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Quantitative Calculations of Fluorescence Polarization and Absorption Anisotropy Kinetics of Double- and Triple-Chromophore Complexes with Energy Transfer

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ABSTRACT A new method is presented for calculation of the fluorescence depolarization and kinetics of absorption anisotropy for molecular complexes with a limited number of chromophores. The method considers absorption and emission of light by both chromophores, and also energy transfer between them, with regard to their mutual orientations. The chromophores in each individual complex are rigidly positioned. The complexes are randomly distributed and oriented in space, and there is no energy transfer between them. The new "practical" formula for absorption anisotropy and fluorescence depolarization kinetics, $P(t) = [3B(t) - 1 + 2A(t)]/[3 + B(t) + 4A(t)]$, is derived both for double- and triple-chromophore complexes with δ-pulse excitation. The parameter $B(t)$ is given by (a) $B(t) = \cos^2(\theta)$ for double-chromophore complexes, and (b) $B(t) = q_{12}(t)\cos^2(\theta_{12}) + q_{13}(t)\cos^2(\theta_{13}) + q_{23}(t)\cos^2(\theta_{23})$ for triple-chromophore complexes, where $q_{12}(t) + q_{13}(t) + q_{23}(t) = 1$. Here θ_{ij} are the angles between the chromophore transition dipole moments in the individual molecular complex. The parameters $q_{ij}(t)$ and $A(t)$ are dependent on chromophore spectroscopic features and on the rates of energy transfer.

INTRODUCTION

Polarization spectroscopy is a powerful method for analyzing the spatial structure and energy transfer in molecular complexes (see, for example, Craver and Knox, 1971; Dale and Eisinger, 1974; Pullerits and Freiberg, 1992). In particular, polarization data contain information about the mutual orientations of interacting molecules. Unfortunately, there is a lack of an appropriate theory to describe polarization phenomena in molecular complexes with incoherent energy transfer, which could provide formulae for practical applications. The "oldest" formula for calculations of the degree of fluorescence polarization in double-chromophore complexes randomly distributed in space: $P = (3\cos^2(\theta) - 1)/(3 + \cos^2(\theta))$, was derived in the 20's by Levshin (1925) and Perrin (1925). The parameter P is defined as $P = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$, where $I_\parallel$ and $I_\perp$ are the fluorescence components parallel to and orthogonal to the polarization of the exciting light. This formula covers the case when only one chromophore can absorb light, only one other can fluoresce, and the angle between their transition dipole moments is equal to θ.

The theory developed in the current work deals with incoherent energy transfer on the femtosecond-nanosecond timescale. Currently many attempts are being made to investigate the femtosecond region of energy transfer (Galli et al., 1993; Gillbro et al., 1993; Du et al., 1993). The general theory of coherent energy transfer was developed in the works by Rahman et al. (1979), Wynne and Hochstrasser (1993), and Knox and Gülen (1993). They obtained the result that at time $t = 0$ the polarization anisotropy $r(0)$ should equal 0.7 when the molecular complexes are excited by δ-pulse laser light, and this anisotropy must decay with a rate of about $(100\text{ fs})^{-1}$. The theory of incoherent energy transfer yields the value $r(0) = 0.4$ at $t = 0$. Thus, here is the principal test for distinguishing between coherent and incoherent energy transfer mechanisms, and a challenge to experimentalists to find out the answer to the question: which kind of energy transfer does take place in a given system under investigation? Recently discovered results include $r(0) = 0.4$ for C-phycocyanin trimers (Gillbro et al., 1993), and $r(0) = 0.7$ for the magnesium derivatives of tetraphenylporphyrin (Galli et al., 1993).

There are no doubts that this edge of ultrafast energy transfer research is very interesting and important, and picosecond-nanosecond and steady-state investigations are clearly important too. Recently, Demidov (1994a) investigated a case of incoherent energy transfer between chromophore pairs more complex than the system considered by Perrin, deriving a formula for determination of the fluorescence degree of polarization for double-chromophore complexes, when both chromophores can absorb light, fluoresce, and exchange energy excitation. It was also shown that Perrin's formula is only a particular case of the formula derived. Then a case of triple-chromophore complexes under steady-state excitation was studied (Demidov, 1994b). We will briefly discuss these results below.

Formulae for the degree of fluorescence polarization P of double- and triple-chromophore complexes under steady-state excitation were derived in the works by Demidov (1994a, b) and the former work (Demidov, 1994a) presented a formula for the fluorescence depolarization kinetics $P(t)$ of double-chromophore complexes with δ-pulse excitation. The present work focuses on derivation of new analytical formulae for fluorescence depolarization and absorption anisotropy kinetics of double-chromophore and triple-chromophore complexes under δ-pulse excitation.

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METHOD

In our calculations we consider molecular complexes consisting either of two or three chromophores. In each individual complex, the chromophores are rigidly oriented in space relative to each other. The angle between i and j chromophores ($i, j = 1, 2$ or $i, j = 1, 2, 3$) is equal to θ_{ij}. Each chromophore i can absorb light with an averaged absorption cross-section $\sigma_i(\lambda_{exc})$, where λ_{exc} is the wavelength of excitation, and fluoresce with a spectrum $f_i(\lambda_{fl})$, where λ_{fl} is the wavelength of fluorescence. Fluorescence spectra are normalized: $f(\lambda)d\lambda = 1$. The rate of energy transfer from the i to j chromophore is equal to K_{ij}. There is a large number of such complexes, randomly distributed and oriented in space. There is no energy transfer between these complexes. Finally, the orientations of the absorption and fluorescence transition dipole moments of individual chromophore are considered to be parallel.

