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Picosecond study of energy transfer within 18-S particles of AN 112 (a mutant of *Synechococcus* 6301) phycobilisomes

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The energy transfer process within 18-S particles of AN 112 (a mutant of *Synechococcus* 6301) phycobilisomes was studied by picosecond absorption and fluorescence techniques. The results are in general agreement with an earlier study on phycobilisomes of the mutant AN 112. Our data indicate that 18 S particles act as functional units with respect to excitation energy transfer. Two kinetic components of 17 ± 2 and 55 ± 5 ps, respectively, were observed in the transfer from C-phycocyanin to allophycocyanin. The fastest component was interpreted as transfer from β-84 and/or α-84 C-phycocyanin chromophores to the APC core. A high anisotropy, $r(\infty) = 0.28 \pm 0.04$, is taken as a further proof of such a process. The 55 ps component most likely represents the transfer from distal β-155 chromophores to the core. When processes in the core were studied at about 650 nm, a fast phase of 15 ± 3 ps and an intermediate phase of 65 ± 9 ps were resolved. These processes, although slightly slower than in AN 112 phycobilisomes, are interpreted as transfer between APC trimers and transfer to the final emitter, a 75 kDa polypeptide, respectively. The chromophore of the 75 kDa protein fluoresces with a lifetime of 1.9 ns, which is similar to that found for intact phycobilisomes.

Introduction

Phycobilisomes are supramolecular light-harvesting pigment-protein complexes of cyanobacteria and red algae situated on the outer surface of the thylakoid membrane. They efficiently collect light energy in the wavelength interval 500–650 nm, which is not well covered by chlorophylls and carotenoids. The excitation energy is rapidly transferred to the photosynthetic reaction centers, mainly that of Photosystem II. The phycobilisome consists of a core, which is directly connected to the thylakoid membrane by special linker polypeptides, and several rods, which are bound to the core. In the cyanobacterium *Synechococcus* 6301, that was used in this work, the phycobilisome core consists of two cylinders, each with four trimeric units of mainly the biliprotein allophycocyanin. There are six rods, each with 1–3 hexameric units of the biliprotein C-phycocyanin, depending on the growth conditions.

The transfer of excitation energy in phycobilisomes has been studied extensively during the last

Abbreviation: 75k, 75 kDa polypeptide in 18-S particle.

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decades using both steady-state and time-resolved fluorescence and absorption techniques (for recent reviews see Refs. 1, 2). From these studies a general picture of the paths of excitation transfer in phycobilisomes has evolved. The main features of this transfer are; (i) a fast (approx. 10 ps) transfer of excitation energy between closely situated chromophores in smaller aggregates, i.e., trimers and/or hexamers, of phycoerythrin, C-phycocyanin and allophycocyanin [3-7]; (ii) a transfer of energy from C-phycocyanin rods to the allophycocyanin core that is 2–3-times faster in rods consisting of one C-phycocyanin hexamer than in rods that are 3–4 hexameric units long [4–6]. It thus appears that the comparatively long transfer time from the rods to the core is controlled by the size of the rod [5]. Recently, Suter and Holzwarth showed [9] that it is possible to account for the rod-size effect if one assumes a fast transfer of excitation energy within and between C-phycocyanin hexamers, and if the transfer from the final C-phycocyanin trimer to the allophycocyanin core is the rate-limiting step. This is an example of trap-limited excitation energy transfer in the model proposed by Pearlstein [10]. The calculations also suggested that the rate constant for transfer from the innermost trimer to the core should be 40 ± 5 ns^{-1}. One question in this context that remains to be addressed experimentally is whether the lifetime of 40–50 ps found when each rod contains only one C-phycocyanin hexamer [5] is limited by the excitation transfer within the hexameric unit itself or if this lifetime is determined by the rate of energy transfer between C-phycocyanin and allophycocyanin at the junction between a rod and the core. A way to tackle this question experimentally would be to study 18-S particles [11,12]. They consist of two separate C-phycocyanin trimers bound to a fragment of the allophycocyanin core (two allophycocyanin trimeric units) in the same manner as phycobilisome rods are attached to the phycobilisome core [13]. Another question that might be addressed in this system is whether hexameric units of C-phycocyanin are a prerequisite for the fast (10 ps) energy transfer and anisotropy relaxation [4,5] in phycobilisomes. A significant change in the lifetime of the fast energy transfer step has been observed for different aggregation states of isolated C-phycocyanin. Generally the transfer rate increases with the size of the aggregate [3,8,1517]. On these grounds, one does not expect to observe the 10 ps transfer component within the C-phycocyanin trimers of the 18-S particle. Furthermore, since 18-S particles contain only the 75 kDa polypeptide (75k) and no allophycocyanin-B, it might also be possible to determine whether allophycocyanin-B or 75k [18,19] acts as the final emitter in intact phycobilisome. Similar fluorescence lifetimes in 18-S particles and whole phycobilisome would indicate that the 75k chromophore is channeling the energy from the phycobilisome to photosystem II in the thylakoid membrane. Finally, a comparison between the kinetics of energy transfer within the allophycocyanin core of intact phycobilisome and the reduced core of 18-S might improve our understanding of the paths of energy transfer within the core itself. So far, very little information is available on the energy transfer pathways in the phycobilisome core [5,20].

