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OPTICAL STUDIES ON COMPLEXES BETWEEN DNA AND PSEUDOISOCYANINE

Bengt NORDÉN
*Division of Inorganic Chemistry 1, University of Lund, Chemical Center,
S-220 07 Lund, Sweden*

and

Folke TJERNELD
*Division of Biochemistry 1, University of Lund, Chemical Center,
S-220 07 Lund, Sweden*

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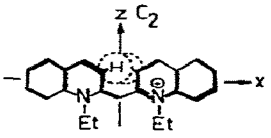
Linear dichroism (LD) results when pseudoisocyanine = PIC (1,1-diethyl-2,2′-cyanine iodide) binds to flow-oriented DNA. LD may be used to follow the complexation both stoichiometrically and structurally, since when specified to unit complex concentration LD provides a measure of the average orientation of the absorbing transition dipole.

Two different types of complexes can be distinguished: I. One strong, ionic-strength insensitive complex with monomeric PIC with an orientation indicating intercalation. II. Several weaker complexes of electrostatic nature (only observed at $I < 0.2$ M NaCl) among which those with dimeric (IIa) and with polymeric (IIb) PIC are concluded both from LD and from circular dichroism (CD). The dimer is probably formed by employing one intercalated PIC as a second site. The polymer complex is characterized by a very sharp absorption band at 553 nm polarized parallel to the DNA-axis (with positive LD and positive CD). Its structure, a right-handed helical array of PIC molecules, is discussed in terms of exciton theory in relation to that of polymeric free PIC ("Scheibe polymer") which is also shown to interact with DNA (IIc) yielding a large aggregate which is degraded at a distinct flow force field. For I a stability constant $K_\mathrm{I} = (9 \pm 3) \times 10^5\ \mathrm{M}^{-1}$ and $n_\mathrm{I} = 0.26 \pm 0.03$ sites per nucleotide residue (formally one site at every second base pair) was obtained; for IIb $K_\mathrm{II} = (2 \pm 1) \times 10^4\ \mathrm{M}^{-1}$, $n_\mathrm{II} = 0.1 \pm 0.5$.

1. Introduction

Linear dichroism spectroscopy was recently employed to study the interaction between oriented DNA and chromophoric small molecules both from stoichiometrical (stability) and structural points of view [1]. We here report that pseudoisocyanine = PIC (1,1-diethyl-2,2′-cyanine) forms complexes with DNA as judged from flow linear dichroism (LD) and circular dichroism (CD). Besides this, results are obtained which are interesting at the background of a number of recent discussions [2–7] on PIC aggregates in relation to photosensitive systems. PIC salts are known to form a thread-like polymer (below referred to as the Scheibe polymer [3,8]) in aqueous solutions at concentrations above 10^{-3} M which is characterized by the appearance of a very sharp absorption band at 573 nm which is polarized parallel to the fibre axis (the J-band [8]). When PIC binds to poly-L-glutamic acid [9] or when being polymerized in an optically active medium [10] this band becomes circularly dichroic which has been ascribed to a helical arrangement of the dye molecules. It is thus often more or less taken for granted (at least for cyanine dyes like PIC) that aggregation is a necessary condition for obtaining induced optical activity. This is understandable if a planar molecule is surmised with little potentiality of gaining rotatory strength in the monomer, but where any chiral arrangement of two or more units can give strong CD by dipole coupling between the intense electric dipole allowed π–π^* transitions in the different units. However, the role of PIC in the literature is ambiguous, many discussions refer to it as a planar dye though both its behaviour as a photosensitizer and the sterical hindrance seen in molecular models suggest it be twisted, which has also recently been found to be the case in crystals [11, 12] ("dihedral" angle φ appr. 50°). A twist can also be expected in solution, the absolute configuration of the enantiomer I will below be referred to as P(C_2).



(I)

P(C_2)-PIC

With this background and in the light of recent claims against [2] and for [6] helical models explaining the optical activity of PIC polymers, we found it valuable to investigate (a) whether CD can be induced in monomeric PIC when complexed to DNA, and (b) whether the Scheibe polymer has any correspondence in a complex. With LD as a sensitive meter of the formation of complexes with non-random orientation, it was further our hope to be able to differentiate between differently structured complexes, of notorious interest in connection with discussions about intercalation and external binding models [13].

2. Experimental method

The LD method is based on a sensitive detection, obtained by the instrumental combination of a Legrand–Grosjean circular dichroism spectrometer (Jasco J-41) and an achromatic quarter-wave accessory, of the differential absorbance [14] LD $= A_{\parallel} - A_{\perp}$ (typical resolution power ± 10^{-6} absorbance units). The polynucleotide solution is in a Couette cell subjected to a constant flow gradient of a magnitude ensuring optimal and time-stable orientation [1]. In the wavelength region characteristic of an added chromophoric molecule a zero LD is observed unless the dye molecule by interaction with the oriented polymer is indirectly given a certain degree of orientation.

If a complex is formed, S(i)L, between a site S(i) on the polynucleotide and the ligand L the observed LD is given by a specific $\Delta\epsilon_i = \epsilon_{\parallel} - \epsilon_{\perp}$ characteristic for the complex and the degree of orientation, $F(\alpha,\beta)$ [1], eqs. (1)–(3), and by the complex concentration and the optical pathlength (d). If only one complex need to be considered at a time (if all $\Delta\epsilon_j = 0$, $i \neq j$ or in a certain concentration range) eq. (3) may reduce to (4). (A is the absorbance and ϵ the decadic extinction coefficient, $\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$). In eq. (2) the total site concentration $C_{\mathrm{S}(i)}$, the total nucleotide concentration C_N (interrelated by the site density number n_i) and the total ligand concentration C_L are related to stability constants K_i.

$$\mathrm{LD} = \sum_i \Delta\epsilon_i [\mathrm{S}(i)\mathrm{L}] d\,, \tag{1}$$

$$\mathrm{S}(i) + \mathrm{L} \rightleftharpoons \mathrm{S}(i)\mathrm{L}\,, \tag{2a}$$

$$K_i = [\mathrm{S}(i)\mathrm{L}]/[\mathrm{S}(i)][\mathrm{L}]\,, \tag{2b}$$

$$C_\mathrm{L} = [\mathrm{L}] + \sum_i [\mathrm{S}(i)\mathrm{L}]\,, \tag{2c}$$

$$C_{\mathrm{S}(i)} = [\mathrm{S}(i)] + [\mathrm{S}(i)\mathrm{L}]\,, \tag{2d}$$

$$C_{\mathrm{S}(i)} = n_i C_\mathrm{N}\,, \tag{2e}$$

$$\mathrm{LD}/(A - \epsilon_\mathrm{L}[\mathrm{L}]d) = \left(\sum_i \Delta\epsilon_i\right) / \sum_i \epsilon_i$$

$$= \tfrac{3}{2} F(\alpha,\beta) \sum_i \epsilon_i (\langle\cos^2\theta_i\rangle - \tfrac{1}{3}) / \sum_i \epsilon_i\,, \tag{3}$$

$$\mathrm{LD}/(A - \epsilon_\mathrm{L}[\mathrm{L}]d) = (\Delta\epsilon_i)/\epsilon_i = \tfrac{3}{2} F(\alpha,\beta)(\langle\cos^2\theta_i\rangle - \tfrac{1}{3})\,. \tag{4}$$

