CIRCULAR DICHROISM OF DNA–PROFLAVINE, DNA–ETHIDIUM AND DNA–DISTAMYCINE FLOW–ORIENTED COMPLEXES

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Abstract. The circular dichroism (CD) spectra of flow-oriented complexes of DNA with proflavine (PF), ethidium (ET) and distamycine (DS) have been studied in the ultraviolet region. The CD spectra with light propagating in parallel to the flow direction were measured by the method of Chung and Holzwarth [1]. \( \Delta \in \parallel \) and \( \Delta \in \perp \) values have been obtained by this method. It was shown that all the complexes studied exhibit a strong CD anisotropy so that ‘isotropic’ CD spectra measured with a conventional procedure can be attributed to the mutual compensation of the two components of opposite signs.

I. INTRODUCTION

Extensive studies of the complexes of nucleic acids with small molecules have been carried out recently in connection with their biological activity. A lot of studies have dealt with the CD method [2-9] owing to its high sensitivity to conformation and to mutual orientation of the interacting molecules. The problem of the interpretation of CD spectra has not been solved yet and obtaining of detailed structural information on the basis of the CD spectra of the complexes proves to be impossible. Using a standard experimental procedure, the average value of the CD tensor can be obtained, whereas the evaluation of individual components gives us more information.

The aim of this study is determination of the CD components of \( \Delta \in \parallel \) and \( \Delta \in \perp \) values for DNA-PF, DNA-ET and DNA-DS complexes in the UV-region to have a better understanding of the complex structures.

II. MATERIALS AND METHODS

The CD spectra of flow-oriented DNA were measured with a flow-cell similar to that described by Chung and Holzwarth [1]. The cell with 35 mm light path constructed by us consists of cen-
ter-piece glass capillaries which have a 0.5 mm inner diameter, 0.1 mm wall thickness and 33 mm length.

To evaluate the degree of orientation $b$, the ratio of flow-induced absorbance change ($\Delta A_f$) of ligand-free DNA to the absorbances without a flow ($A$) was used [1]:

$$\frac{\Delta A_f}{A} = b(1-3 \sin^2 \alpha)/2,$$

where $\alpha$ is the angle between the normal to the base plane and the helix axis which nearly approaches zero for the B-form of DNA. For ligand-free DNA, $b$ was equal to 0.12 $\pm$ 0.02. In all calculations a possible increase in $b$, due to DNA stiffness produced by the dye molecules [10, 11], was not taken into account.

The specific CD effects measured along the helix axis ($\Delta \varepsilon_\| )$ and perpendicular to it ($\Delta \varepsilon_\perp$) were obtained from the expressions [1]:

$$\Delta \varepsilon_\| = \frac{\Delta \varepsilon_f - (1-b)\Delta \varepsilon}{b},$$

$$2\Delta \varepsilon_\perp = 3\Delta \varepsilon - \Delta \varepsilon_\|,$$

where $\Delta \varepsilon_f$ and $\Delta \varepsilon$ are experimentally measured CD values (molar) obtained at various fixed wavelengths with a flow and without it.

DNA from *E. coli* with a molecular weight of 5-6 $\times$ 10$^6$ daltons was used. Proflavine and ethidium were purchased from BDH (England) and distamycine A from Calbiochem (U.S.A.). The CD spectra were measured with dichrograph ‘Jobin-Yvon’ Mark III (France).

### III. RESULTS AND DISCUSSION

Figure 1A presents the CD spectra of DNA (curve 1), the DNA-PF complex (curve 2), the DNA-PF flow-oriented complex (curve 3) and their difference $- \Delta \varepsilon_f - \Delta \varepsilon$ (curve 4), molar values for the differential spectrum being scaled on the right ordinate. On orientation, the shape of the CD effect changes dramatically; the above change is negative and non-conservative (curve 4).

A similar effect may also be observed with the DNA-ET complex (Figure 1B). In the longwave region of the spectrum (300-350 nm) a small increase in positive CD bands occurs (Figure 1B).

CD changes induced by the flow-orientation of the DNA-DS complex (figure 1C) in the region below 300 nm are very similar to those observed for the DNA-PF and DNA-ET complexes. In the longwave region (from 300 to 350 nm) the sign inversion of the CD effect occurs.

$\Delta \varepsilon_\|$ and 2 $\Delta \varepsilon_\perp$ values for the DNA-PF complex obtained from spectrum 2 and spectrum 3 of Figure 1A are represented on Figure 2A. Both components are nearly symmetrical and exceed the dichroism of isotropic spectra by an order. So it is clear, that a small isotropic spectrum appears as a result of the mutual cancellation of large components, the negative parallel $\Delta \varepsilon_\|$ and the positive perpendicular $\Delta \varepsilon_\perp$ ones. This result is just indicating that quite a number of the interpretations based on CD measurements of the unordered complexes may be of limited value only.