CIRCULAR DICHROISM OF FLOW-ORIENTED DNA–PROFLAVINE COMPLEX

Andrey I. POLETAYEV
Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, 117 312, USSR

Received 5 June 1976

1. Introduction

The biological activity of complexes of the nucleic acids with small molecules has been studied extensively in recent years, particularly using the CD* method [1–10], because of its high sensitivity to the conformation and mutual orientation of interacting molecules. But still the problem of CD spectra interpretation is not solved and it is impossible to obtain most of the required structural information from CD spectra of the complexes. By coventional experimental procedures one can determine the average value of the components of the CD-tensor only, whilst evaluation of the individual components would give us much more information.

This work aims to determine the CD-components- $\Delta\epsilon_{\parallel}$ and $\Delta\epsilon_{\perp}$ - for the DNA-PF complex. We oriented the complex along the light beam by means of a multi-capillaries flow-cell by the method used by Chung and Holzwarth [11]. The CD effect of the DNA–PF complex in the visible region shows an essential anisotropy – $\Delta\epsilon_{\parallel}$ and $\Delta\epsilon_{\perp}$ have opposite signs. The component perpendicular to the DNA helix axis – $\Delta\epsilon_{\perp}$ – has rather a big amplitude, therefore the PF chromophore cannot be perpendicular to the DNA axis. First results concerning anisotropy of the DNA–dye complexes in u.v.-region will appear elsewhere [12].

2. Experimental

2.1. *Materials*

DNA isolated from *E. coli* with mol. wt ~ $3 \cdot 10^7$ was used for the experiments. Proflavine was purchased from BDH (England). All measurements were done in 0.01 M phosphate buffer, pH 6.9.

**Abbreviations:* CD, circular dichroism; PF, proflavine, 3,6-diaminoacridine.

2.2. *CD measurement*

CD spectra were recorded on a Mark III dichrograph 'Jobin Yvon' (France). CD spectra of the oriented DNA-PF complexes were measured with a flow cell, similar to that of Chung and Holzwarth [11]. The only difference being that metallic capillaries nontransparent to visible light were used. The capillaries had 0.45 mm inner diameter, 0.05 mm wall thickness, and 28 mm length. The space between the inner surfaces of the two windows was 30 mm, leaving a 1 mm gap at each end for the fluid to enter and leave the capillaries.

The specific CD effects measured with light propagating parallel to the helix axis – $\Delta\epsilon_{\parallel}$ – and perpendicular to this axis – $\Delta\epsilon_{\perp}$ – were evaluated from two expressions [11]:

$$\Delta\epsilon_f = (1 - b)\,\Delta\epsilon + b\Delta\epsilon_{\parallel} \quad (1)$$

$$\Delta\epsilon = (\Delta\epsilon_{\parallel} + 2\Delta\epsilon_{\perp})/3 \quad (2)$$

where $\Delta\epsilon_f$ and $\Delta\epsilon$ are CD amplitudes measured with flow-orientation and without it; b - the parameter indicating the degree of orientation was estimated for the DNA without dye by absorbance measurements [11].

2.3. *Absorbance measurement*

Absorbance spectra of the complexes were recorded on a 'Specord UV–VIS' spectrophotometer (Carl Zeiss, Jena). The degree of orientation was

obtained using a Cary-16 spectrophotometer from the following expression:

$$\triangle A_f/A_{260} = b\,(1 - 3\sin^2\alpha)/2 \qquad (3)$$

where α is the inclination angle between the normal of base plane and the helix axis; in practice b is small and for DNA in the B-form can be ignored. The accuracy of the b-value was about 0.02; so the uncertainties in $\triangle\epsilon_{\parallel}$ and $\triangle\epsilon_{\perp}$ amplitudes are about 10–15%. In calculations of the $\triangle\epsilon_{\parallel}$ and $\triangle\epsilon_{\perp}$ values we used the b parameter for the ligand-free DNA, so we ignore the possible changes of b due to the stiffening of DNA caused by the absorption of the dye molecules. The control experiment showed that this effect did not influence the b-value [17].