Under the described conditions, the degree of fluorescence polarization in double-chromophore complexes ($i, j = 1, 2$) under steady-state excitation (Demidov, 1994a) is equal to

$$P = \frac{3\cos^2(\theta_{12}) - 1 + 2A}{3 + \cos^2(\theta_{12}) + 4A},$$
$$A = \frac{\tau_2^{-1} + K_{21} + (\tau_1^{-1} + K_{12})\,\alpha\gamma}{\alpha K_{21} + \gamma K_{12}}, \qquad (1)$$

Here τ_1 and τ_2 are the intrinsic lifetimes of chromophore excitation deactivations, i.e., deactivation by any processes other than energy transfer; $\alpha = \sigma_2(\lambda_{exc})/\sigma_1(\lambda_{exc})$; $\gamma = (\tau_1\eta_2 f_2(\lambda_{fl}))/(\tau_2\eta_1 f_1(\lambda_{fl}))$; η_1, η_2 are the fluorescence quantum yields of the first and second chromophores. Formula (1) converts into Perrin's formula when A is equal to 0. This corresponds to the case when only the first chromophore absorbs light ($\alpha = 0$) and only the second one fluoresces ($\gamma = \infty$).

For triple-chromophore complexes (Demidov, 1994b), the analogous formula for fluorescence polarization degree is more complicated:

$$P = \frac{3(q_{12}\cos^2\theta_{12} + q_{13}\cos^2\theta_{13} + q_{23}\cos^2\theta_{23}) - 1 + 2A^\circ}{3 + (q_{12}\cos^2\theta_{12} + q_{13}\cos^2\theta_{13} + q_{23}\cos^2\theta_{23}) + 4A^\circ}$$
$$q_{12} + q_{13} + q_{23} = 1, \qquad (2)$$

although the parameters q_{ij} and A° are again dependent on the same spectroscopic features of the chromophores as in formula (1): the intrinsic lifetimes τ_k ($k = 1, 2, 3$), the relative absorption $\alpha_{k1} = \sigma_k(\lambda_{exc})/\sigma_1(\lambda_{exc})$ and fluorescence $\gamma_{k1} = (\tau_1\eta_k f_k(\lambda_{fl}))/(\tau_k\eta_1 f_1(\lambda_{fl}))$ parameters ($k = 1, 2, 3$), and the rates of energy transfer K_{km}. For details, see Appendix 1.

It can be shown (Demidov, 1994b) that formula (1) is a particular case of formula (2). Formulae (1, 2) were derived for determination of the degree of fluorescence polarization (Demidov, 1994a, b). They could easily be modified for determination of the absorption anisotropy. The procedure is simple: if one excites molecular complexes by polarized light at the wavelength λ_{exc} and detects absorption anisotropy at the "probe" wavelength λ_{pr}, then the value of absorption anisotropy $r = (A_\parallel - A_\perp)/(A_\parallel + 2A_\perp)$ can be calculated from formulae (1) or (2) by a consequent substitution of γ_{kl} by $\alpha_{kl}^{pr} = \sigma_k(\lambda_{pr})/\sigma_1(\lambda_{pr})$, bearing in mind the relation $r = 2P/(3 - P)$. Here, by the parameter P we mean $P = (A_\parallel - A_\perp)/(A_\parallel + A_\perp)$, where $A_\parallel$ and $A_\perp$ are absorption recoveries.

The presented formulae offer a unique opportunity for conducting a quantitative deconvolution of overlapped spectra, allowing extraction of the partial absorption and fluorescence spectra if one has data on the mutual orientations of the chromophores and the polarization spectra, as well as the overlapped absorption and fluorescence spectra.

Calculations of depolarization kinetics

In the work by Demidov (1994a), it was shown that with δ-pulse excitation the formula for the fluorescence depolarization kinetics of double-chromophore complexes is equal to

$$P(t) = \frac{3\cos^2\theta - 1 + 2A(t)}{3 + \cos^2\theta + 4A(t)},$$
$$A(t) = \frac{\xi(1 - \alpha\gamma) + (t_2^{-1} - t_1^{-1})(1 + \alpha\gamma)F(t)}{2\,(\alpha K_{21} + \gamma K_{12})}, \qquad (3)$$
$$\xi = \tau_2^{-1} - \tau_1^{-1} + K_{21} - K_{12},$$
$$F(t) = \frac{e^{-t/t_1} + e^{-t/t_2}}{e^{-t/t_1} - e^{-t/t_2}},$$

where the time parameters t_1 and t_2, respectively, reflect a redistribution of excitation between the chromophores and overall energy decay

$$t_1^{-1} = 0.5(\tau_1^{-1} + \tau_2^{-1} + K_{12} + K_{21} + \sqrt{\xi^2 + 4K_{12}K_{21}}),$$
$$t_2^{-1} = 0.5(\tau_1^{-1} + \tau_2^{-1} + K_{12} + K_{21} - \sqrt{\xi^2 + 4K_{12}K_{21}}). \qquad (4)$$

These time parameters are presented in the fluorescence decay kinetics. The fluorescence kinetics display biexponential behavior: $\phi(t) = C_1\exp(-t/t_1) + C_2\exp(-t/t_2)$, where C_1 and C_2 are constants dependent on the excitation conditions, whereas the fluorescence depolarization kinetics $P(t)$ has the behavior as described in (3).

The extreme values $P(0) = P(t = 0)$ and $P(\infty) = P(t \gg t_1t_2/(t_1 - t_2))$ are equal to

$$P(0) = 0.5,$$
$$P(\infty) = \frac{3\cos^2(\theta_{12}) - 1 + 2A(\infty)}{3 + \cos^2(\theta_{12}) + 4A(\infty)},$$
$$A(\infty) = \frac{K_{21} + K_{12}\alpha\gamma}{\alpha K_{21} + \gamma K_{12}}.$$

The value $P(\infty)$ is practically equal to P at steady-state excitation (see (1)) if $\tau_{ij}^{-1} \ll K_{km}$ ($i, j, k, m, = 1, 2$).