From the observed LD signal eq. (1) may provide information about the complex concentration. Eq. (3) can be used to evaluate the "optical factor", $(\langle\cos^2\theta\rangle - \frac{1}{3})$, yielding the average orientation of the active transition dipole moment with respect to the orientation axis of the polynucleotide (= helix axis of DNA), or if this is known, to extract hydrodynamic information (stiffness of DNA, chain conformation) by theoretical correlation of $F(\alpha,\beta)$ to the velocity gradient, G, for a model molecule [1]. In the present study we have evidence for that the existence range of S(1)L is essentially separated from those of S(i)L,

$i \geqslant 2$ and constant ϵ_i and $\Delta\epsilon_i$ may be assumed at very low C_L. Actually a certain spectral change suggests the formation of a DNA complex with dimer L_2. Since we have no possibility to distinguish this complex stoichiometrically, both monomer and dimer will be included in the notation S(1) L. The site manifold S(1) appears to be completely occupied at about $C_L = C_N/2$ where LD shows saturation and a primitive ligand number $\bar{n}_i$ may be assumed according to eq. (5), which also defines our site concept:

$$\bar{n}_i = [\mathrm{S}(i)\,\mathrm{L}]/C_{\mathrm{S}(i)}$$

$$\approx \mathrm{LD}(\lambda_i)/\mathrm{LD}(\lambda_i)_{\max}\,, \qquad 0 \leqslant \bar{n}_i \leqslant 1\,. \tag{5}$$

K_1 is estimated from eq. (6), straightforwardly derived from eqs. (2), (5).

$$1/(1-\bar{n}_1) = K_1 C_L/\bar{n}_1 - K_1 n_1 C_N - K_1 \bar{n}_2 C_{S(2)}/\bar{n}_1\,. \tag{6}$$

By plotting $1/(1-\bar{n}_1)$ versus $C_L/\bar{n}_1$ a straight line should be obtained when the last term of eq. (6) can be neglected, that is when [S(2) L] is negligible, with K_1 as the slope and $n_1 C_N$ as the intercept at $1/(1-\bar{n}_1) = 0$.

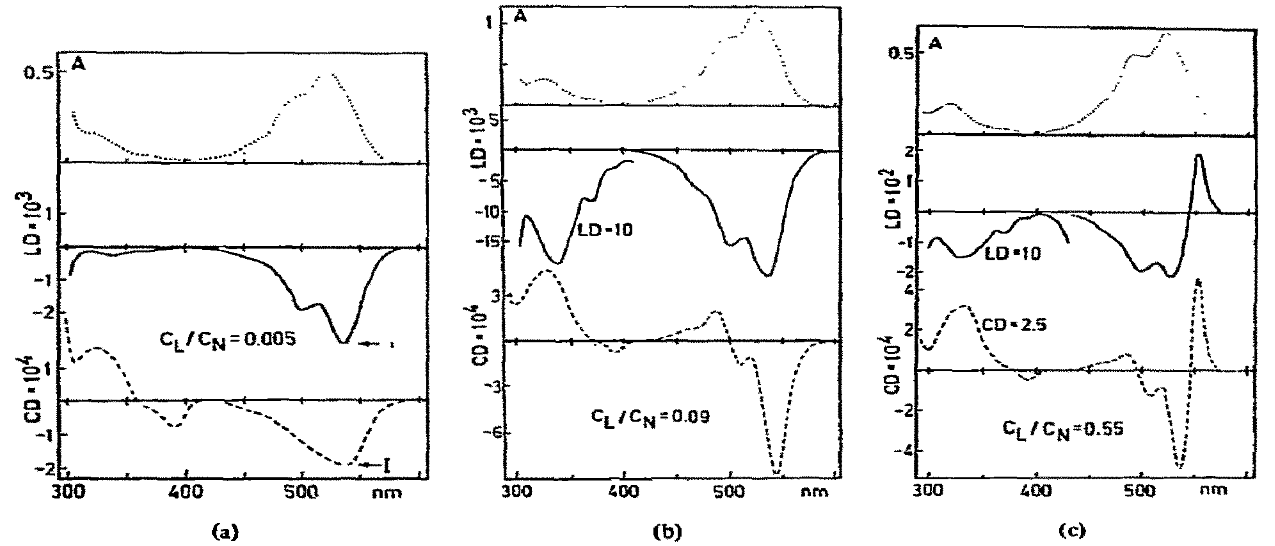
A Scatchard plot was also employed according to eq. (7) [15], where $r = n_1\,\mathrm{LD}/\mathrm{LD}_{\max}$ and $[\mathrm{L}] = C_L - n_1 C_N\,\mathrm{LD}/\mathrm{LD}_{\max}$.

$$r/[\mathrm{L}] = n_1 K_1 - rK_1\,. \tag{7}$$

Confidence limits when given are subjective estimates from apparent uncertainty rather than statistical standard deviations, since the latter appeared throughout negligible at the side of the possible systematic errors due to model assumptions. Typical noise levels have been represented by vertical bars in some spectra. No correction for optics or electronics was made (for absolute LD accuracy see [14]).

Calf thymus DNA (Sigma type I) was used. The commercial pseudo-isocyanineiodide (Koch-Light, No. 33047) was used without purification. An absorption coefficient of PIC at 520 nm, $\epsilon = 6.0 \times 10^4\,\mathrm{M}^{-1}\mathrm{cm}^{-}$ was determined for the 10^{-4} M concentration range in aqueous solution. The DNA concentration (C_N) was determined by the absorption at 260 nm using $\epsilon = 6600\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$.

Circular dichroism is given in absorbance units, observed as $\mathrm{CD} = A_l - A_r = (\epsilon_l - \epsilon_r)\,Cd$, where $\epsilon_l - \epsilon_r$ is in $\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$. Ionic strength 0.01 M NaCl has been used.



(a) (b) (c)

Fig. 1. *A*, LD and CD for $C_L/C_N = 0.005$ (a, 6.4×10^{-4} M) $C_L/C_N = 0.09$ (b, 5.1×10^{-4} M) and $C_L/C_N = 0.55$ (c, 2.5×10^{-4} M). DNA concentrations (C_N) within parentheses. Pathlengths: (*A*, CD) $d_a = 3$ cm, $d_b = 0.5$ cm, $d_c = 0.1$ cm. LD 0.1 cm. Ionic strength 0.01 M NaCl.

