Polarized absorption picosecond kinetics as a probe of energy transfer in phycobilisomes of *Synechococcus* 6301

Tomas Gillbro, Åke Sandström, Villy Sundström and Alfred R. Holzwarth*

Department of Physical Chemistry, University of Umeå, S-901 87 Umeå, Sweden

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The energy transfer between C-phycocyanin chromophores in intact phycobilisomes of *Synechococcus* 6301 is shown to lead to an anisotropy relaxation with a lifetime of 10 ± 2 ps. However, due to the molecular order within the hexameric units of C-phycocyanin the anisotropy does not decay to zero. The Förster dipole–dipole mechanism of energy transfer can qualitatively explain these data provided that there is no back transfer of excitation energy and that the chromophore distribution is non-random. The rate of energy transfer in phycobilisomes between C-phycocyanin and allophycocyanin can best be described by a double exponential with lifetimes of 12 ± 3 and 84 ± 8 ps.

Synechococcus *6301* *Phycobilisome* *C-phycocyanin* *Absorption anisotropy* *Picosecond spectroscopy* *Energy transfer*

1. INTRODUCTION

The detailed composition and structure of phycobilisomes in several red algae and cyanobacteria including *Synechococcus* 6301 have been determined [1,2]. It has also been shown that excitation energy transfer between different phycobiliproteins occurs in <100 ps [3–6]. As a further proof of the efficient energy transfer within phycobilisomes one has found that the fluorescence from the emitting chromophore of allophycocyanin is highly depolarized [7]. The depolarization seems to occur during the first transfer steps within a phycobiliprotein aggregate [8].

We have chosen to study the energy transfer in phycobilisomes of *Synechococcus* 6301 (*Anacystis nidulans*) since their structure is well known [2,9] and because of their relatively simple protein composition; i.e., the phycobilisome core consists mainly of allophycocyanin, and the 6 rods of hexameric discs that are bound to the core are composed of C-phycocyanin.

Here, we focus our attention on the kinetics of energy transfer within C-phycocyanin complexes. For this purpose we have measured the decay of the absorption anisotropy induced in the phycobilisome sample by a laser pulse. The kinetics of the anisotropy relaxation will give us an understanding how fast and by which mechanism the excitation energy is transferred within this important biological antenna system.

We have also studied the rate at which C-phycocyanin loses its excitation energy by transfer to allophycocyanin, by observing the isotropic component of the C-phycocyanin absorption recovery.

2. MATERIALS AND METHODS

Synechococcus 6301 was grown under continuous white light illumination (17.5 $\mu E.m^{-2}.s^{-1}$) at 37°C in a standard growth medium [10]. The phycobilisomes were isolated by centrifugation at room temperature from cells broken in a French

* Permanent address: Max-Planck-Institut für Strahlenchemie, 4330 Mülheim a.d. Ruhr, FRG

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pressure cell (20000 lb.in^{-2}) and following the method in [11] with minor modifications. The purity was checked by the ratio of the absorbances at 622 and 275 nm. This ratio was about 5, which according to [1] indicates that the phycobilisomes are intact and essentially free of contamination; e.g., free phycobiliproteins. The fluorescence spectrum had a maximum at 675 nm which indicates an effective energy transfer between C-phycocyanin and allophycocyanin [1,7].

The ps absorption experiments were done at ~23°C with fresh samples in a flow cell to prevent build-up of photoproducts. A detailed description of the mode-locked, cavity-dumped dye laser system used in these experiments has been presented in [12]: The picosecond pulse-train from the dye laser was split into two parts. About 95% of the intensity was used to excite the sample and ~2% was used as analyzing light. Time resolution was achieved by a motor-driven delay in the analyzing light beam. The modulation imposed on the analyzing light through the chopping of the excitation beam (~1 kHz) was detected by a photomultiplier, whose output was fed into a phase-sensitive amplifier. The output of the amplifier was recorded on a chart recorder as a function of optical delay (time). The signal is proportional to the transmission change in the sample. At the small transmission change in this work the signal is proportional to the absorbance change.