The formula for the fluorescence depolarization kinetics of triple-chromophore complexes is more complicated:

$$P(t) = \frac{\mathcal{A} - 1 + 2A^\circ(t)}{\mathcal{B} + 4A^\circ(t)}, \quad q_{12}(t) + q_{13}(t) + q_{23}(t) = 1. \quad (5)$$

where

$$\mathcal{A} = 3(q_{12}(t)\cos^2\theta_{12} + q_{13}(t)\cos^2\theta_{13} + q_{23}(t)\cos^2\theta_{23})$$

$$\mathcal{B} = 3 + (q_{12}(t)\cos^2\theta_{12} + q_{13}(t)\cos^2\theta_{13} + q_{23}(t)\cos^2\theta_{23})$$

Here the parameters $q_{ij}(t)$ and $A^\circ(t)$ are dependent on the chromophore spectroscopic parameters and energy transfer. For particular details, see Appendix 2. Now, the number of specific time parameters for energy redistribution and decay involved in the $P(t)$ calculations is equal to three. All of them are presented in the fluorescence decay kinetics as $\phi(t) = C_1\exp(-t/t_1) + C_2\exp(-t/t_2) + C_3\exp(-t/t_3)$.

Thus, the formulae (3, 5) enable one to calculate the fluorescence depolarization kinetics at various excitation (λ_{exc}) and registration (λ_{fl}) wavelengths. The absorption anisotropy kinetics can be derived in the same manner as in the case of steady-state excitation, i.e., by substitution of $\gamma(\lambda_{fl})$ by $\alpha(\lambda_{pr})$, including the case when $\lambda_{pr} = \lambda_{exc}$.

APPLICATION TO C-PHYCOCYANIN AGGREGATES

We have tried to apply the derived formulae to a well known natural object: C-phycocyanin (C-PC) aggregates of the blue-green algae *Agmenellum quadruplicatum*. This natural pigment forms aggregates such as β-subunits and monomers. The first aggregates are typical double-chromophore complexes consisting of β-84 and β-155 chromophores, and the latter are triple-chromophore complexes consisting of α-84, β-84, and β-155 chromophores (Gantt, 1981; Scheer, 1981; Glazer, 1984; Holzwarth, 1986). The spectroscopic features and energy migration rates of these complexes were determined in works by Mimuro et al. (1986), Sauer et al. (1987, 1988), and Demidov and Borisov (1993); the mutual positions and orientations of the chromophores were precisely calculated by Schirmer et al. (1985, 1987) using x-ray spectroscopy. The following data are used in the calculations below: $\tau_{\alpha 84} = \tau_{\beta 84} = \tau_{\beta 155} = 1.5$ ns, $\eta_{\alpha 84} = \eta_{\beta 155} = 0.72$, $\eta_{\beta 84} = 0.48$ (Sauer et al., 1987, 1988); $K_{\alpha 84,\beta 84} = 12.4\ \text{ns}^{-1}$, $K_{\alpha 84,\beta 155} = 0.445\ \text{ns}^{-1}$, $K_{\beta 84,\alpha 84} = 10.5\ \text{ns}^{-1}$, $K_{\beta 84,\beta 155} = 7.54\ \text{ns}^{-1}$, $K_{\beta 155,\alpha 84} = 2.04\ \text{ns}^{-1}$, $K_{\beta 155,\beta 84} = 34.1\ \text{ns}^{-1}$ (Demidov and Borisov, 1993); $\theta_{\alpha 84,\beta 84} = 15.5^\circ$, $\theta_{\alpha 84,\beta 155} = 61.9^\circ$, $\theta_{\beta 84,\beta 155} = 46.4^\circ$ (our calculations on the basis of data by Schirmer et al. (1985, 1987)); $\sigma_i(\lambda)$ and $f_i(\lambda)$ are borrowed from the work by Sauer et al. (1987).

The C-phycocyanin aggregates of the blue-green algae *Agmenellum quadruplicatum* and *Mastigocladus laminosus* having a similar structure have been under intense investigation over the last 10 years, but we found that only three groups tried to measure the polarization kinetics of C-PC β-subunits and monomers: (a) Hefferle et al. (1984a, b)—subunits, monomers, and trimers; (b) Gillbro et al. (1993), Sandström et al. (1988), and (c) Xia et al. (1993)—monomers and trimers. The results of the first group contradict the results of the second and third groups, whereas the latter two coincide. In particular, in the case of C-PC monomers Hefferle et al. (1984a, b) measured the fast depolarization component of about $\tau_{fast} = 150$ ps, whereas Sandström et al. (1988) measured $\tau_{fast} = 57 \pm 4$ ps and Xia et al. (1993) measured $\tau_{fast} = 52$ ps. The measurements of Gillbro's (Sandström et al., 1988) and Xia's et al. (1993) groups of the C-PC monomers are more reliable, but still bear some uncertainties.

In particular, it is obvious that the absorption anisotropy (investigated by both groups) should depend on the excitation distribution over the monomer chromophores, and as soon as quasi-equilibrium is established ($t < 1$ ns), the anisotropy should be stable. Sauer et al. (1988) and Demidov and Borisov (1994c) have calculated that there are two characteristic timescales for excitation equilibration in C-PC monomers: $t_1 = 21$ ps and $t_2 = 47$ ps. Thus, at times greater than 100 ps the value of the absorption anisotropy should be stable. Thus, the permanent decrease in absorption anisotropy with component $t = 2{,}5$–3 ns observed in the works by Sandström et al. (1988) and Xia et al. (1993) looks strange and could be explained by the Brownian rotation of the C-PC monomers in solution. The possibility of Brownian rotation by the C-PC monomers was admitted in the work by Xia et al. (1993).