3. Results

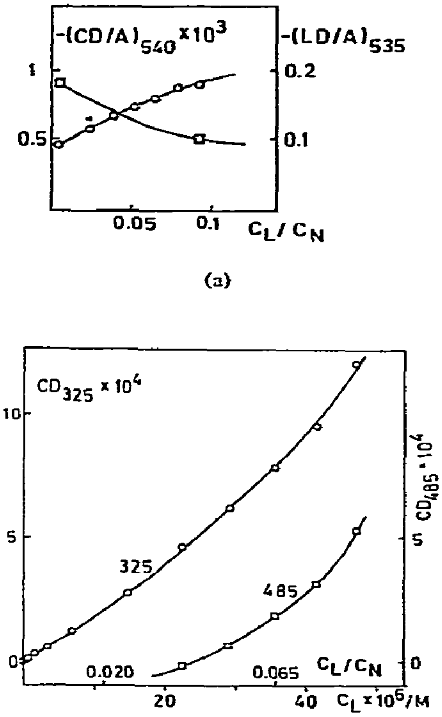
Fig. 1 gives A, LD and CD spectra for DNA/PIC solutions at some selected representative ratios C_L/C_N in 0.01 M NaCl as an ionic medium. A first important observation is a nonzero LD and a non-zero CD at very low C_L ($C_L/C_N = 0.005$) indicating the presence of monomerically bound PIC (dimer influence was excluded from the typically monomeric property of the CD spectrum and from an estimate of the dimerisation constant [4]) and the fact that PIC can exhibit optical activity as a monomer, respectively. The negative LD is in qualitative agreement with an intercalation concept, LD/A at 500 nm is −0.18, which value is almost the same as that obtained for the DNA–ethidiumbromide complex [1] and of the same magnitude as was observed for the DNA purine–pyrimidine $\pi \rightarrow \pi^*$ transitions.

As the next representative we have chosen $C_L/C_N = 0.09$ and here a new (positive) CD band at 485 nm indicates the presence of a complex with dimeric PIC. A decrease of the 525 nm absorption band by almost one half and a well defined high intensity band at 483 nm (observed in 4×10^{-4} M PIC water solution) have been ascribed as characteristics of the dimer [16].

In fig. 2 a values of LD/$A = (\epsilon_\parallel - \epsilon_\perp)/\epsilon_{\text{complex}}$ (because free L can be neglected) and CD/$A = (\epsilon_l - \epsilon_r)/\epsilon_{\text{complex}}$ at about 540 nm have been plotted versus C_L/C_N. This graph further visualizes the appearance of at least a second species (the dimer) which is characterized by a lower linear dichroism to absorption ratio and a higher dissymmetry ratio than of the monomer complex. This behaviour is consistent with the increased possibility, in the dimer, of gaining rotary strength by dipole–dipole coupling and a decreased average order, respectively, when intercalation sites are no longer exclusively employed. Fig. 2b compares the dimeric CD band (appearing above $C_L/C_N = 0.04$) with a CD band at 325 nm belonging to the monomer, but which is probably also present in the dimer.

In the next representative in fig. 1 $C_L/C_N = 0.55$ and here a new band, visible both in LD and CD, has arisen at 553 nm. This wavelength does not agree with that known for the Scheibe polymer (J-band 573 nm), which we take as evidence for that we have a specific polymer PIC$_N$–DNA complex. This conclusion is supported when LD versus flow gradient is studied (fig. 6a): the 553 nm and 528 nm LD band curves by following each other closely, strongly indicate that they originate from a single hydrodynamical species.

In fig. 3 LD versus C_L was plotted for the dye bands (a) as well as for the intrinsic DNA chromophore band (b), PIC has zero absorption at 257 nm. The constancy of the latter observable indicates that the helix is not severely perturbed by the intercalation. At higher C_L ($C_L/C_N > 1$) a certain decrease can be seen in |LD| at 257 nm, which is the effect to be expected at an expansion of the helix, yielding a decreased θ in eq. (4). However, LD is very sensitive to changes in θ and a decrease in LD/A from −0.12 to −0.10, corresponds formally only to the change from 59° to 58°, if a constant $F(\alpha,\beta) = 0.43$ is assumed (vide infra). LD of the dye bands increases linearly with C_L which may suggest a large stability of the



(a)

(b)

Fig. 2. (a) (CD/A)$_{540}$ (○) and (LD/A)$_{535}$ (□) versus C_L/C_N at low C_L. (b) CD at 325 nm (○) and at 485 (□) versus C_L at $C_N = 6.52 \times 10^{-4}$ M.

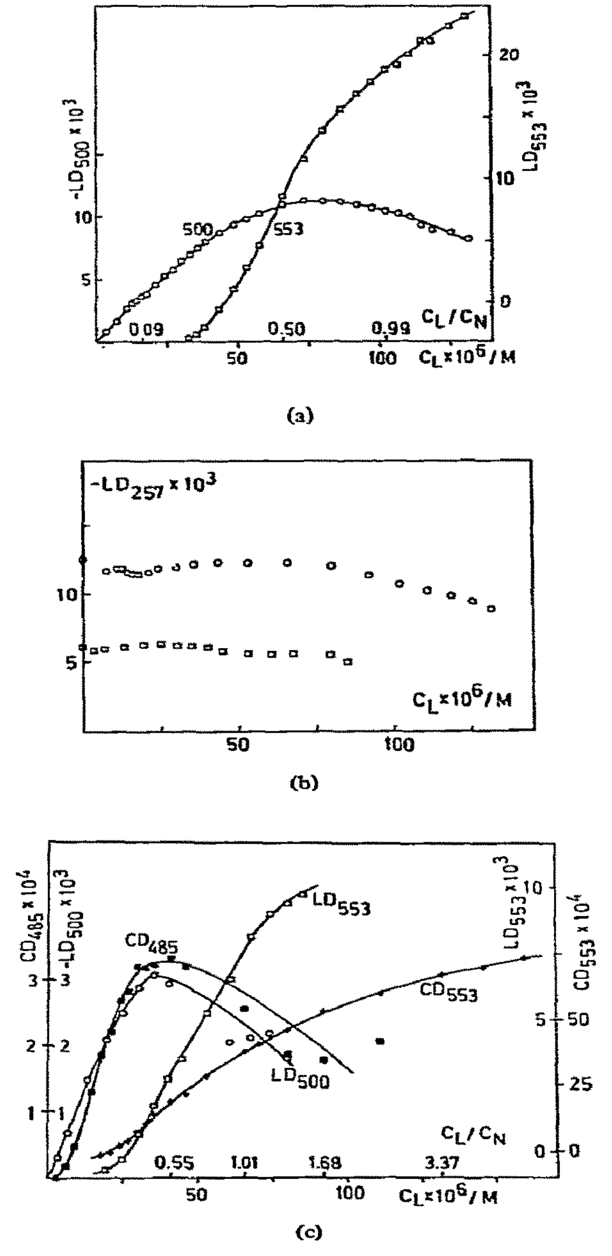


Fig. 3. (a) LD at 500 nm (o) and at 553 nm (□) versus C_L. C_N = 1.86 × 10^{-4} M.

(b) LD of the intrinsic DNA chromophore at 257 nm versus C_L. C_N = 1.86 × 10^{-4} M (o) and C_N = 0.91 × 10^{-4} M (□).

(c) LD at 500 nm (o), 553 nm (□), CD at 485 nm (■) and at 553 nm (+) versus C_L. C_N = 0.91 × 10^{-4} M.

complex. Unfortunately PIC, like many other dyes "disobeying Beer's law" due to aggregation, is very sensitive to the nearest neighbour distance and a constant ϵ_1 for S(1)L cannot be expected when new molecules approach. This effect may be the reason for why the 500 nm band after reaching a maximum then decreases simultaneously with the appearance of the 553 nm band. However, a more plausible explanation is a positive $\Delta\epsilon$ at 500 nm exhibited by the polymer. The position of the maximum, at C_L/C_N = 0.53 ± 0.05 is not changed if C_N is changed between 0.186 mM and 0.091 mM (fig. 3c) which supports the conclusion of a high stability of the first PIC–DNA complex. In fig. 3c it is seen that similar titration curves are obtained when based on either the 485 nm CD band (belonging to the dimer) or on the 500 nm LD band (covering monomer as well as dimeric PIC). The 553 nm LD and CD curves are also similar to each other.