The pulses used in this work were ~6 ps long and the excitation wavelength was varied between 550 and 650 nm.

To avoid singlet–singlet annihilation effects which might occur at high pulse intensities [3,5] we used an excitation degree of ~1%. When this was reduced further by a factor of 2 or 10 by inserting neutral density filters in the excitation beam, no change in the recorded kinetics within experimental error (± 10%) was noticed.

3. RESULTS AND DISCUSSION

The relaxation of the anisotropy induced by the excitation pulse was probed with the analyzing light polarized parallel ($\theta = 0°$) or perpendicular ($\theta = 90°$) to the excitation light. The corresponding signal intensities were $I_{\parallel}(t)$ and $I_{\perp}(t)$, respectively. Fig.1 shows these signals at 630 nm. The absorption anisotropy function, $r(t)$, is defined as:

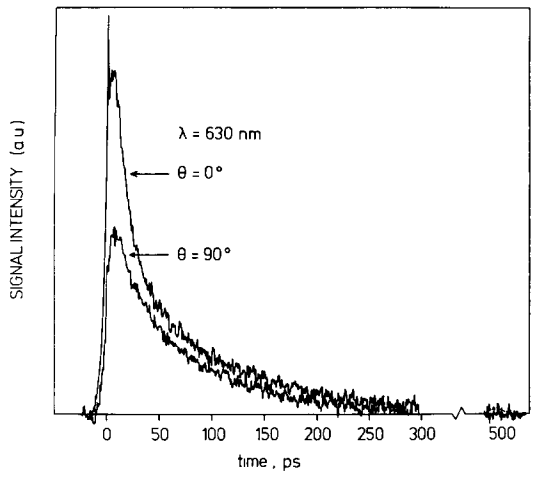

Fig.1. The absorption recovery kinetics of phycobilisomes of *Synechococcus* 6301 at 630 nm with the polarization of the analyzing light parallel ($\theta = 0°$) or perpendicular ($\theta = 90°$) to the excitation light.

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \quad (1)$$

which is shown as a semilogarithmic plot in fig.2. From fig.2, it is clear that the anisotropy does not relax according to a single exponential function of time. However, it is possible to fit the data with a function of the form [13–15]:

$$r(t) = [r(0) - r(\infty)] \cdot e^{-t/\tau_r} + r(\infty) \quad (2)$$

where $r(\infty)$ is the constant value of $r(t)$ at $t > 50$ ps. This function, although only a simplification of a more general expression [13], is typical of anisotropic systems (e.g., proteins in biological membranes) where $r(t)$ never decays to zero, but reaches a value determined by the order parameter, S, of the system according to [14,15]:

$$r(\infty) = r(0)S^2, \text{ where } S = \left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle$$

where θ is the angle between the symmetry axis of a hexameric disc of C-phycocyanin and the direction of the absorption transition moment of a chromophore. With $r(\infty) = 0.07$ (mean value of 3 measurements) and $r(0) = 0.4$ we calculate