Now Gillbro's group has equipment with 70 fs resolution (Gillbro et al., 1993), and they focus efforts on the processes of energy transfer faster than those having a place in C-PC monomers. Thus, we come to conclusion that unfortunately there is now a lack of experimental data on either the fluorescence depolarization or the absorption anisotropy kinetics of C-PC β-subunits and monomers. The only available data by Sandström et al. (1988) and Xia et al. (1993) could be employed on a time interval less than 200 ps, where rotational depolarization does not significantly influence the depolarization kinetics, which is caused mainly by the excitation equilibration.

Nevertheless, we consider C-PC aggregates as suitable for examination of our formulae because the C-PC structure, energy migration rates and chromophore spectroscopic features are some of the best investigated. We believe that after the present work special experiments will be undertaken for testing the derived formulae.

C-PC β-subunits

Let us assign the index '1' to the β-84 chromophore and index '2' to the β-155 chromophore. Using the data listed above and formula (4), one can calculate $t_1 = 24$ ps and $t_2 = 1500$ ps. Unfortunately, there is no reliable depolarization kinetics data measured either experimentally or calculated theoretically to compare with our calculations. The comparison of our determinations, formula (1) (Demidov, 1994a), with the experimental data by Mimuro et al. (1986) obtained at steady-state excitation show good fit.

Our calculations of fluorescence depolarization and absorption anisotropy kinetics are presented in Figs. 1-3. The stationary values of the curves in Figs. 1 and 2 at $t > 100$ ps

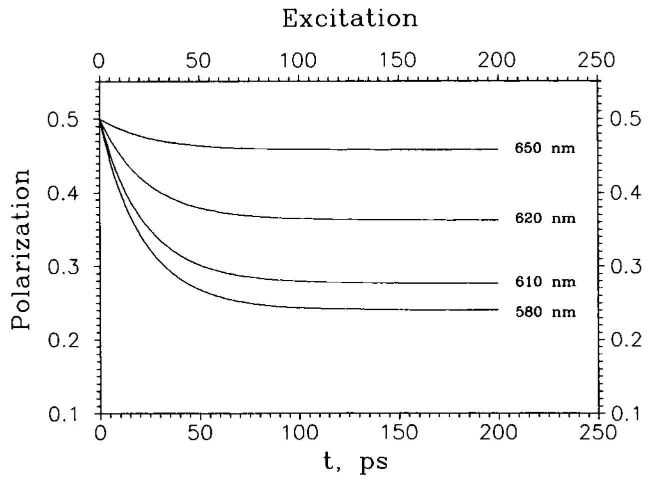

FIGURE 1 Kinetics of C-PC β-subunit polarized fluorescence at various wavelengths of excitation; the wavelength of fluorescence detection is $\lambda_{fl} = 670$ nm.

coincide with the experimental data by Mimuro et al. (1986) and earlier results by Demidov (1994a). Thus, as predicted above, because the condition $\tau^{-1} \ll K_{12}, K_{21}$ applies, the value $P(\infty) \approx P(t > 100 \text{ ps})$ is practically equal to P for steady-state excitation. The low levels of $P(\infty)$ at $\lambda_{exc} = 580$ nm, $\lambda_{fl} = 670$ nm (Fig. 1), and at $\lambda_{exc} = 550$ nm, $\lambda_{fl} = 670$ nm, (Fig. 2) are quite understandable: at these wavelengths the complexes are preferentially excited through the β-155 chromophores, whereas the fluorescence quanta are emitted mainly by the β-84 chromophores. The angle between these chromophores is equal to 46.4°. The opposite situations when λ_{exc} is close to λ_{fl} ((a) $\lambda_{exc} = 650$ nm, $\lambda_{fl} = 670$ nm (Fig. 1) and (b) $\lambda_{exc} = 550$ nm, $\lambda_{fl} = 590$ nm (Fig. 2)) generally yield higher degrees of polarization because the same chromophores absorb light and fluoresce in these cases, i.e., in the "a" case, β-84 chromophores, and in the "b" case, β-155 chromophores.

The graphs of the absorption anisotropy kinetics presented in Fig. 3 are calculated for $\lambda_{exc} = \lambda_{pr}$. One can see that the curves for $\lambda = 580$ and $\lambda = 670$ nm have different behaviors. According to Sauer et al. (1987), at the excitation wavelength $\lambda = 670$ nm only the β-84 chromophores absorb light and participate in formation of absorption anisotropy, and this yields $r = 0.4$, whereas at $\lambda = 580$ nm both chromophores can absorb light. In the latter case, the excitation energy distribution changes via energy transfer from $n_{\beta 155} > n_{\beta 84}$ to $n_{\beta 155} < n_{\beta 84}$ after exciting by a δ-pulse. This yields a decrease of absorption anisotropy from $r(t = 0) = 0.4$ to $r(t > 100 \text{ ps}) \approx 0.24$. One of the most striking results is that, whereas a naive interpretation of the data presented in the figures could lead to a conclusion that they represent a distribution of chromophores with different emissions and lifetimes; in fact, there are only two components.

C-PC monomers

Now we assign indices "1" to the α-84, "2" to the β-84, and "3" to the β-155 chromophores. Using the data presented above and Eq. A2.1, we calculate $t_1 = 21.3$ ps, $t_2 = 47$ ps, and $t_3 = 1500$ ps (Demidov and Borisov, 1994c). These values match well the theoretical evaluations by Sauer et al. (1988): 21 ps, 45 ps, and 1500 ps; experimental data ($\tau_{fast} = 46$ ps) by Holzwarth et al. (1987); and Monte-Carlo simulation ($\tau_{fast} \approx 33$–48 ps) by Demidov and Borisov (1993).