3. Results and discussion

The cell system used in our experiments was first tested for CD-measurement of free DNA in the B and C forms. The results thus obtained were quite analogous to those of Chung and Holzwarth [11].

CD spectra of the isotropic sample of the DNA–PF complex (curve 1) and flow-oriented complex (curve 2) are presented in fig.1. Curve 3 of the same figure is the difference $\triangle\epsilon_f - \triangle\epsilon$ (of curve 1 and curve 2), the right ordinate gives the molar values for curve 3. From the data shown in fig.1 it is evident that the orientation causes an increase of both CD-components in the spectrum of the DNA–PF complex.

Based on b = 0.12 and eqs. (1) and (2), the resolved specific CD spectra parallel to the helix axis, $\triangle\epsilon_{\parallel}$, and perpendicular to the helix axis, $\triangle\epsilon_{\perp}$, are presented in fig.2. The accuracy of the amplitude determination is about 15%. The possible increase of b value (0.12) which may be caused by stiffening of the DNA by the dye molecules [13,14]

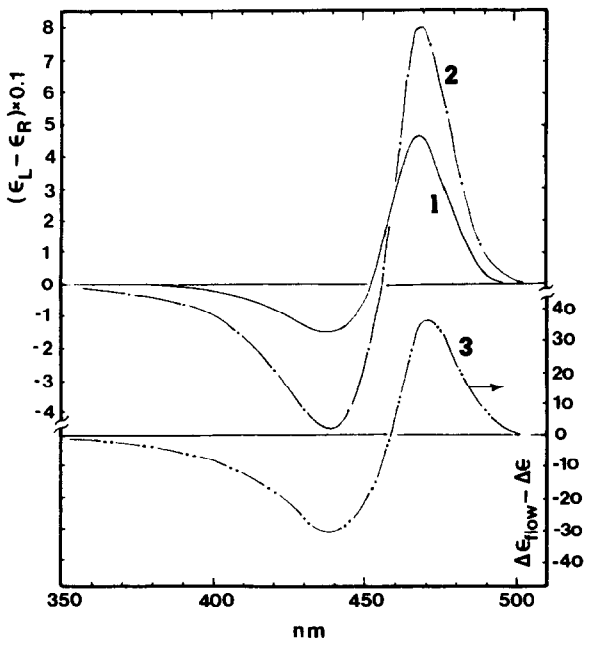

Fig.1. Circular dichroism of DNA–PF complex ([P] = $1.62\cdot10^{-5}$ M, [PF] = $2.91\cdot10^{-6}$ M) - curve 1; flow-oriented complex (b = 0.12 ± 0.015) - curve 2; difference $\triangle\epsilon_f - \triangle\epsilon$ - curve 3, the right hand ordinate gives molar values for curve 3. Light-path of the cell – 30 mm, DNA from *E. coli* in 0.01 M phosphate buffer pH 6.9, 22°C.

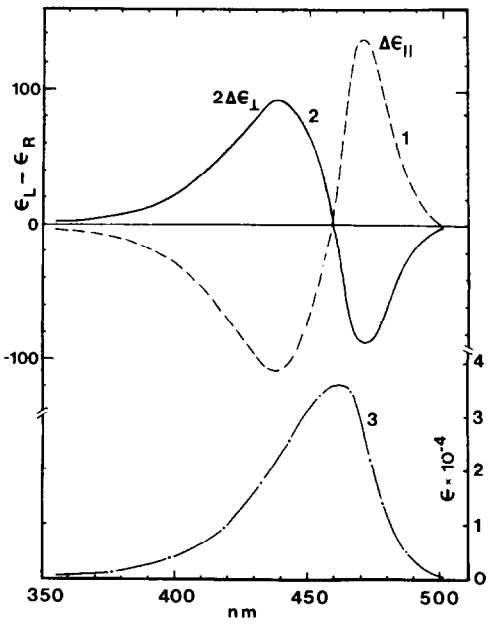

Fig.2. Specific circular dichroism spectra of the DNA–PF complex: curve 1 – CD spectrum parallel to the DNA axis; curve 2 – CD spectrum perpendicular to the DNA axis; curve 3 – absorption spectrum of PF in complex with DNA, the right hand ordinate corresponds to curve 3.

