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Davydov Splitting in Spectra of Cyanine Dye J-Aggregates, Formed on the Polynucleotides

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It was found in our previous work (T. Y. Ogul'chansky *et al.* (2001) *Spectrochimica Acta Part A* **57**, 2705–2715) that the carbocyanine dye Cyan βiPr forms J-aggregates in the groove of poly(dA)-poly(dT). In the present paper we study in detail the spectral properties and energy levels structure of the Cyan βiPr J-aggregates by means of absorption and fluorescence spectroscopy and polarization measurements. The energy structure of an aggregate consists of at least two exciton zones, and dipole moments of the absorption transitions to these zones are oriented at an angle of about 90° one to another. It was supposed that the transition moment of the lower zone is parallel to the polynucleotide axis and the moment of the upper zone is perpendicular. The fluorescent transitions are possible only from the lower exciton zone, while the excitations of higher zone undergo nonradiative transitions to the lower one.

KEY WORDS: Aggregate; cyanine dye; polynucleotide; fluorescence anisotropy.

INTRODUCTION

Carbocyanine dyes show promise as fluorescent probes for nucleic acid detection. The study of spectral properties of carbocyanine dye Cyan βiPr (Fig. 1) in the presence of polynucleotide poly(dA)-poly(dT) revealed the formation of the dye J-aggregates on the polynucleotide [1]. The free dye in water solution and the dye in the presence of $[poly(dG\text{-}dC)]_2$, exist mainly in monomeric form, whereas in the presence of chicken erythrocyte DNA, both J-aggregate and monomeric forms of dye are present. So a conclusion was made that the AT-sequence serves as template for the formation of Cyan βiPr J-aggregates [1]. In the present work we studied in more detail the spectral properties and the energy levels structure of the Cyan βiPr J-aggregates.

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EXPERIMENTAL

The carbocyanine dye Cyan βiPr was synthesized in our laboratory as described in [2]. The polynucleotide poly(dA)-poly(dT) was purchased from Sigma. The length of the polynucleotide molecules was not less than 400 nucleotide base pairs. The 0.05 M TRIS-HCl buffer (pH 7.9) was used as a solvent. All measurements were performed at room temperature (25°C). The absorption titration of the polynucleotide by the dye was performed by addition to the buffer solution of $3{,}5 \cdot 10^{-5}$ M base pairs poly(dA)-poly(dT) of 10 μl of one of the dye stock solutions (the dye stock solutions of the concentrations 10^{-3}, $5 \cdot 10^{-4}$, $2{,}5 \cdot 10^{-4}$ and $1{,}25 \cdot 10^{-4}$ M were obtained by the consequent dissolving of the initial stock solution with the concentration of $2 \cdot 10^{-3}$ M).

The absorption spectra were obtained with the help of the spectrophotometer Specord M 40 (Carl Zeiss,

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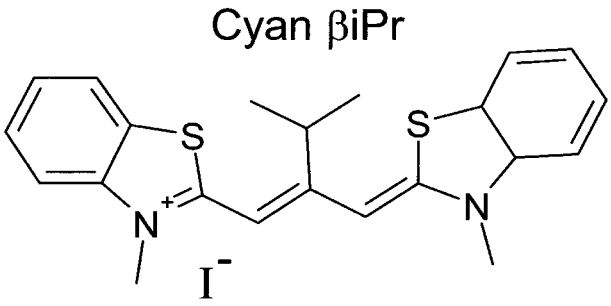

Fig. 1. Chemical structure of carbocyanine dye Cyan βiPr.

Germany). The fluorescence spectra were recorded on the Cary Eclipse fluorescence spectrophotometer (Varian, Australia). The fluorescence excitation anisotropy spectrum was obtained on the Hitachi 850 fluorescence spectrophotometer (Japan).

RESULTS AND DISCUSSION

To investigate the structure of the Cyan βiPr J-aggregates formed on the poly(dA)-poly(dT), we studied the absorption spectra of different dye concentrations in the presence of $3.5 \cdot 10^{-5}$ M base pairs of poly(dA)-poly(dT) (Fig. 2, solid lines). At low dye concentrations the spectrum consists of the band with the maximum at 550 nm (M-band) corresponding to the dye monomer bound to poly(dA)-poly(dT) [1]. With increasing dye concentration, the M-band decreases and the aggregate spectrum with the maxima at 574 (J1-band) and 603–606 nm (J2-band) grows up. Though the clearly distinguished isobestic point is absent, we see that near 563 nm all the spectra are very close (Fig. 2). This can indicate that the aggregates of different size are formed on the polynucleotide, and the aggregates consisting of a different number of dye molecules have different but rather close absorption spectra. The latter is also supported by the fact that with increasing dye concentration from $1{,}5 \cdot 10^{-5}$ M up to $4 \cdot 10^{-5}$ M the J2-band wavelength shifts from 603 nm to 606 nm. Moreover, the J1-band can be only distinguished beginning from the dye concentration $1{,}25 \cdot 10^{-5}$ M (J2 can be distinguished from $5 \cdot 10^{-6}$ M). All the above facts permit us to suppose that the increase in dye concentration leads to the increase in the dye molecules number in an aggregate. The latter leads to the long-wave shift and narrowing of the J2 band, to the appearing of the 574-nm J1-band, and to the better separation of two aggregate bands (Fig. 2).