The comparison of our calculations for steady-state excitation with experimental data by Mimuro et al. (1986) for C-PC monomers shows a good fit for the excitation spectrum and some discrepancy in the case of emission spectra (Demidov, 1994b). Analysis of this discrepancy yields a proposition about the necessity of revising the chromophore spectra used (Demidov, 1994b).

The following set of dimensionless parameters are calculated using equations (A2.2) and the intrinsic lifetimes τ_i and K_{ij} listed above: $y_{01} = -3.71$, $y_{02} = -0.679$, $y_{03} = 1.17$, $z_{01} = 2.71$, $z_{02} = -0.312$, $z_{03} = 0.257$, $a_1 = 0.026$, $a_2 = -0.$

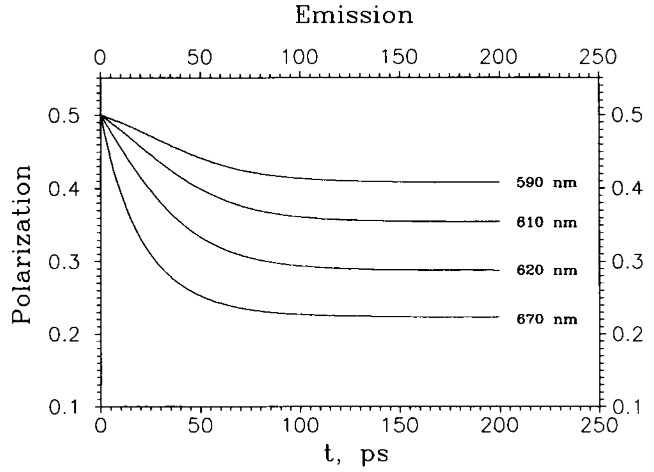

FIGURE 2 Kinetics of C-PC β-subunit polarized fluorescence at various wavelengths of fluorescence detection; the wavelength of excitation is $\lambda_{exc} = 550$ nm (see Demidov, 1994a).

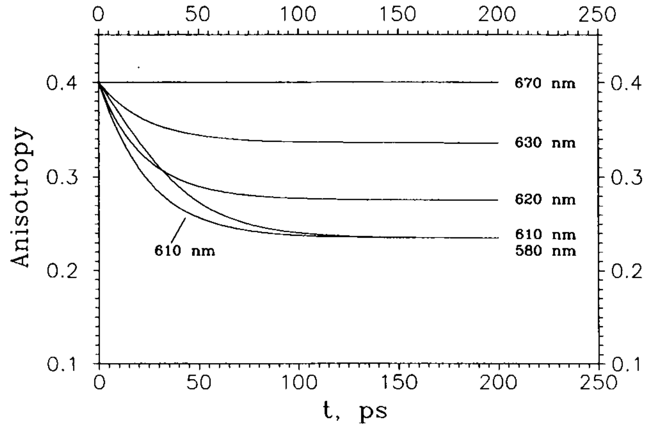

FIGURE 3 Kinetics of C-PC β-subunit absorption anisotropy at various detection wavelengths; the wavelength of the "pump" pulse λ_{exc} is equal to the wavelength of the "probe" pulse λ_{pr}: $\lambda = \lambda_{exc} = \lambda_{pr}$.

078, $a_3 = 0.253$, $b_1 = 0.564$, $b_2 = -0.335$, $b_3 = -0.667$, $d_1 = 0.409$, $d_2 = 0.413$, $d_3 = 0.414$. Then Eqs. A2.4–2.5 yield the parameters $q_{ij}(t)$ and $A^{\circ}(t)$ used in Eq. 5.

Now we have the whole set of data necessary for calculations of fluorescence depolarization and absorption anisotropy kinetics. We use the chromophore spectra presented by Sauer et al. (1987) and Mimuro et al. (1986) despite the discrepancies discussed above because our main purpose here is a demonstration of the applications of formula (5). The calculated fluorescence depolarization and absorption anisotropy kinetics are presented in Figs. 4-6. The curves presented in these figures are more complicated to analyze, but they still have same features as the kinetics shown in Figs. 1–3 and could be interpreted in the same manner. The values $P(\infty) \approx P(t > 100 \text{ ps})$ presented in Fig. 4 fit well the experimental data by Mimuro et al. (1986) for steady-state excitation and results obtained by Demidov (1994b). The analogous values $P(\infty) \approx P(t > 100 \text{ ps})$ presented in Fig. 5 coincide with calculations by Demidov (1994b) based on the accepted spectroscopic data (see above), but contradict the experimental data by Mimuro for steady-state excitation (private information). We have mentioned this discrepancy above. Soon we plan to conduct a special study based on our formulae to revise the C-PC chromophore spectroscopic data.

The graphs of anisotropy kinetics presented in Fig. 6 have similar features to those on the β-subunit graphs, see Fig. 3. With 670 nm excitation, it is mainly the α-84 and β-84 chromophores that participate in the formation of absorption anisotropy. The angle between these chromophores is equal to 15.5° ($\cos^2(15.5°) \approx 0.93$), and they behave as essentially parallel. This yields a high level of anisotropy: $r \geq 0.38$. The significant decrease in absorption anisotropy after δ-pulse excitation at the wavelength $\lambda = 580$ nm is associated with the same processes of energy redistribution between the α-84, β-84, and β-155 chromophores as discussed above in the analysis of Fig. 3.