Fig. 4 illustrates an attempt to estimate a stability

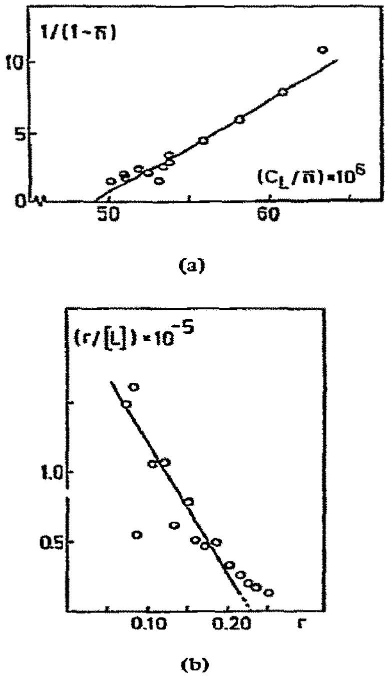


Fig. 4. (a) Plot according to eqs. (5) and (6) for the 500 nm LD band, yielding n_1 = 0.26 and K_1 = 7 × 10^5 M^{-1}.

(b) Plot of $r/[L]$ versus r, eq. (7) yielding n_1 = 0.23 and K_1 = 9 × 10^5 M^{-1}.

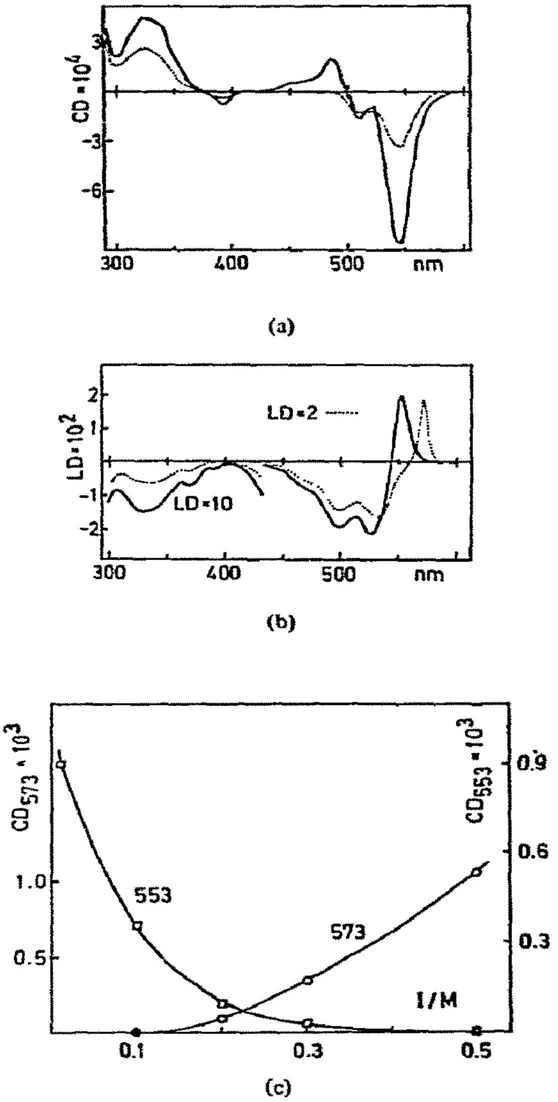


Fig. 5. Effects of ionic medium.

(a) In the existence range of the PIC–DNA and PIC_2–DNA complexes. C_L/C_N = 0.09 (C_N = 5.1 × 10^{-4} M). CD spectrum at I = 0.01 M NaCl (——) and at I = 0.21 M NaCl (----).

(b) In the existence range of the $(PIC)_N$–DNA polymer. C_L/C_N = 0.55 (C_N = 2.5 × 10^{-4} M). LD spectrum at I = 0.01 M NaCl (——) and at I = 0.21 M NaCl (----).

(c) CD at 573 nm (o) and at 553 nm (□) versus ionic strength (M NaCl), C_L/C_N = 1.4 (C_N = 0.85 × 10^{-4} M).

constant of the first complex from the 500 nm LD at low C_L by means of eqs. (6) and (7), under assumption according to eq. (5). $K_1 = (9 \pm 5) \times 10^5$ M^{-1} and $n_1 = 0.26 \pm 0.03$ was obtained. The latter number should mean that every second base-pair slot can be occupied. As will be seen (Discussion) the observed circular dichroism for the 530 nm band system (negative) as well as shorter wavelength CD bands (positive at 330 nm, negative at 390 nm) is consistent with a monomer PIC with $P(C_2)$ configuration (I), which is also the conformation expected to be preferred sterically by the right-handed helical strands (fig. 7a). The idea of an intercalated monomer and a weak dimer–DNA complex is in agreement with the disappearance of the 490 nm "dimeric" CD band in 0.2 M NaCl, fig. 5a. At higher C_L a corresponding extinction by increased ionic strength of the 553 nm CD band, assigned to the PIC_N-DNA complex, shows that also this complex is of a weak (electrostatic) nature. In LD (fig. 5b) the mentioned effect by ionic strength is also clearly visible and here also a certain formation of the Scheibe polymer occurs recognized by the J-band at 573 nm (I = 0.2 M). Only a small fraction (ca. 0.8% at 1.32 × 10^{-4} M PIC) of the total C_L, however, is employed by this polymer and the J-band is therefore in practice only visible in LD and CD due to its opposite sign contra the surrounding monomeric contributions, while in the absorption spectrum it is completely drowned. In fig. 5c the increasing and decreasing Scheibe polymer and PIC_N–DNA concentrations, respectively, by ionic strength are visualized.

With the aim of estimating to which extent the Scheibe polymer is in contact with DNA (an electrostatic attraction can be expected), LD versus flow gradient curves [1] were traced for transitions specific for DNA (257 nm), for the monomerically bound PIC (500 nm) and for the Scheibe polymer (573 nm), respectively. Absence of binding forces should be revealed by different curves due to then uncoupled hydrodynamic properties of DNA and Scheibe polymer. A most amazing result was however obtained (fig. 6b). For the 257 nm LD bands a monotonic growth curve was obtained closely resembling that of free DNA in consistency with a DNA molecule only slightly perturbed by complexation. A similar shape for the 500 nm band curve was an independent proof of the monomer PIC–DNA complex. As expected no