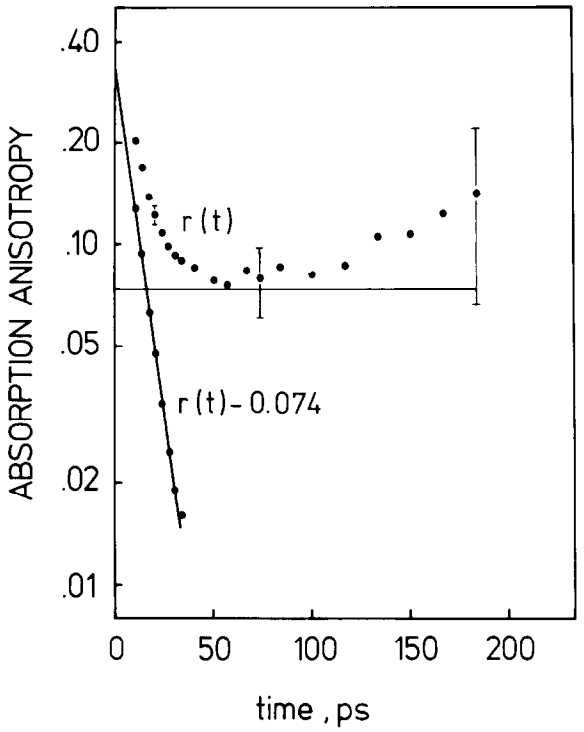

Fig.2. A semilogarithmic plot of the absorption anisotropy, $r(t)$, *vs* time, t. The function $r(t) - 0.074$ is also shown.

$S = \pm 0.418$ and $\langle\cos^2\theta\rangle = 0.612$ or 0.055. From these numbers we cannot calculate how the individual chromophores are ordered, since there are 3 chromophores/protein unit but they show that the transition moments are not randomly oriented.

The initial fast decay of $r(t)$ in fig.2 is close to exponential with a lifetime, τ_r, of 10.4 ps (mean value of 3 measurements at λ = 605, 620 and 630 nm is 10 ± 2 ps). Theoretically, however, it is difficult to justify a single exponential relaxation, since the relaxation is caused by energy transfer from excited C-phycocyanin chromophores situated in different parts of the phycobilisomes to unexcited chromophores, whose orientations and distance relative to the initially excited chromophores are widely distributed. One would rather expect the relaxation to be multiexponential [13,15]. It is not possible to evaluate $r(t)$ exactly, however, since we do not know the exact position of all the chromophores. We will therefore compare our data to theoretical calculations of energy transfer by the Förster dipole–dipole mechanism [16] in random systems [17–19]. Two cases will be considered: in the first case back-transfer of the excitation energy to the originally excited molecule is allowed [18]. In the second case the excitation energy is trapped after the first transfer and never returns to the original site of excitation [17,19]. For the calculations we need the reduced concentration, C, which is the number of C-phycocyanin chromophores within the Förster radius, R_o, for the energy transfer. Since R_o = 47 Å (random distribution [20]) for the energy transfer between two C-phycocyanin molecules, we obtain C = 11.5 (there are 18 chromophores in a 6 nm high hexameric disc with a diameter of 12 nm [1,2]). This value of C is considered to be an upper limit, since the C-phycocyanin chromophores in a hexamer have slightly different absorption spectra. At 630 nm, for instance, the C-phycocyanin α-units absorb much more than the β-units. The fluorescence lifetime, τ_f, of the C-phycocyanin chromophores, which is 2.1 ns [20,21], is also needed.

The probability $G^S(t)$ in [18] that the initially excited molecule in a system of donors is still excited at t is proportional to $r(t)$, i.e., $r(t) = 0.4\ G^S(t)$. We have calculated $G^S(t)$ and some values are plotted in fig.3 *vs* $\sqrt{t}$. The deviation of $G^S(t)$ from a function of the form $\exp(-2A\sqrt{t})$ with $2A = 0.27$ is indistinguishable for $t < 50$ ps.

For a system of randomly distributed donors and acceptors authors in [19] have calculated a probability, $G^T(t)$, that the excited state is on a donor. $G^T(t)$ is a function of the reduced donor (C_D) and acceptor (C_T) concentrations, the lifetime, τ_f, and the Förster radius for donor–donor (R_o^{DD}) and donor–acceptor (R_o^{DT}) transfer. Values of $G^T(t)$ for $C_D = 0$, $C_T = 11.5$, $\tau_t = 2.1$ ns and $R_o^{DD} = R_o^{DT}$ are shown in fig.3. As expected in this case [19], which is similar to that giving the expression $\exp[-0.845 \cdot C_T(\pi t/\tau_f)^{1/2}]$ according to [17,22], the data fall close to a function $\exp[-2A\sqrt{t}]$ with $2A = 0.45$. Förster's solution gives $2A = 0.38$. Our experimental data for $r(t) - 0.074$ have also been plotted in fig.3 and in the limited useful time interval (10–25 ps) they are close to a $\exp(-2A\sqrt{t})$ function with $2A = 0.75$. At $t = 0$, however, we obtain $r(0)$ larger than the theoretical value 0.4. We believe that this deviation can at least partially be explained by a distortion in the experimental signal due to the finite length of