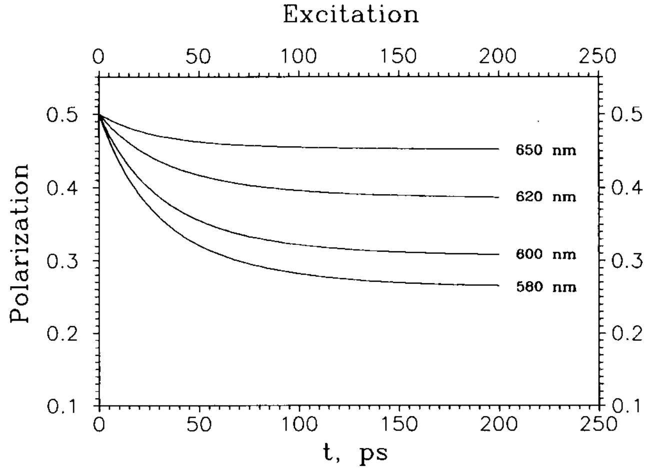

FIGURE 4 Kinetics of C-PC monomer polarized fluorescence at various wavelengths of excitation; the wavelength of fluorescence detection is $\lambda_{fl} = 670$ nm.

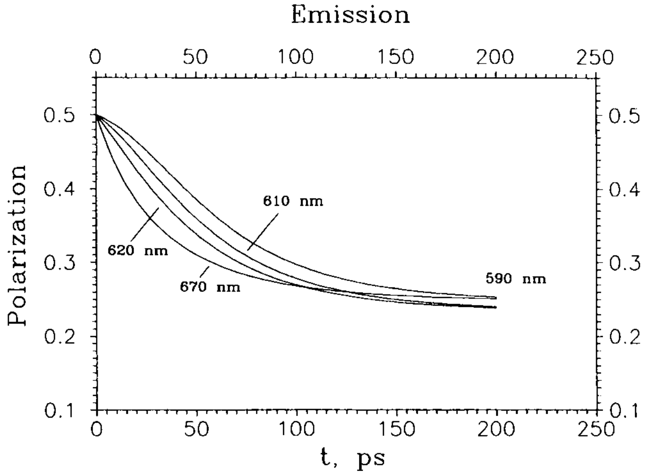

FIGURE 5 Kinetics of C-PC monomer polarized fluorescence at various wavelengths of fluorescence detection; the wavelength of excitation is $\lambda_{exc} = 550$ nm.

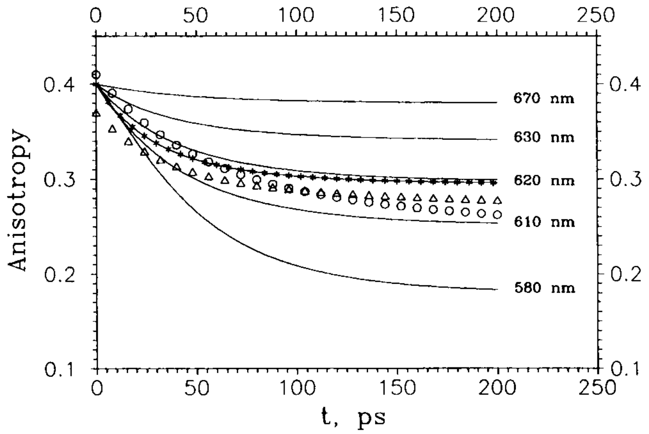

FIGURE 6 Kinetics of C-PC monomer absorption anisotropy at various detection wavelengths (——); the wavelength of the "pump" pulse λ_{exc} is equal to the wavelength of the "probe" pulse λ_{pr}: $\lambda = \lambda_{exc} = \lambda_{pr}$. Kinetics calculated for "revised" chromophore absorptions at $\lambda = 635$ nm (see text) is marked as -*-*-*-. Absorption anisotropy kinetics measured by Sandström et al. (1988) are marked as ○ for $\lambda = 580$–590 nm and △ for $\lambda = 635$–640 nm. For the presentation of experimental data, the content of Table II of the work by Sandström et al. (1988) was used.

For comparison with our calculations, the kinetics of monomer absorption anisotropy is currently available only in the experimental data of Sandström et al. (1988) and Xia et al. (1993). In Fig. 6 we present data by Sandström et al. (1988); the data obtained by Xia et al. (1993) are similar. Generally, these experimental data fit our calculations. One has to bear in mind that we had to base our calculations on the spectroscopic data available, and no free parameters were used to match our calculations to experimental results. Some differences between the experimental data and our calculations could be explained by a difference between the real spectroscopic properties of the chromophores and those employed in our calculations (see above). In the work by Demidov (1994b), the conclusion was made that the spectroscopic data of C-PC chromophores should be revised. This work is now in progress in collaboration with Dr. M. Mimuro (Japan). Our deconvolution of the C-PC chromophore spectra is based on the re-processing of "old" data obtained by Mimuro et al. (1986) in the light of a new theory of the degree of fluorescence polarization under steady-state excitation (Demidov et al., 1994a, b). The application of our new preliminary data obtained for the wavelength $\lambda = 635$ nm yields a better match between the experimental and calculated kinetics (see Fig. 6). In particular, the "old" values of the absorption efficiencies for this wavelength were equal to 51,000, 50,000, and 2,800 $\mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$ for the α-84, β-84, and β-155 chromophores, respectively (according to Sauer et al., 1987), whereas our recent calculations yielded 66,000, 57,700, and 26,600 $\mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$ for the same chromophores. The absorption efficiency of the β-155 chromophores is 10 times higher, and this explains why $r(\infty)$ is lower in the case of the "new" data.

CONCLUSION

Thus, we come to a conclusion that the fluorescence depolarization (absorption anisotropy) kinetics of chromophore complexes with energy transfer has three major features.

(1) At the first moment of δ-pulse excitation, all chromophores behave quasi-independently and the degree of fluorescence polarization (anisotropy) is equal to $P(t = 0) = 0.5$ ($r(t = 0) = 0.4$).