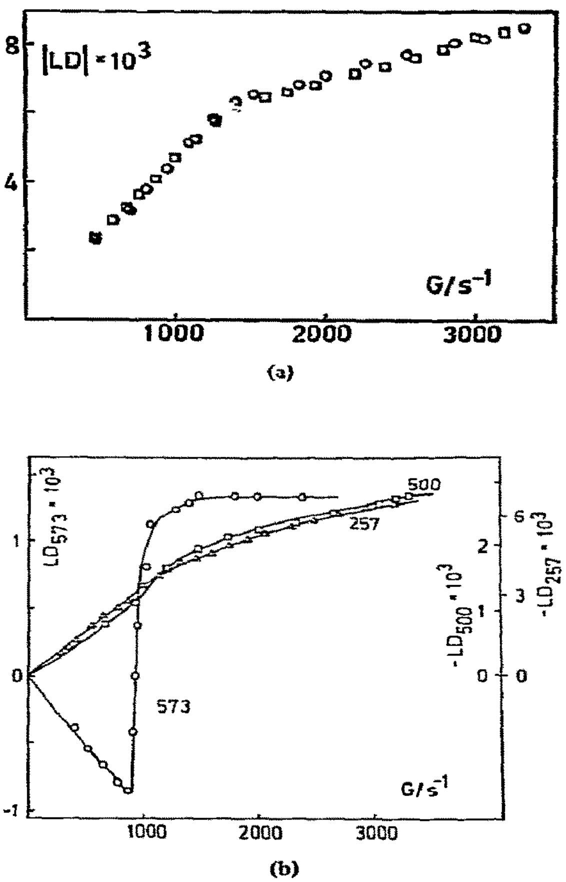


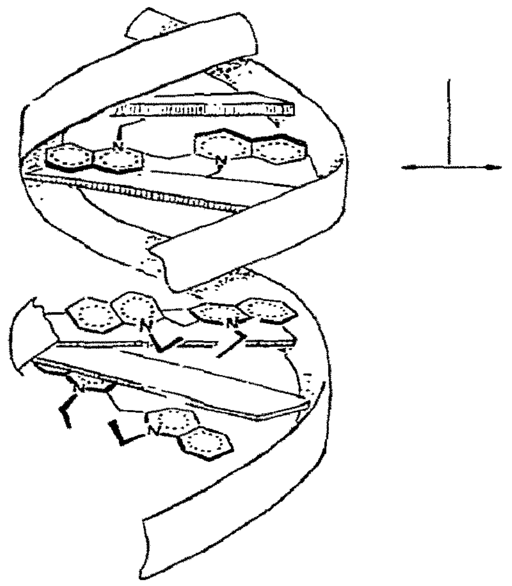
Fig. 6. (a) LD at 528 nm (□) and at 553 nm (○) versus flow gradient at $C_I/C_N = 0.47$ ($C_N = 1.09 \times 10^{-4}$ M). $I = 0.01$ M NaCl.

(b) LD versus flow gradient at 257 nm (△), 500 nm (□) and 573 nm (○) for the Scheibe PIC$_\infty$–DNA sample at $C_I/C_N = 0.91$ ($C_N = 1.6 \times 10^{-4}$ M). $I = 0.21$ M NaCl.

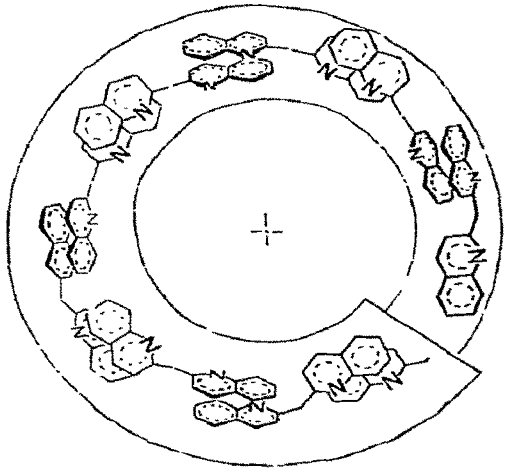
effect on the stability of the complex by the velocity gradient could be detected. However, judging from the 553 nm LD, the Scheibe polymer has apparently required a small amount of DNA to form extremely large particles with a remarkable force-field dependent stability: At low gradients ($G < 900$ s^{-1}) these particles are oriented to give a strong negative LD which, if the DNA axis is still the orientating axis, implies that the fibre axis of the Scheibe polymer is almost perpendicular to the DNA axis. Such an arrangement can be realized in a PIC "super-coil" with low pitch (cf. Discussion, table 1) coaxially surrounding the DNA fibre or in some extended net-work aggregate. If the flow is interrupted a slow relaxation ($\tau_{1/2} = 20$ s) proves a very large particle. At a gradient $G = 990$ s^{-1} (well reproducible at different DNA and PIC proportions) a strikingly distinct transition takes place, probably leading to a disruption of the particle into free DNA and Scheibe polymer, as judged from the positive LD at 573 nm and the now instantaneous relaxation to zero orientation. Furthermore, the relaxation, when the flow gradient is rapidly changed downwards just across the transition limit, leads immediately to the negative stationary LD value observed for the "macro-particle" which shows that the latter is readily formed. The possibility that the transition field defines the particular binding force seems very interesting and not unreasonable in view of the large dimensions of the participating molecules and their ability of acting as levers. However, to our best knowledge this is the first example of a reversible molecular disruption by a mechanical force exerted on the macrosystem.

4. Discussion

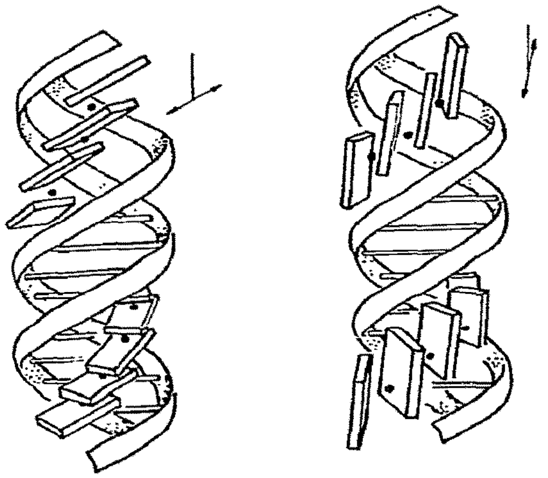
It is known that the long-wavelength (400–550 nm) absorption in PIC is essentially x-polarized (I) [16, 17], the 525 nm band is the 0–0 band and the other bands are members in a progression (0–1 at 500 nm) with successively decreasing intensities, characteristic of the symmetric stretching vibration of the conjugated C=C, C=N bonds. Hence the negative LD proves that the molecular long axis is forming a large angle to the helix axis, in the monomeric PIC–DNA complex. $\Delta\epsilon/\epsilon = -0.18$ yields a formal $\theta = 57°$ if $F(\alpha,\beta) = 1$, but taking into account the possibly uncomplete DNA orientation and elongation, a larger value (61°) may be estimated, fig. 7a, in perfect agreement with an intercalation structure, when referred to the base pair orientation judged from the LD of the intrinsic DNA chromophore [1]. From the observation of $\Delta\epsilon/\epsilon = +1.3$ for the 553 nm polymer band expected to have



(a)



(d)



(b) (c)

Fig. 7. (a) Monomeric PIC–DNA complex, and (b), (c) sketched structures for PIC$_N$–DNA, (d) arrangement at low pitch and r = 11 Å.

completely parallel polarization we get $F(\alpha,\beta) = 0.43$ from eq. (4) with $\theta = 0°$.