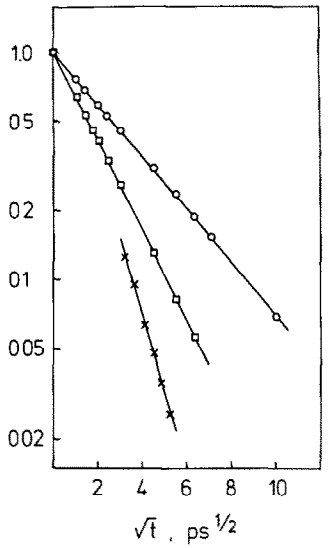

Fig.3. Comparison between experiment and theory: (×) $r(t) - 0.074$ (experimental data at 630 nm); (○) $G^S(t)$ according to [14] ($C = 11.5$, $\tau_f = 2.1$ ns, $\gamma = 0.846$); (□) $G^T(t)$ according to [19] ($C_T = 11.5$, $C_D = 0$, $\tau_f = 2.1$ ns, $R_o^{DD} = R_o^{DT}$).

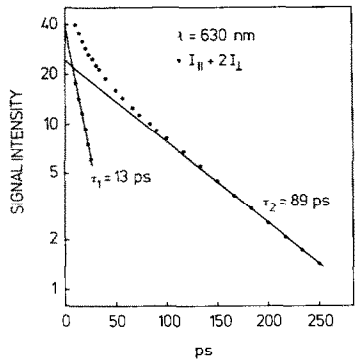

Fig.4. The isotropic absorption recovery in phycobilisomes at 630 nm obtained from the sum of $I_{\parallel}$ and $2\,I_{\perp}$ in fig.1.

the excitation pulse. A comparison between experiment and theory indicates that the excitation energy probably migrates without back transfer. This result is appealing since it means that the excitation energy reaches its final destination very efficiently. This is expected, since the C-phycocyanin chromophores depending on their position have slightly different absorption spectra [2], which should make an energy transfer towards the chromophore with a lower excitation energy more likely than the reverse process. From the theoretical estimates we can also draw the conclusion that the energy transfer is more efficient than in a system of randomly distributed chromophores. This means that R_o is larger than 47 Å due to favourable orientation of the transition moments. An effective R_o of 56 Å was calculated from our experimental data using the expression [17] above with $C_T = 11.5$. This Förster radius should, however, be regarded as a lower limit, since as mentioned above the β-units are excited less than the α-units at 630 nm [23] and thus might not take part in the energy transfer process observed at this wavelength.

The isotropic component of the C-phycocyanin absorption recovery at 630 nm can be obtained by calculating the sum of $I_{\parallel}$ and $2I_{\perp}$ from fig.1. The result is shown in fig.4. The signal does not decay according to a single exponential or an $\exp(-2A\sqrt{t})$ function. The best fit in the time range 10–250 ps is obtained for a double exponential decay with the lifetimes of 13 and 89 ps. The mean values of 8 measurements at different wavelengths are 12 ± 3 and 84 ± 8 ps, respectively. The kinetics is a measure of the energy transfer from C-phycocyanin in the rods to allophycocyanin in the core of the phycobilisome, since other relaxation mechanisms as, e.g., the radiative process ($\tau = 2.1$ ns), can be neglected. Presently, we cannot give a conclusive interpretation of the kinetics observed. One possibility is that there are two kinds of C-phycocyanin rods which couple different strengths to the allophycocyanin core.

A second more likely possibility is that the energy transfer to allophycocyanin is faster when a C-phycocyanin chromophore close to the core is excited than when the excited chromophore is in the periphery of a rod of C-phycocyanin hexamers.

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