(2) After δ-pulse excitation, the values of $P(t)$ and $r(t)$ decrease to the stationary values $P(\infty)$ and $r(\infty)$, respectively. Both functions $P(t)$ and $r(t)$ include the exponential functions $\exp(-t/t_i)$, where the number of components i is equal to the number of chromophores (or the size of the "rate" matrix, see Appendix 1). The decay of depolarization (anisotropy) kinetics reflects processes of energy redistribution among the chromophores. The time parameters t_i are the parameters presented in the multiexponential deconvolution of the fluorescence (absorption) decay kinetics. Actually, in the function $P(t)$ ($r(t)$), only the differences $\Delta t_{ij}^{-1} = t_i^{-1} - t_j^{-1}$ are presented: $\exp(-t/\Delta t_{ij})$. The condition $t \to \infty$ is roughly equal to condition $t \gg \Delta t_{km}$, where Δt_{km} is the "longest" time component.

(3) If the intrinsic deactivation rates τ_i^{-1} are much smaller than the energy transfer rates K_{ij}, then the value $P(\infty)$ ($r(\infty)$) is equal to the steady-state excitation value $P(r)$.

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APPENDIX 1

The excitation population n_i ($i = 1,2,3$) and energy migration in each individual triple-chromophore complex are described by the system $\mathbf{Gn} + \mathbf{b} = 0$, where $\mathbf{n}$ is the vector of chromophore excitation populations, $\mathbf{b}$ is the vector of excitation conditions proportional to $\sigma_i(\lambda_{exc})$, and $\mathbf{G}$ is the following "rate" matrix:

$$G = \|g_{ij}\|$$

$$= \begin{vmatrix} (-1/\tau_1 - K_{12} - K_{13}) & K_{21} & K_{31} \\ K_{12} & (-1/\tau_2 - K_{21} - K_{23}) & K_{32} \\ K_{13} & K_{23} & (-1/\tau_3 - K_{31} - K_{32}) \end{vmatrix}.$$

The matrix equation can be solved for the vector $\mathbf{n}$: $\mathbf{n} = -\boldsymbol{\Omega}\mathbf{b} = -\|\omega_{ij}\|\mathbf{b}$, where $\|\omega_{ij}\|$ is the inverse of the matrix $\|g_{ij}\|$: $\boldsymbol{\omega}\mathbf{G} = \mathbf{E}$. Here $\mathbf{E}$ is the unit matrix (the identity matrix). The ω_{ij} elements are equal to:

$$\omega_{11} = \frac{g_{22}g_{33} - g_{23}g_{32}}{\det g} = \frac{(1/\tau_2 + K_{23} + K_{21})(1/\tau_3 + K_{31} + K_{32}) - K_{32}K_{23}}{\det g}$$

$$\omega_{12} = \frac{g_{13}g_{32} - g_{12}g_{33}}{\det g} = \frac{K_{31}K_{23} + K_{21}(1/\tau_3 + K_{31} + K_{32})}{\det g}$$

$$\omega_{13} = \frac{g_{12}g_{23} - g_{22}g_{13}}{\det g} = \frac{K_{21}K_{32} + K_{31}(1/\tau_2 + K_{23} + K_{21})}{\det g}$$

$$\omega_{21} = \frac{g_{31}g_{23} - g_{21}g_{33}}{\det g} = \frac{K_{13}K_{32} + K_{12}(1/\tau_3 + K_{31} + K_{32})}{\det g}$$

$$\omega_{22} = \frac{g_{11}g_{33} - g_{31}g_{13}}{\det g} = \frac{(1/\tau_1 + K_{12} + K_{13})(1/\tau_3 + K_{31} + K_{32}) - K_{13}K_{31}}{\det g}$$

$$\omega_{23} = \frac{g_{21}g_{13} - g_{11}g_{23}}{\det g} = \frac{K_{12}K_{31} + K_{32}(1/\tau_1 + K_{12} + K_{13})}{\det g}$$

$$\omega_{31} = \frac{g_{21}g_{32} - g_{22}g_{31}}{\det g} = \frac{K_{12}K_{23} + K_{13}(1/\tau_2 + K_{21} + K_{23})}{\det g}$$

$$\omega_{32} = \frac{g_{12}g_{31} - g_{11}g_{32}}{\det g} = \frac{K_{21}K_{13} + K_{23}(1/\tau_1 + K_{12} + K_{13})}{\det g}$$

$$\omega_{33} = \frac{g_{11}g_{22} - g_{21}g_{12}}{\det g} = \frac{(1/\tau_1 + K_{12} + K_{13})(1/\tau_2 + K_{21} + K_{23}) - K_{12}K_{21}}{\det g}$$

$$\det g = g_{11}(g_{22}g_{33} - g_{23}g_{32}) - g_{12}(g_{21}g_{33} - g_{31}g_{23}) + g_{13}(g_{21}g_{32} - g_{31}g_{22}).$$

These ω_{ij} parameters are used in calculations of the degree of fluorescence polarization under steady-state excitation. The parameters $A°$ and q_{ij} (see (2)) are given by; $A° = B/D$, $q_{ij} = Q_{ij}/D$, $D = Q_{12} + Q_{13} + Q_{23}$, $B = \omega_{11} + \omega_{22}\alpha_{21}\gamma_{21} + \omega_{33}\alpha_{31}\gamma_{31}$, $Q_{12} = \omega_{12}\alpha_{21} + \omega_{21}\gamma_{21}$, $Q_{13} = \omega_{13}\alpha_{31} + \omega_{31}\gamma_{31}$ and $Q_{23} = \omega_{23}\gamma_{21}\alpha_{31} + \omega_{32}\gamma_{31}\alpha_{21}$ (Demidov, 1994b).