Since any z-polarized component in PIC should be very expressive in the rotational strength in a scewed conformation it is of interest to more closely analyse the LD/A spectrum (fig. 8). A variation in LD/A immediately tells that θ is not constant so components with different polarizations must be present. A positive tendency is pronounced at the 320 nm absorption

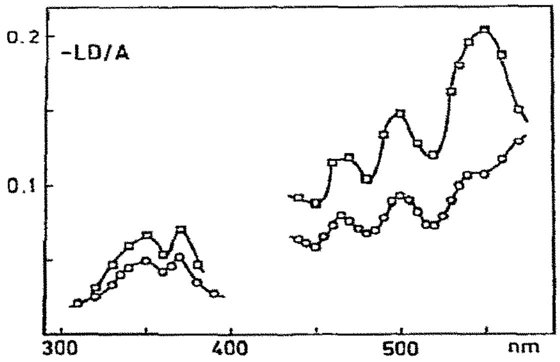


Fig. 8. Analysis of the LD/A spectrum, LD/A at C_L/C_N = 0.005 (□) and 0.09 (○) (C_N = 6.4 × 10^{-4} M and 5.1 × 10^{-4} M).

band where LD/A is almost nil. If no x-polarized component is interfering here and the configuration according to fig. 7a is considered, i.e., the PIC x-axis perpendicular to helix axis, a zero LD/A should be explained by a z-polarized (in the quinoline ring planes [18]) transition in the monomeric PIC skewed to a dihedral angle $\varphi = 2(90° - 54.7°) = 71°$ (68° if LD/A = −0.05 is used). From fig. 7a (or by molecular models) it is obvious that the helical strands imply sterical restrictions (at least in the case of "partial intercalation") leading to that the configuration above denoted $P(C_2)$ will be preferred. With this configuration and a $\varphi < 90°$ a z-polarized component is expected to give rise to an exciton couplet with a positive CD band at the high-energy side of the original transition and a negative on the low-energy side. Thus the 330 nm positive CD band and the negative 390 nm band may be the result of exciton coupling among z-polarized components connected with the 360 nm absorption. The high dissymmetry factor observed for the 390 nm band (5×10^{-3} for the resolved band) is in harmony with this type of origin of optical activity. A similar, strongly dissymmetric, CD band was reported in DNA–ethidiumbromide at 378 nm and was ascribed to an n–π* transition [19]. However, regarding the ethylenes on the nitrogens this cannot be the explanation here.

Also in the long-wavelength region variations in LD/A (fig. 8) indicate the presence of z-polarized vibronic components. However, the main oscillator strength is here x-polarized which, either by exciton theory or by the rule formulated for cisoid dienes [20], implies an overall negative CD in the $P(C_2)$ configuration, in agreement with the observed 400–580 nm CD spectrum.

The positive CD band at 490 nm observed at intermediate C_L and low ionic strength may be assigned either to a high-energy x-polarized exciton component in the dimer, being allowed by the presence (in a first approximation) of an inversion center [3] or to the high-energy monomeric exciton band from a reinforced (in the dimer) vibronic z-polarized transition, which should explain the strong and positive CD. On the basis of crystal studies [11, 12] we suggest the arrangement II in the dimer, which can probably also represent the linking in the polymers as well (vide infra).

(II)

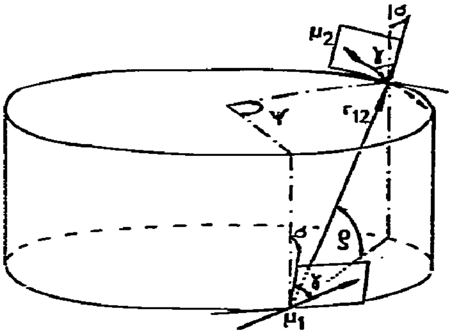
The perhaps most interesting observation in the present study is the J-band-like sharp and strongly anisotropic band at 553 nm (LD/A = 1.3 ± 0.5, CD/A = 0.02 ± 0.01 when corrected for the background absorption). The different position as compared with the J-band is evidence for a $(\mathrm{PIC})_N$–DNA complex with a specific structure. In the light of the large number of different theories about the structure of the Scheibe polymer and about the origin of its optical activity it seems important to discuss the structure of the present polymer which is probably closely related to the Scheibe polymer. Hence, the first observation is that the positive circular dichroism can be connected with a right-handed helix (this has been an open question for the Scheibe polymer). To this conclusion one may arrive by tentatively considering the array of polarizable PIC units as an infinite helical conductor. According to Czikkely, Försterling and Kuhn [5], actually a one-dimensional electron gas model should be preferable to exciton theory since there is evidence for that the latter should break down in the point dipole approximation for molecules in van der Waals contact [21]. First let us however scrutinize the case in terms of an exciton formalism.

According to the cyclic exciton theory of Moffitt [22] (considering a helix of a number of amide residues) only two allowed transition combinations are observed, one with transition moment directed along the helix axis (with a rotational strength $R_\parallel$) and one doubly degenerate with a moment perpendicular to the helix ($R_\perp$). If the monomer transition moments have zero radial components, as in fig. 7b and 7c the rotational strength of the parallel 553 nm band can be related to the radius, r, of the helix and to the inclination angle, γ, of each unit, in III defined with sign for the case of a right-handed helix, eq. (8):

$$R_\parallel = +\pi\nu r|\mu|^2 \sin\gamma\,, \qquad 0 \leqslant \gamma \leqslant 90°\,, \tag{8a}$$

$$(\epsilon_l - \epsilon_r)/\epsilon \approx 4R/|\mu|^2\,, \tag{8b}$$

(ν is the frequency, cm^{-1}, μ is the electric transition dipole moment in the monomer). In the case of nonzero radial component, i.e., when the "chromophoric plane" forms an angle σ with the tangential plane, eq. (8a) should be multiplied by $\cos\gamma\cos\sigma$. By the approximation of the dissymmetry factor (8b) CD/A may be used to estimate one of γ and r if the other is known. Assuming a helix diameter of 20 Å, our $CD/A = 0.02 \pm 0.01$ implies $26° \leqslant \gamma \leqslant 90°$ by eq. (8), which may fit either model b or c in fig. 7.



(III)

According to exciton theory by Tinoco et al. for helical oligomers [23] the energy shift from the monomerical energy is essentially equal to the dipole–dipole coupling energy between adjacent units, modified by a factor containing the unit number, N, according to:

$$\nu_{\parallel,N} - \nu_{\mathrm{monomer}} = \frac{1}{hc}\left(\frac{\boldsymbol{\mu}_1\cdot\boldsymbol{\mu}_2}{r_{12}^3} - \frac{3(\boldsymbol{\mu}_1\cdot\boldsymbol{r}_{12})(\boldsymbol{\mu}_2\cdot\boldsymbol{r}_{12})}{r_{12}^5}\right) 2\cos\frac{\pi}{N+1}. \tag{9}$$

Here $\boldsymbol{r}_{12}$ ($|\boldsymbol{r}_{12}| = r_{12}$) denotes the vector connecting the two adjacent PIC transition dipoles $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ ($|\boldsymbol{\mu}_1| = |\boldsymbol{\mu}_2| = \mu$). By simple geometry eq. (9) is transformed to eq. (10) introducing the coordinates according to III [γ = inclination angle of the transition moment μ in the chromophoric plane which has an angle of tilt σ to the tangential plane, ρ = pitch angle of helix, pitch is $(360/\psi) r_{12}\sin\rho$ if ψ is in degrees, r = radius of helix, r_{12} = inter-dye distance, obviously $\sin(\psi/2) = r_{12}(\cos\rho)/2r$].