In brief, the results of this work show that the energy transfer in 18-S is very similar to the transfer in phycobilisome of the mutant AN 112 [5]. Thus 18-S can be considered as the smallest functional unit of a phycobilisome comprizing both phycocyanin and allophycocyanin. We find, for instance, a lifetime of about 50 ps for the energy transfer from C-phycocyanin to allophycocyanin and a 60–70 ps transfer time from allophycocyanin to 75k. This is in close agreement with the transfer rates observed in AN 112 phycobilisome [5]. The main differences in the energy transfer kinetics, as compared to AN112 phycobilisome, is a lengthening of the fast process at 590–600 nm from about 10 to 15–20 ps and an increase of the anisotropy at $t > 50$ ps from about 0.08 to about 0.28 in the 18-S particle. These results are discussed in terms of couplings between different C-phycocyanin chromophores and the core.

Experimental

Preparation of phycobilisome and 18-S particles

Cultures of AN 112 (a mutant of *Synechococcus* 6301) were grown as previously described [5].

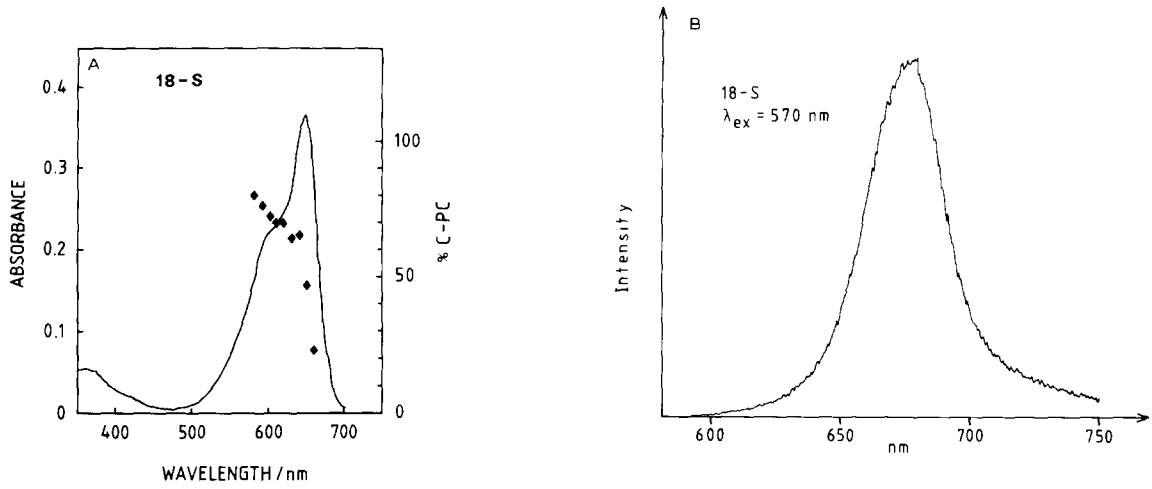

Fig. 1. (A) Absorption spectrum of 18-S particles (for preparation see text). The percentage of light absorbed by the C-phycocyanin chromophores as calculated from Ref. 12 is also shown. (B) Fluorescence spectrum of 18-S particles excited at 570 nm.

