transition dipole moment responsible for the absorption.

$$\Delta\epsilon/\epsilon = \tfrac{3}{2}\,(3\langle\cos^2\theta\rangle - 1)\,F(\alpha,\beta) \qquad (12)$$

At random orientation $F(\alpha,\beta) = 0$, and $\Delta\epsilon = 0$. At complete orientation $F(\alpha,\beta) = 1$ and by assuming that this is the case, $\theta = 57°$ is obtained from the observation of a saturation value of $\Delta\epsilon/\epsilon \approx -0.20$ at increasing gradient ($\epsilon \approx 9500$ M^{-1} cm^{-1}, $\lambda = 330$ nm).

It has been claimed that the phenanthridinium nucleus should be hydrogen-bonded via its amino groups to opposite phosphate groups on the DNA strands [13]. In such a case a θ of at least 80° should be expected, since the transition moment seems to be polarised parallel to the long-axis of the phenanthridinium nucleus [12]. Hence, a complete orientation is not achieved or is the plane of the base pair at a smaller angle to the helix axis than expected from the Watson–Crick model. The effective $F(\alpha,\beta)$ may be reduced if the molecule is fluttering, behaving like a chain, but the angle between the base pair and the helix axis might also be changed, due to a changed pitch when the DNA helix is subject to the hydrodynamic forces. Both effects should tend to reduce $|LD/A|$ at increasing G.

$F(\alpha,\beta)$ may be obtained according to eq. (13) presuming the hydrodynamic theory of Peterlin and Stuart [19] for rigid prolate spheroids (axial ratio = p), where the average values $\langle\sin^2\alpha\rangle$, $\langle\sin^2\alpha\cos^2\beta\rangle$ via Legendre functions are related to the series of $a_i R_i$, $R = (p^2-1)/(p^2+1)$. From numerical evaluations of these [17] as functions of the "reduced" velocity gradient, G/D (D = rotatory diffusion constant) we obtain $F(\alpha,\beta)$, table 1. Obviously, it is sufficient to use $F(p = \infty)$ for practical purposes when $p > 25$.

Table 1
$F(\alpha,\beta)$ as a function of G/D for $p \geq 25$

G/D	$F(\alpha,\beta)$ $p = 25$	$F(\alpha,\beta)$ $p = \infty$
1	0.00767	0.00767
3	0.05479	0.05478
10	0.21320	0.21323
25	0.35631	0.35738
150	0.59056	0.60559
300	0.66164	0.66412
500	0.70531	0.70779
700	0.74504	0.74752
1000	0.77163	0.77412
1500	0.78044	0.78292
2000	0.78139	0.78345

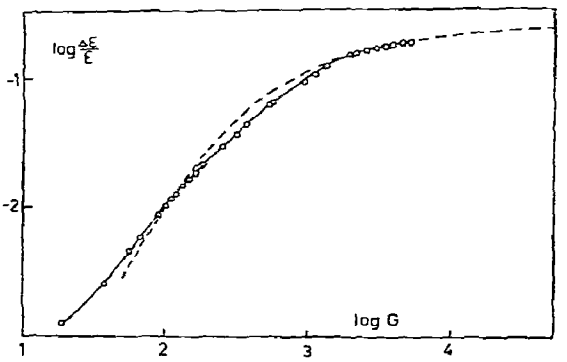

Fig. 8. $\log_{10}(\Delta\epsilon/\epsilon)_{EB-DNA}$ versus $\log G$ (—o—). The theoretical $\log_{10} F(\alpha,\beta) + a$ versus $\log_{10} G + b$ (table 1) has been fitted to the experimental curve by choosing $a = -0.55$ and $b = 1.7$ (– – – –). By means of eq. (12) and table 1, $\langle\theta\rangle = 60°$ and $D = 50\ s^{-1}$. $\Delta\epsilon/\epsilon$ is calculated as $LD/(A - [L]\,\epsilon_L d)$, $[L] = 16 \times 10^{-6}$ M from eq. (6).

$$F(\alpha,\beta) = \tfrac{1}{2}\,(3\langle\sin^2\alpha\rangle - \langle\sin^2\alpha\cos 2\beta\rangle - 2)$$

$$= \tfrac{2}{5}\left(4\sum_i^{\infty}(-a_{11\,i})R^i + \sum_i^{\infty}(-a_{10\,i})R^i\right). \qquad (13)$$