$$\Delta = \nu_{\parallel,N} - \nu_{\mathrm{monomer}} = \frac{\mu^2}{hcr_{12}^3}\{\cos^2\sigma[\sin^2\gamma\cos\psi + \cos^2\gamma - 3(\cos\rho\cos\psi/2\sin\gamma + \sin\rho\cos\gamma)^2]\} + \sin^2\sigma(\cos\psi + 3\cos^2\rho\sin^2\psi/2)\}2\cos[\pi/(N+1)]\,. \tag{10}$$

With five independent structural parameters ($\gamma, \sigma, \rho, r, r_{12}$) and only two observables, eqs. (9) and (10), there is no hope of getting any explicit structure. The most reasonable relaxation of the starting condition is to assume μ be polarized in a tangential plane ($\sigma = 0°$), which assumption is justified for two reasons: Firstly, the effect on $R_{\parallel}$ by a not too large σ of any sign is negligible regarding the uncertainty of the determined CD. Secondly, the existence of a polarization component parallel to the helix axis eliminates the possibility of the ideal "card peak" structure (or structures B, B′ in ref. [5]) and any alternative polymer structure must then almost certainly rely on joints of the type II, that is by engaging both quinoline rings. Hence the x-axis of each PIC unit in a helical array is approximately tangenting the helix cylinder at the internuclear carbon, i.e., at the presumed center of gravity of the point dipole (see for instance fig. 7d).

The dipole strength μ^2 was estimated to about $3.4 \times 10^{-35}\ \mathrm{esu}^2\ \mathrm{cm}^2$ from the absorption spectrum, for the J-band of the Scheibe polymer. We have used this couplet dipole strength instead of the monomeric value with the idea of thereby better accounting for the effective coupling. In table 1 we have used eq. (10) to calculate Δ to be expected by exciton theory for some possible combinations of the structural parameters γ, ρ, r and r_{12}. These values should be compared with the experimental Δ, which is $930\ \mathrm{cm}^{-1}$ for the PIC_N–DNA polymer and $1559\ \mathrm{cm}^{-1}$ for the PIC_N Scheibe polymer.

Obviously Δ is insensitive to modest variations of the helix radius. Also the N-dependence must be considered "ineffective" and we found it very unlikely that the difference in Δ between the Scheibe and DNA cases should be due to a change in only r or N. The distinct band position which remained at 553 nm during the course of the titration also apparently rules out N as any important modulating factor in Δ.

Table 1

Structural examples of PIC_N–DNA complexes and PIC_N Scheibe polymers and corresponding shifts Δ according to eq. (10), σ assumed to be zero

Model	γ (deg)	ρ (deg)	r (Å)	r_{12} (Å)	ψ (deg)	$r_{12}^{-3} \times 10^{-21}$ (cm^{-3})	$\sin^2\gamma(\cos\psi - 1) + 1$ $-3[\sin\gamma\cos\rho\cos(\psi/2) + \cos\gamma\sin\rho]^2$	$\cos[\pi/(N+1)]$	$-\Delta$ (cm^{-1})
	45	45	10	9	37	1.37	−1.95	1	915
	45	45	7	9	54	1.37	−1.89	1	887
	45	45	5	9	79	1.37	−1.75	1	826
	45	45	10	8.9	37	1.42	−1.95	1	948
	45	45	10	8.0	33	1.95	−1.96	1	1309
	45	45	10	7.5	31	2.37	−1.96	1	1591
	45	45	10	7	29	2.92	−1.97	1	1970
Model (b)	45	35	10	9	43	1.37	−1.81	1	849
	45	25	10	9	48	1.37	−1.51	1	708
	45	15	10	9	52	1.37	−1.10	1	516
	45	50	10	9	34	1.37	−1.95	1	914
	45	54.73	10	9	30	1.37	−1.90	1	891
	45	65	10	9	22	1.37	−1.65	1	774
	45	70	10	9	18	1.37	−1.47	1	689
Model (c)	15	35	10	9	43.3	1.37	−0.71	1	333
	25	35	10	9	43.3	1.37	−1.17	1	549
	35	35	10	9	43.3	1.37	−1.56	1	732
	50	35	10	9	43.3	1.37	−1.88	1	882
	55	35	10	9	43.3	1.37	−1.91	1	896
	60	35	10	9	43.3	1.37	−1.89	1	886
Changed CD	−5	35	10	9	43.3	1.37	+0.23	1	−108
	−10	35	10	9	43.3	1.37	+0.43	1	−202
	−25	35	10	9	43.3	1.37	+0.83	1	−389
	−45	35	10	9	43.3	1.37	+0.81	1	−380
$N = \infty$	45	45	10	7.5	31	2.37	−1.96	1	1591
$N = 3.4$	45	45	10	7.5	31	2.37	−1.96	0.76	1209
PIC_NDNA from CD and LD data	54	35	11	8.5	37	1.63	−1.93	1	1077 ↓ × 1.68
Expected for Scheibe	?	?	?	?	?	?			1809
Scheibe alternatives:									
Linear brickstone	0	30	6	15 [a)]	180	0.2963	+0.25	1	−25
Double layer	0	30	3.4	8	180	1.95	+0.25	1	−167

Table 1 (continued)

Model	γ (deg)	ρ (deg)	r (A)	r_{12} (A)	ψ (deg)	$r_{12}^{-3} \times 10^{-21}$ (cm^{-3})	$\sin^2\gamma(\cos\psi - 1) + 1$ $-3[\sin\gamma\cos\rho\cos(\psi/2) + \cos\gamma\sin\rho]^2$	$\cos[\pi/(N+1)]$	$-\Delta$ (cm^{-1})
Slow pitch helices:									
coin pile, model (b)	80	10	11	8.5	45	1.63	−1.86	1	1037
	50	10	11	8.5	45	1.63	−1.13	1	630
	45	10	11	8.5	45	1.63	−0.91	1	507
	40	10	11	3.5	45	1.63	−0.67	1	374
ladder model (c)	30	10	11	8.5	45	1.63	−0.17	1	95
Steep pitch helices	−60	45	11	8.5	32	1.63	+0.72	1	−402
	30	45	11	8.5	32	1.63	−1.76	1	982
	60	45	11	8.5	32	1.63	−1.78	1	993
Expanded helix "PIC/NDNA model"	54	35	20	8.5	20	1.63	−1.98	1	1105
Compound-change effect (pitch, r_{12}, model (c))	45	30	11	7.6	35	2.28	−1.73	1	1350
	45	50	11	7.6	26	2.28	−1.95	1	1522
	45	50	11	7.2	24	2.68	−1.96	1	1798

a) According to structure suggested in [5].

Actually this low uncertainty in the experimental Δ ($\pm$ 0.2 nm) formally implies $N > 10^2$ as a consequence of the correspinding maximation of uncertainty of $\cos[\pi/(N+1)]$ with the reasonable assumption that the J-band properties are exhibited at least during a ten-fold increase in molecular weight of the polymer (i.e., $\cos[\pi/(10N_{\min}+1)]-\cos[\pi/(N_{\min}+1)] < 4 \times 10^{-4}$). For the Scheibe polymer it has been suggested that $N > 10^6$ [8]. Examples are given in the table in which the stacking can account for the energy shift between the Scheibe and PIC_N–DNA polymers. It may also be noted that a small change in the internuclear distance r_{12} (e.g., from 7.5 to 8.9 Å) is sufficient to explain this shift.