To resolve the aggregate absorption spectrum from the monomer component, we subtracted the spectrum

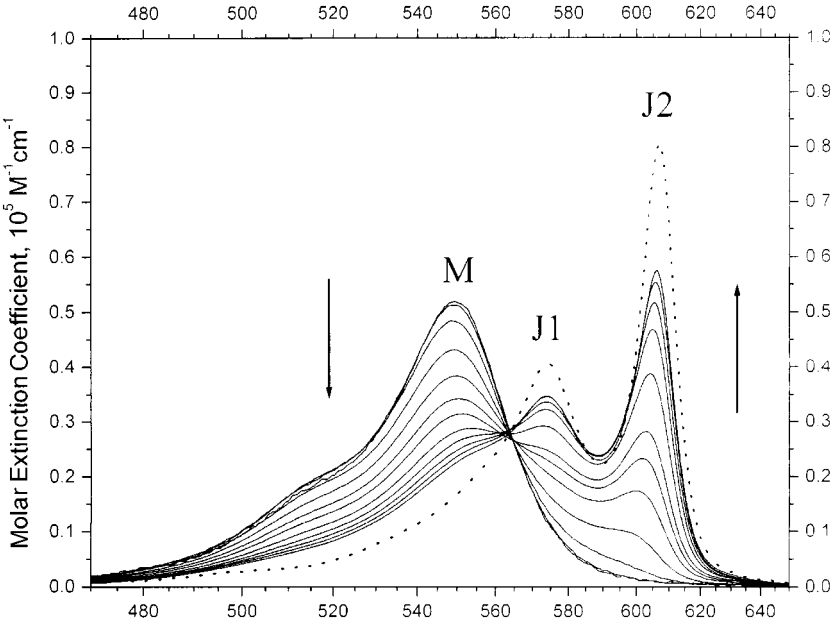

Fig. 2. Solid lines: absorption spectra of the $1{,}25 \cdot 10^{-6} - 4 \cdot 10^{-5}$ M of Cyan βiPr in the presence of $3{,}5 \cdot 10^{-5}$ M base pairs of poly(dA)-poly(dT); arrows show the increase in dye concentration. Dashed line: the difference between the spectra corresponding to the $3 \cdot 10^{-5}$ M and $3{,}5 \cdot 10^{-5}$ M of dye. All the spectra presented in molar extinction units.

corresponding to the dye concentration $3 \cdot 10^{-5}$ M from the one corresponding to $3{,}5 \cdot 10^{-5}$ M (see Fig. 2, dashed line). As shown in Fig. 2, the aggregate spectrum consists of two well-resolved bands situated at 574 nm and 607 nm. It is natural to suppose that the obtained spectrum corresponds to the J-aggregate fraction with the high number of molecules, compared to the whole assembly of aggregates formed in this concentration range. Thus we can conclude that the position of the J1-band (at 574 nm) does not depend on the aggregate size, whereas the J2-band shifts to the long-wave region when increasing the number of dye molecules in an aggregate.

Whereas the absorption spectrum of Cyan βiPr J-aggregates consists of at least two well-resolved bands, its fluorescence spectrum contains only one band (Fig. 3), and the shape of this band is independent of the excitation wavelength. This means that the both absorption transitions excite the same fluorescent transition. Figure 3 shows that the J2 absorption band of an aggregate is symmetrical to the aggregate fluorescence spectrum. So fluorescence is emitted from the energy level excited by the J2 transition.

Earlier it was supposed that the presence of two bands in Cyan βiPr J-aggregate absorption spectrum could be connected with Davydov splitting of aggregate exciton zone [1]. To confirm this suggestion, we performed the fluorescence polarization study of the J-aggregates. The fluorescence excitation anisotropy spectrum of the Cyan βiPr J-aggregates on poly(dA)-poly(dT), is presented in Fig. 4. The anisotropy has the value $r = 0{,}16$ when excited at the J2-band and the value $r = -0{,}04$ when excited at the J1-band. The obtained results could mean that the dipole moments of the two absorption transitions of the aggregate are oriented at a considerable angle one to another. To find this angle (α) we use the formula $\frac{r_{574}}{r_{607}} = \frac{3\cos^2\alpha - 1}{2}$ [3]. The calculation gives the value $\alpha = 66°$. But it is highly possible that the anisotropy value at 574 nm is the sum of anisotropy values of different bands; hence the real angle α must be more than 66°. We can suppose that the moments of the two transitions are oriented at an angle of about 90° one to another, as is often the case with Davydov splitting. Kasha [4] examined the helical aggregate and concluded that one allowed absorption transition in such an aggregate is oriented parallel to the helix axes and the other(s) (oriented) perpendicularly. Our results agree with the energy level structure of a helical aggregate predicted in [4].