Phycobilisomes were prepared by dissolving the harvested cells (3–4 g wet weight) in 0.8 M Na_2SO_4 buffered with 50 mM Tris-HCl (pH 8.0) (according to Ref. 12), to a volume of 7 ml/g of cells. 2% (w/v) Triton X-100 and 0.3% (w/v) lysozyme were added and the mixture was stirred at room temperature overnight. Centrifugation at 20000 rpm, SS-34 rotor, for 30 min pelleted membrane fragments and remaining whole cells. The supernatant containing the phycobilisome was then passed through a 8 μm Millipore filter in order to remove scattering particles. The phycobilisomes were pelleted by centrifugation at 55000 rpm, 70 Ti rotor, for 2.5 h.

The 18-S particles were prepared according to Ref. [12]. The sucrose step gradient centrifugation was, however, slightly modified for the pump and probe experiments, where a 70 Ti rotor (50000 rpm, 7.5 h) was used. The integrity of the 18-S particles was checked by studying their absorption and fluorescence spectra (Fig. 1). The 18-S particles had a fluorescence spectrum identical to that of intact phycobilisome of AN 112 when excited at 570 nm. The absorption spectrum had a maximum at 649 nm and a pronounced shoulder at 600 nm [11]. 18-S particles prepared by the two methods had identical absorption and fluorescence spectra.

Picosecond absorption measurements

Tunable picosecond light pulses were generated by a cavity-dumped dye laser, synchronously pumped by an argon ion laser. This system produced pulses of typically 10 ps duration with a pulse energy of a few nanojoules. We used the so-called pump and probe method, where the sample is excited by a strong pump pulse and its ground state recovery is analysed with a weak probe pulse. The time resolution is obtained by varying the optical pathlength between the pump and probe pulses. By changing the angle of polarization between the excitation and probing light, different kinds of information can be obtained. When the angle is 54.7° an isotropic signal is obtained, which is free from depolarization effects due to molecular rotation and/or energy transfer between identical chromophores with differently oriented transition dipole moments. In order to study the anisotropy decay of the sample, two experiments, with parallel and perpendicular polarization, respectively, are required. The time-dependent anisotropy, which is defined as $r(t) = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ can then be calculated. The sum $I_{\parallel} + 2I_{\perp}$ is proportional to the isotropic signal. All measurements were performed at room temperature in a 0.5 mm flow cell with an optical absorbance of 0.5–0.2, depending on excitation

wavelength. The number of photons absorbed in the sample was less than $10^{14}/cm^2$ per pulse, which means that typically less than 0.2 C-PC chromophores per 18-S particle were excited. Decreasing the intensity by a factor of 10 did not alter the kinetics of the energy transfer. Thus, artifacts due to excitation annihilation can be ruled out in our measurements. The curve fitting was done on an IBM PC-AT microcomputer using a nonolinear least-squares fitting program [24] based on the Marquardt algorithm [25]. We have used open circles in the figures to show the experimental data points taken from kinetic traces and a solid line for the theoretical best fits.

Fluorescence measurements

The time-resolved fluorescence measurements were performed with a single photon timing apparatus described earlier [5,21]. This sensitive technique makes it possible to analyse multi-exponential kinetics of up to four components. The isotropic fluorescence decay functions were obtained by setting the analyzing polarizer at an angle of 54.7° relative the polarization of the excitation light. All measurements were carried out at room temperature in flow cells of either 1.5 or 3 mm path length. The photon density at the sample was no greater than 10^{11} per cm^2 per pulse, which is far below the level where annihilation effects might influence the fluorescence kinetics (see above).