Regarding the reported failures of the exciton theory for molecules in contact [5,21] we might expect a large systematic error in Δ obtained by means of eq. (10). It is therefore interesting to approach the structural problem from another side: From the estimated stability constant (vide infra) of the PIC_N–DNA complex it is possible make a rough estimate of $\Delta\epsilon/\epsilon$ at 500 nm of this complex, being about +0.059 which result is also qualitatively obtained from fig. 3. This is not in disagreement with the extremely large LD/A observed at 553 nm, since the latter band has, by selection rules within the exciton concept, a purely parallel polarization. On the other hand, for the 500 nm band we have reason to believe that the transition has essentially monomeric polarization, that is along the x-axis, which means that $\gamma = \theta$. By eq. (4) we then get $\gamma = 54°$. From this value $r = 11 \pm 6$ Å is obtained by eq. (8). Any reasonable PIC_N–DNA model must adopt the pitch of DNA, that is 34 Å per turn and $\rho = 35°$. Of the two models in fig. 7 we are now closer to b than to c and it lies near at hand to imagine a structure based on dimeric units like II, and in the point-dipole approximation we therefore put $r_{12} = 8.5$ Å. Feeding these data into eq. (10) a Δ somewhat above the experimental is obtained (table 1). After scaling by 1.68 (the observed ratio between the shifts of the Scheibe and complex polymers) this Δ may be used to characterize the Scheibe polymer in the language of eq. (10). In this way the effect of systematic errors in Δ is thought to be minimized. From the different structural alternatives tried in table 1 it may be concluded that the larger energy shift of the Scheibe polymer may either be due to steeper pitch or to a decreased inter-dye distance (r_{12}). A pitch change has probably minor importance since with a larger ρ of the Scheibe polymer a larger γ should be expected. No such effect is manifested in the linear dichroism of the 0–0 band, on the contrary $(LD/A)_{500} = -0.026$ and +0.059 for the Scheibe and PIC_N–DNA polymers indicate $\gamma = 55°$ and $54°$, respectively.

Recently a reversal of circular dichroism with time was reported for the Scheibe polymer in tartrate solution [6] and it has furthermore been claimed [7] that the sign of accidental optical activity, when PIC is polymerized in absence of an optically active medium, can be correlated to if the sense of stirring is clockwise or anti-clockwise *. A reversed helicity was suggested as the explanation for sign reversal. However, we feel that this should first require the complete dissociation of the polymer and we suggest as a simpler mechanism a changed sign of γ instead. By symmetry reasons it is realized that the equivalent effect is acquired by exceeding 90°, and the reversal may readily occur at minimum activation energy from very small as well as from very large (close to 90°) γ.

The present discussion within the exciton model should be taken for what it is worth. It may thus be noted that Nolte [24] recently also obtained reasonable values both for shift and rotational strength for the Scheibe polymer by applying the one dimensional electron gas model of Czikkely et al. [5] to scewed PIC molecules in a brickwork arrangement. According to their model the PIC polymer should constitute a helical band with a pitch directly determined by the dihedral angle, φ, between the quinoline planes. φ could vary between 10°–50° and the result be still in qualitative agreement with experiment.

It is interesting to compare the calculated rotational strength for the long-wavelength transition of the scewed PIC with the here for the first time available optical activity of monomeric PIC. The CD presented in fig. 1a for the region 430–580 nm yields a value $R = -5.8 \times 10^{-39}$ esu^2 cm^2 with respect to monomeric PIC bound to DNA. This result is indeed compa-

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* The proposal by these authors may seem absurd since the sense of stirring cannot be dissymmetric. However, it may define a dissymmetric system after coupling with some specific property of the surrounding vessel or due to the geo-rotational Coriolis effect – in the latter case anticorrelation to that reported should be obtained on the northern hemisphere.

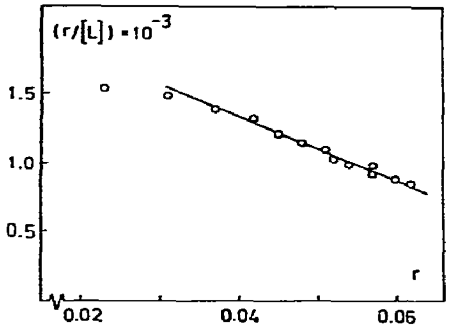

Fig. 9. Estimate of a stability constant for the (PIC)$_N$–DNA complex by means of a Scatchard plot: $K_{II} = (2 \pm 1) \times 10^4 \mathrm{M}^{-1}$, $n_{II} = 0.1 \pm 0.05$.

tible with the rotational strength $R = -3.2 \times 10^{-39}$ esu^2 cm^2 predicted by Nolte for a dihedral angle $\varphi = 25°$ [24].

Finally, a Scatchard plot (fig. 9, eq. (7)) was applied to obtain an approximate value of the stability constant for the PIC$_N$–DNA complex from the 553 nm LD band (fig. 3a). $\Delta\epsilon = 2 \times 10^4$ M^{-1} cm^{-1} was used, on the basis of the observed $\Delta\epsilon/\epsilon$ and an estimate of ϵ being about 2×10^4 M^{-1} cm^{-1} (which is probably rather too low than too high in view of a maximum value 10^5 noted for the J-band). $K_{II} = (2 \pm 1) \times 10^4$ M^{-1} and $n_{II} = 0.1 \pm 0.05$ was obtained.

Attempts to obtain the stability of the first PIC–DNA complex at 0.2 M ionic strength (with the aim of suppressing the formation of the dimer) failed due to a pronounced formation of Scheibe polymer above $C_L/C_N = 0.5$. However, with its clear spectroscopic manifestation there is no reason to doubt about a complex with a dimer, which phenomenon is not unique for PIC but has been described for instance in the case of acridine orange [25] with a similar ionic strength sensitivity.

5. Concluding remark

The present results leave the impression of two distinctly different types of complexes between PIC and DNA. The first one fulfills the criteria for an intercalated molecule, with a large apparently ionic-strength independent stability and a large angle between the PIC long-axis and the helix axis. This angle (60°) as well as that obtained from linear dichroism for the purine–pyrimidine plane (59°) is in contradiction [1] with the Watson–Crick model for solid DNA ($\theta = 90°$) and suggests that the base-pairs have considerable tilt. The second type of complexes have lower binding affinities and their ionic-strength dependency suggests predominantly electrostatic bonding. The space demanding properties of polymeric PIC as well as the spectral observations indicate that the PIC$_N$–DNA complex is not formed by employing already intercalated PIC but involves a separate peripheral right-handed helix with 8–12 PIC units per turn (fig. 7d) probably on the negatively charged surface of a strand.

For the first complex holds that every slot between two successive base-pairs constitutes a site for one intercalated PIC molecule, if the two slots immediately adjacent to one already occupied are excluded. Further, every intercalated PIC appears to be a possible binding site for an additional, nonintercalated molecule, the pair so formed being revealed by the spectroscopic properties of dimeric PIC.

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