We can conclude that the space structure of the Cyan βiPr J-aggregates formed in poly(dA)-poly(dT) groove causes the Davydov splitting of the aggregate energy structure into several (two at least) exciton zones. The absorption transitions to these zones are possible, but fluorescence occurs only from the lowest one; from the higher exciton zones the nonradiative transitions to

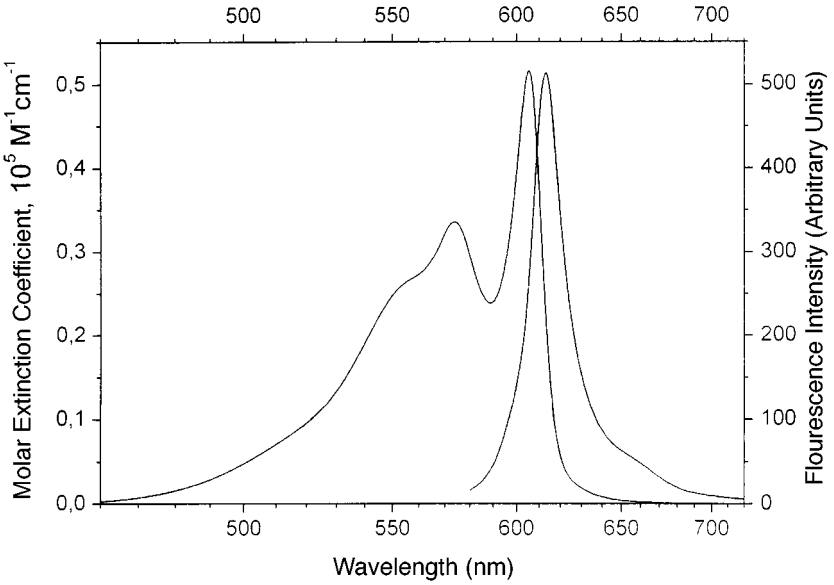

Fig. 3. Absorption and fluorescence spectra of $3 \cdot 10^{-5}$ M of Cyan βiPr in the presence of $3{,}5 \cdot 10^{-5}$ M base pairs of poly(dA)-poly(dT). Fluorescence spectrum presented in arbitrary units.

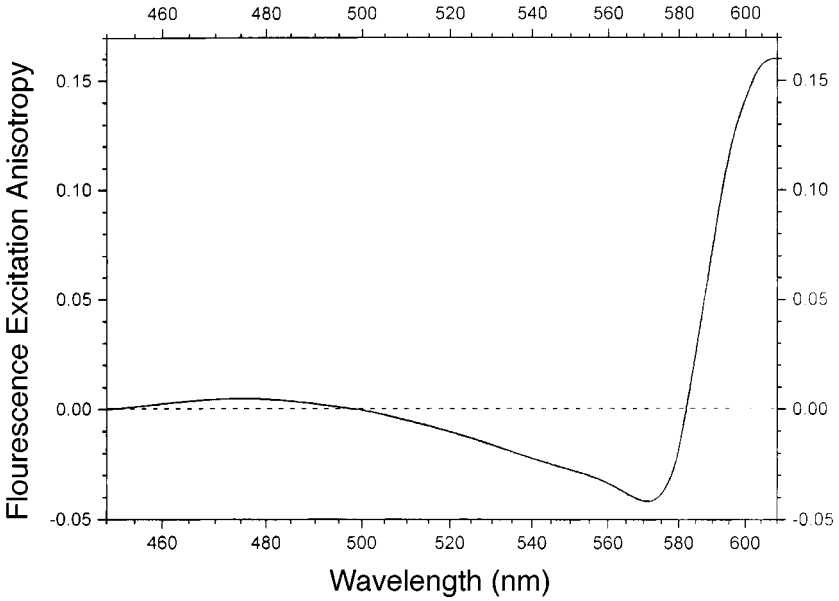

Fig. 4. Fluorescence excitation anisotropy spectrum of $3 \cdot 10^{-5}$ M of Cyan βiPr in the presence of $3.5 \cdot 10^{-5}$ M base pairs of poly(dA)-poly(dT).

the lowest zone take place, from where the fluorescent transition is possible.

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