For analysis of the fluorescence decay curves recorded at various excitation/emission wavelength pairs, we applied a deconvolution procedure based on a global optimization algorithm [8,22,23]. Instead of analyzing individual decay curves recorded at one wavelength, all decay curves recorded at different wavelengths are analysed simultaneously in a single run. The algorithm is based on the assumption that the decay constant (lifetime) of a particular decay component should be independent of wavelength, while the pre-exponential factors vary. The fluorescence decay is described by a sum of exponential functions. The quality of the fits was judged by a global $(chi)^2$ value, individual $(chi)^2$ values, and plots of the weighted residuals. The iteration procedure applied in our program is a semi-linear Marquardt algorithm [25]. The feasibility and reliability of the four-exponential analysis has been tested on a number of simulated data sets with three and four decay components, which mimicked the experimental time-resolved spectra. Poissonian noise had been added to these simulated decay data. These tests confirmed the expected drastic improvement of the global analysis as compared to the conventional single-decay analysis. In all these simulations the theoretical parameters (amplitudes and lifetimes) were recovered very closely, which makes us confident that the present analysis is both reliable and accurate.

Results

Representative results of picosecond absorption recovery experiments at different wavelengths, using different polarization of the excitation light, are illustrated in Figs. 2–6. The isotropic absorption recovery signal of isolated 18-S particles recorded at 600 nm is shown in Fig. 2A. In Fig. 2B we present a fit of these data to a sum of three exponentials. A good fit is obtained with the lifetimes, 15, 54 and 1800 ps, respectively. The longest lifetime contributes only 5% of the total intensity. The intensity ratio, R_1/R_2, of the two fast components is about 1.2. In Fig. 3A we show the absorption recovery data at 600 nm with the polarization of the excitation light parallel or perpendicular to the polarization of the analyzing light. A plot of the anisotropy, $r(t)$, calculated from these data is shown in Fig. 3B. A very fast decay of the anisotropy occurs, with a lifetime of about 10 ps, until a steady-state value of 0.29 is reached after about 50 ps. We could not observe any further decay of the anisotropy at later times within the experimental error. It should also be noted that from the data in Fig. 3B we get $r(0) \approx 0.40$ within the experimental error, as expected when there is no additional anisotropy decay with a lifetime much shorter than 10 ps. Results from pump-probe experiments at 590 nm gave lifetimes similar to those at 600 nm, i.e., 19, 40 and 1800 ps. However, the ratio R_1/R_2 decreases significantly from about 1.2 to 0.25, while R_3 remains small (6%) (Fig. 4).

Fig. 5 shows the absorption recovery results at 653 nm, where about 60% of the excited chromophores are located in the allophycocyanin core as

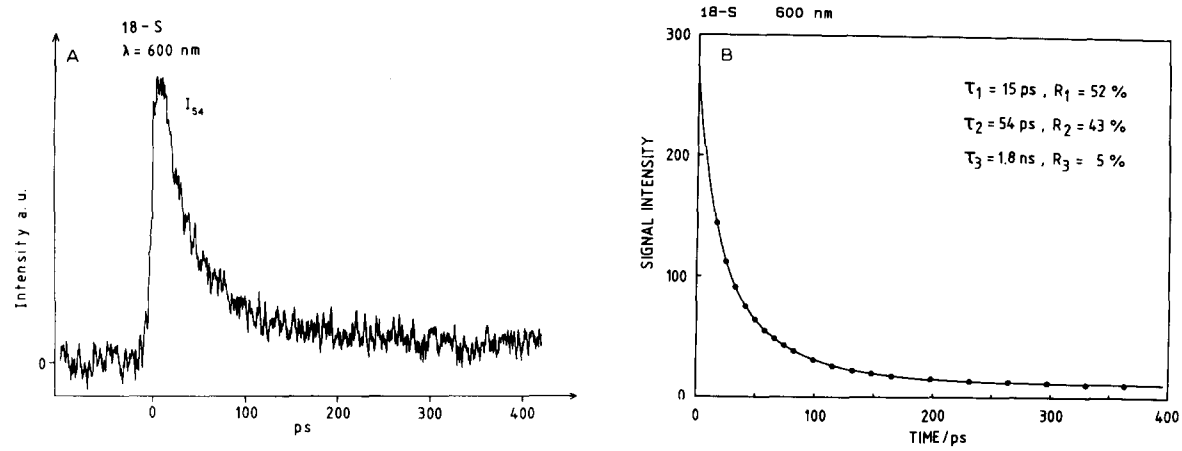

Fig. 2. (A) Pump-probe experiment on 18-S particles at 600 nm using magic angle (54.7°) polarization between the excitation and probe light pulses. (B) The theoretical fit (solid line) of a sume of three exponential functions to the experimental data from Fig. 2A (open circles). The lifetimes and amplitudes for the best fit are shown in the figure.