APPENDIX 2

The parameters t_1, t_2, and t_3 of energy redistribution and overall decay can be calculated from the equation $det(\mathbf{G} + \nu\mathbf{E}) = 0$, or in equivalent form:

$$\det \begin{vmatrix} g_{11} + \nu & g_{12} & g_{13} \\ g_{21} & g_{22} + \nu & g_{23} \\ g_{31} & g_{32} & g_{33} + \nu \end{vmatrix} = 0, \tag{A1}$$

where "det" means determinant and $\nu = 1/t$. This is a cubic equation having three roots: ν_1, ν_2, and ν_3 (or t_1, t_2, and t_3). One can find the elements g_{ij} of the $\mathbf{G}$ matrix in Appendix 1. For calculations of $q_{ij}(t)$ and $A°(t)$, it is necessary to conduct a set of calculations of intermediate parameters:

$$y_{0i} = \frac{g_{13}g_{21} - g_{23}(g_{11} + \nu_i)}{g_{12}g_{23} - g_{13}(g_{22} + \nu_i)},$$

$$z_{0i} = \frac{\nu_i^2 + (g_{11} + g_{22})\nu_i + (g_{11}g_{22} - g_{12}g_{21})}{g_{12}g_{23} - g_{13}(g_{22} + \nu_i)}, \qquad i = 1, 2, 3,$$

$$\Delta = (y_{01} - y_{03})(z_{02} - z_{03}) - (y_{02} - y_{03})(z_{01} - z_{03}), \tag{A2}$$

$$a_1 = \frac{y_{02}z_{03} - y_{03}z_{02}}{\Delta}, \qquad a_2 = \frac{z_{02} - z_{03}}{\Delta}, \qquad a_3 = \frac{y_{03} - y_{02}}{\Delta},$$

$$b_1 = \frac{y_{03}z_{01} - y_{01}z_{03}}{\Delta}, \qquad b_2 = \frac{z_{03} - z_{01}}{\Delta}, \qquad b_3 = \frac{y_{01} - y_{03}}{\Delta},$$

$$d_1 = \frac{y_{01}z_{02} - y_{02}z_{01}}{\Delta}, \qquad d_2 = \frac{z_{01} - z_{02}}{\Delta}, \qquad d_3 = \frac{y_{02} - y_{01}}{\Delta}.$$

All of these parameters are involved in calculations of the time-dependent matrix $\omega_{ij}(t)$, which is analogous to matrix ω_{ij} in Appendix 1:

$$\begin{aligned}
\omega_{11}(t) &= a_1 e^{-t/\tau_1} + b_1 e^{-t/\tau_2} + d_1 e^{-t/\tau_3} \\
\omega_{12}(t) &= a_2 e^{-t/\tau_1} + b_2 e^{-t/\tau_2} + d_2 e^{-t/\tau_3} \\
\omega_{13}(t) &= a_3 e^{-t/\tau_1} + b_3 e^{-t/\tau_2} + d_3 e^{-t/\tau_3} \\
\omega_{21}(t) &= a_1 y_{01} e^{-t/\tau_1} + b_1 y_{02} e^{-t/\tau_2} + d_1 y_{03} e^{-t/\tau_3} \\
\omega_{22}(t) &= a_2 y_{01} e^{-t/\tau_1} + b_2 y_{02} e^{-t/\tau_2} + d_2 y_{03} e^{-t/\tau_3}. \qquad \text{(A3)} \\
\omega_{23}(t) &= a_3 y_{01} e^{-t/\tau_1} + b_3 y_{02} e^{-t/\tau_2} + d_3 y_{03} e^{-t/\tau_3} \\
\omega_{31}(t) &= a_1 z_{01} e^{-t/\tau_1} + b_1 z_{02} e^{-t/\tau_2} + d_1 z_{03} e^{-t/\tau_3} \\
\omega_{32}(t) &= a_2 z_{01} e^{-t/\tau_1} + b_2 z_{02} e^{-t/\tau_2} + d_2 z_{03} e^{-t/\tau_3} \\
\omega_{33}(t) &= a_3 z_{01} e^{-t/\tau_1} + b_3 z_{02} e^{-t/\tau_2} + d_3 z_{03} e^{-t/\tau_3}
\end{aligned}$$

Now at last we approach the calculations of the major parameters $q_{ij}(t)$ and $A^\circ(t)$:

$$\begin{aligned}
q_{12}(t) &= \frac{\omega_{12}(t)\alpha_{21} + \omega_{21}(t)\gamma_{21}}{D(t)} \\
q_{13}(t) &= \frac{\omega_{13}(t)\alpha_{31} + \omega_{31}(t)\gamma_{31}}{D(t)} \\
q_{12}(t) &= \frac{\omega_{23}(t)\gamma_{21}\alpha_{31} + \omega_{32}(t)\gamma_{31}\alpha_{21}}{D(t)} \qquad \text{(A4)} \\
A^\circ(t) &= \frac{\omega_{11}(t) + \omega_{22}(t)\gamma_{21}\alpha_{21} + \omega_{33}(t)\gamma_{31}\alpha_{31}}{D(t)} \\
D(t) &= \omega_{12}(t)\alpha_{21} + \omega_{21}(t)\gamma_{21} + \omega_{13}(t)\alpha_{31} + \omega_{31}(t)\gamma_{31} \\
&\quad + \omega_{23}(t)\gamma_{21}\alpha_{31} + \omega_{32}(t)\gamma_{31}\alpha_{21}
\end{aligned}$$

The parameters α_{i1} and γ_{i1} are the same as described above. Obviously, $q_{12}(t) + q_{13}(t) + q_{23}(t)$ is equal to 1 at any time t. Of course, the described calculation procedure is a routine one, and computers should be used for convenience.

Now let us evaluate the extreme of $P(t)$ as $t \rightarrow 0$. One can easily find that under these conditions $\omega_{ij} \rightarrow 0$ $(i \neq j)$ and, thus, $P(t \rightarrow 0) = 0.5$. This result is understandable because at the first moment of δ-pulse excitation there is as yet no energy redistribution, and all the chromophores behave quasi-independently.

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