According to eq. (12) the function $\log(\Delta\epsilon/\epsilon)$ versus $\log G$ may be easily fitted by the theoretical $\log F(\alpha,\beta)$ versus $\log(G/D)$ yielding the possible $\langle\cos^2\theta\rangle$ and D values. This procedure was employed by Wada in a study of the intrinsic LD of DNA, [18], however with a considerably smaller spectrum of G values. With the present practically total range a perfectly consistent fitting seems impossible. This is probably due to the fact that the helix is not sufficiently stiff

to be described by the Peterlin–Stuart model. However, the values $\theta = 60°$, $D = 50\ s^{-1}$ yield a reasonable fitting (fig. 8) and as a first approach this procedure must be considered satisfactory.

We may now consider the possibility of flow perturbations of the "optical factor" in eq. (12). If we may neglect an extention of the helix, i.e., assuming that θ is intrinsically fixed (pitch is fixed), DNA excists as a flexible coiled structure at rest which is more or less easily developed at flow. This case is considered by a model of Kuhn [20,21] for the birefringence of a general flexible chain. Then the change in the "optical factor" may be written as in eq. (14), where $\langle\theta_a\rangle$ is the apparent "chromophoric" angle at a certain gradient and $\langle\theta_0\rangle$ the real value, e.g., referring to the axis of a local helix segment, l is the end-to-end distance of the chain and l_0 the total length of the chain (θ_0 and l_0 should be observed for a completely extended chain).

$$(3\langle\cos^2\theta_a\rangle-1)/(3\langle\cos^2\theta_0\rangle-1)$$

$$= \tfrac{3}{5}\,(l/l_0)^2 + \tfrac{36}{175}\,(l/l_0)^4 + \ldots. O[(l/l_0)^6]\ . \qquad (14)$$

The break of the expansion requires that $l \ll l_0$, i.e., that we have a sufficiently low G. According to Kuhn [20,21], Peterlin [22] and Wada [18] the LD may then be expected to depend quadratically on G; with their theory and notations we obtained eq. (15).

$$\Delta\epsilon/\epsilon G = \tfrac{1}{2}\,(3\langle\cos^2\theta_0\rangle-1)$$

$$\times\ \tfrac{2}{5}\,[M[\eta]\eta_0/NkT]^2\,G\ . \qquad (15)$$

With this background it is interesting to observe $\Delta\epsilon/\epsilon G$ plotted against G at small gradients, fig. 9. The behaviour between $G = 0$–$100\ s^{-1}$ may thus tentatively be interpreted in terms of an extension of a coiled chain. From the slope of the line eq. (15) should theoretically offer us a value of $\langle\theta_0\rangle$, however, N = the average segment number may be difficult to obtain [22]. Actually, if we assume $\theta_0 = 80°$ and that $D = 50\ s^{-1}$ eqs. (12), (13) give us a theiretical $(\Delta\epsilon/\epsilon)_0 = -1.36 \times 0.0288 = -0.0392$, to be compared with the observed $\Delta\epsilon/\epsilon = -0.0103$. The behaviour in fig. 9 may thus be explained if the coil is successively unfolded towards an equilibrium extension ratio $l/l_0 \approx 0.63$, eq. (14), during $G = 0$–$100\ s^{-1}$, but then behaves almost stiffly at higher gradients. The ratio $(\Delta\epsilon/\epsilon)_{max}/(\Delta\epsilon/\epsilon)_{\theta=80°} = (-0.2)/(-1.36) = 0.147$ requires formally, $l/l_0 = 0.50$. Hence, if this difference is significant we can not exclude that the helix itself is perturbed yielding a smaller θ_0 by a larger pitch.

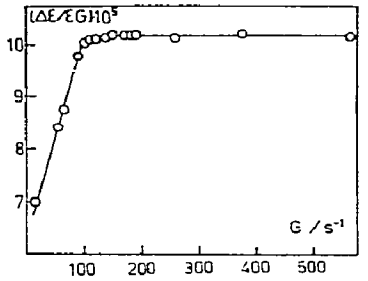

Fig. 9. A possible application of eq. (15) at low gradients.

The essential experience from the present work is not the estimate of a stability constant but rather the establishment of the linear dichroism method as a direct and sensitive tool for measuring a polynucleotide – ligand complex concentration. This fact and the possibility of easily employing the specific linear dichroism of the complex in a structural discussion may suggest that sensitive LD be used as an important complement to other spectroscopic methods in studies of polynucleotides.

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