TABLE I

ISOTROPIC AND ANISOTROPIC DECAY LIFETIMES FOR 18-S PARTICLES IN DIFFERENT WAVELENGTH REGIONS

λ (nm)	τ_1 (ps)	τ_2 (ps)	τ_3 (ns)	τ_r (ps)	$r(\infty)$
590–600	17±2	55±5	~1.8	13±3	0.28±0.04
647–600	15±3	65±9	1.6±0.2	10±2	0.09±0.02

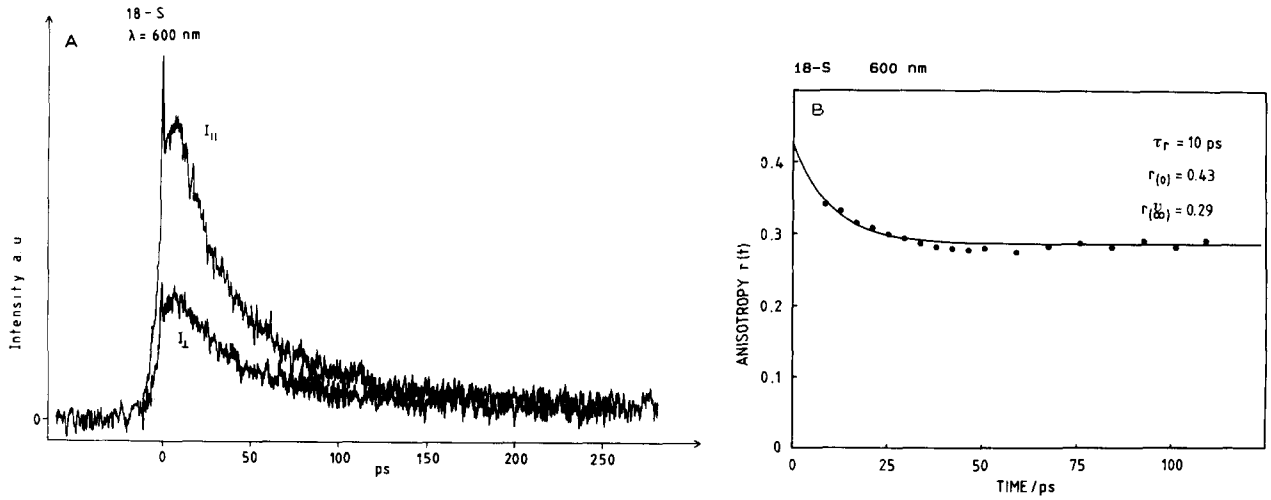

Fig. 3. (A) Pump-probe experiments on 18-S with parallel and perpendicular polarization of excitation light at 600 nm. (B) The anisotropy, $r(t)$, calculated from the traces of Fig. 3A. The best fit to the sum of an exponential function and a constant term $r(\infty)$) is also shown.

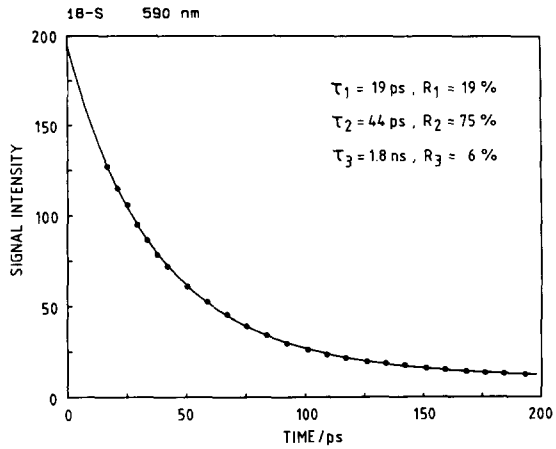

Fig. 4. The data are from a pump-probe measurement on 18-S particles at 590 nm. The solid line represents the best fit of three exponentials to the experimental points. The lifetimes and amplitudes are shown.