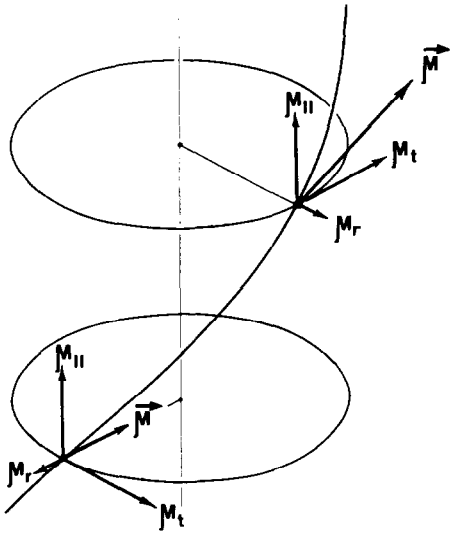

Fig.3. Transition dipole moments of two dye molecules bound to a helical polymer, $\mu_{||}$ – component of the moment parallel to the polymer helix axis, μ_r – radial and μ_t – tangential components.

does not influence significantly the shape of the specific CD components but slightly changes their amplitudes.

These components $\Delta\epsilon_{||}$ and $\Delta\epsilon_{\perp}$ are of opposite sign, the amplitude of $\Delta\epsilon_{\perp}$ is nearly one third that of $\Delta\epsilon_{||}$. Fig.2 shows the CD effect of the DNA–PF complex to be strongly anisotropic, which leads to the conclusion that the CD spectrum of unoriented samples of DNA–PF complex is a result of cancellation between two components of opposite signs. The same phenomenon was found by Chung and Holzwarth [11] with pure DNA molecules.

The CD effect in the longwave band of the DNA–PF complex is due to chromophore–chromophore interaction between the adjacent dye molecules on the DNA [2–4]. This chromophore–chromophore interaction splits the band of PF producing the CD-effect of conservative mode [2,4]. It is known, that the electric transition moment for the longwave adsorption band of PF lies parallel to the long axis of the PF chromophore [15]. The scheme with two PF molecules in helical arrangement is shown in fig.3. The electric transition moments in this case can be resolved in three components: $\mu_{||}$ – vertical, along the axis; μ_r – radial; μ_t – tangential. The sum of μ_r and μ_t we may denote as $\mu_{\perp}$.

The CD effect measured with light propagating parallel to the helix axis – $\Delta\epsilon_{||}$ – is due to the square of the $\mu_{\perp}$ component; while $\Delta\epsilon_{\perp}$ arise from two components – $\mu_{||}$ and $\mu_{\perp}$ – and is proportional to its product: $\mu_{||}\cdot\mu_{\perp}$ [16,17]. It is known that the DNA molecule possesses C_2 symmetry and the PF molecule – C_{2v}; it would be quite natural to suppose that in the complex both axes must coincide and the complex will possess C_2 symmetry [18]. For this reason μ_r must be equal to zero and $\Delta\epsilon_{\perp}$ is caused only by the product of $\mu_{||}$ and $\mu_{\perp}$.

If the dye chromophores are perpendicular to the DNA helix and are situated between the adjacent base pairs – intercalated in DNA [19] – $\mu_{||}$ must be equal to zero and $\Delta\epsilon_{\perp} = 0$. If the dye chromophore is not exactly perpendicular to the helix axis, and the deviation from perpendicular is not big (3–4°), $\Delta\epsilon_{\perp} \neq 0$, but its amplitude will be much smaller than the amplitude of $\Delta\epsilon_{||}$. In fig.2 we can see an opposite situation – $|\Delta\epsilon_{\perp}| \sim |\Delta\epsilon_{||}|/3$. For this reason we must conclude that the dye chromophore is not perpendicular to the DNA axis. A detailed theoretical consideration of this situation will appear elsewhere [17], but preliminary results give a value of 25–30° for the inclination angle between the normal of the dye plane and the helix axis. This value is slightly bigger than in the model of Gursky [18].

The experimental results presented above are in contradiction to those obtained by Mason and McCaffery in 1964 with the DNA–Acridine Orange complex [20]. The most probable reason of this discrepancy, the two dyes being similar is the low sensitivity of the CD-instrument used by Mason and McCaffery, and the ignoring of the real value of the degree of orientation. Our preliminary results with the DNA–Acridine Orange complex are in accord with those obtained with DNA–PF: both complexes exhibit similar effects of orientation.

The results presented here can principally give us information about the orientation of the dye molecules in the complex [17], and in any case show that the configuration of the DNA–PF complex deviates from that which had been proposed in the initial model of intercalation [19] and probably from those which were proposed later by Fuller and Waring [21] and Prichard, Blake and Peacock [22].

Acknowledgements

I am deeply grateful to Dr. V. L. Makarov for fruitful discussion and to Dr A. S. Zasedatelev for critical remarks on this paper.

References

[1] Yamaoka, K. and Resnik, R. (1966) J. Phys. Chem. 70, 4051–4066.
[2] Gardner, B. J. and Mason, S. F. (1967) Biopolymers 5, 7994.
[3] Dalgleish, D. G., Fugita, H. and Peacock, A. R. (1969) Biopolymers 11, 2415–2422.
[4] Li, H. J. and Crothers, D. M. (1969) Biopolymers 8, 217–235.
[5] Jackson, K. and Mason, S. F. (1971) Trans. Farad. Soc. 67, 966.
[6] Poletayev, A. I. (1972) Abstract of IV Intern. Biophys. Congress, E VII, b 4/4.
[7] Aktipis, S. and Kindelis, A. (1973) 12, 1213–1220.
[8] Zasedatelev, A. S., Gursky, G. V., Zimmer, Ch. and Thrum, H. (1974) Mol. Biol. Reports 1, 337–342.
[9] Houssier, C., Hardy, B. and Fredericq, E. (1974) Biopolymers 13, 1141–1160.
[10] Parodi, S., Kendall, F., Nicolini, C. (1975) Nucleic Acid Res. 2, 477–486.
[11] Chung, S. Y. and Holzwarth, G. (1975) J. Mol. Biol. 92, 449–466.
[12] Poletayev, A. I., Zavriev, S. K. and Minchenkova, L. E (1976) Mol. Biol. Reports, in press.
[13] Cohen, G. and Eisenberg, H. (1969) Biopolymers 8, 45.
[14] Moroshina, E. B., Shisho, A. K., Krivtsova, M. A., Zshadin, N. N. and Frisman, E. V. (1975) Molekul. Biol. 9, 836.
[15] Ballard, R. E., McCaffery, A. J. and Mason, S. F. (1966) Biopolymers 4, 97.
[16] Tinoco, I., Woody, R. W. and Bradley, R. F. (1963) J. Chem. Phys. 36, 1317; Biopolymers 1, 238.
[17] Makarov, V. L., Poletayev, A. I., Sveshnikov, P. G. and Kondratyeva, N. O. (1976) Molekul. Biol., in press.
[18] Gursky, G. V. (1966) Biofizika 11, 737.
[19] Lerman, L. S. (1961) J. Mol. Biol. 3, 18.
[20] Mason, S. F. and McCaffery, A. J. (1964) Nature 204, 468.
[21] Fuller, W. and Waring, M. J. (1964) Ber. Bunsenges Physik. Chem. 68, 805–808.
[22] Pritchard, N. J., Blake, A., Peacock, A. R. (1966) Nature 212, 1360.