calculated by the absorption ratios of phycobilisome and C-phycocyanin [12] (see also Fig. 1A). In Fig. 5B the best fit to a sum of three exponentials of the kinetic data is presented. We find that a long lifetime of about 1.7 ns contributes 12% to the isotropic signal. The remaining fast signal can be resolved into two components with the lifetimes 17 and 73 ps, respectively, and with the intensity ratio $R_1/R_2 = 1.4$. The anisotropy decay at 653 nm and a fit to two exponentials are displayed in Fig. 6. We observe a very fast (about 8 ps) decay of the initial anisotropy to a constant value of about 0.07 at times longer than 50 ps. Mean values of the isotropic and anisotropic lifetimes together with $r(\infty)$, observed in the wavelength intervals 590–600 nm and 647–660 nm, respectively, are shown in Table I.

Results of the time-resolved fluorescence measurements from the 18-S particles at 595 nm are shown in Figs. 7 and 8. In Fig. 7 the fluorescence kinetics at 595 nm with excitation at 580 nm are shown. In a single decay analysis the fluorescence decay curve could be fitted well ($(chi)^2 = 1.047$) to a sum of three exponentials. The lifetimes were 45 ps (94%), 0.67 ns (2%) and 1.70 ns (4%). When the emission wavelength was shifted to 675 nm, while keeping the excitation wavelength at 580 nm, the result shown in Fig. 8 was obtained. In this case, a fast initial risetime of 45 ps was observed followed by a slow decay. The latter was resolved into two components, with lifetimes 2.71 ns (3%) and 1.85 ns (97%). In this case, the residual plot ($(chi)^2 = 1.135$) for a three component fit shows significant deviations near the maximum of the exciting pulse, which indicates a more complex kinetics (see global analysis).

When a global analysis [8] of the fluorescence kinetics at several different excitation and emission wavelengths was performed, the fast 40 ps kinetic component in Figs. 7 and 8 was resolved

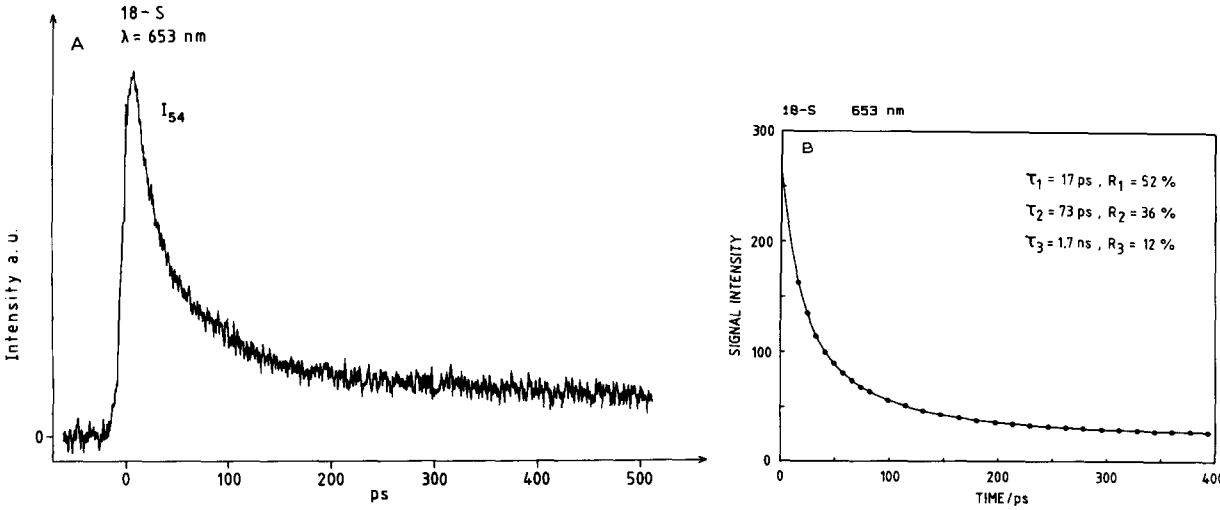

Fig. 5. The absorption recovery of 18-S particles at 653 nm. (B) A theoretical fit of data from (A) to a sum of three exponentials. Lifetimes and amplitudes are displayed in the figure.

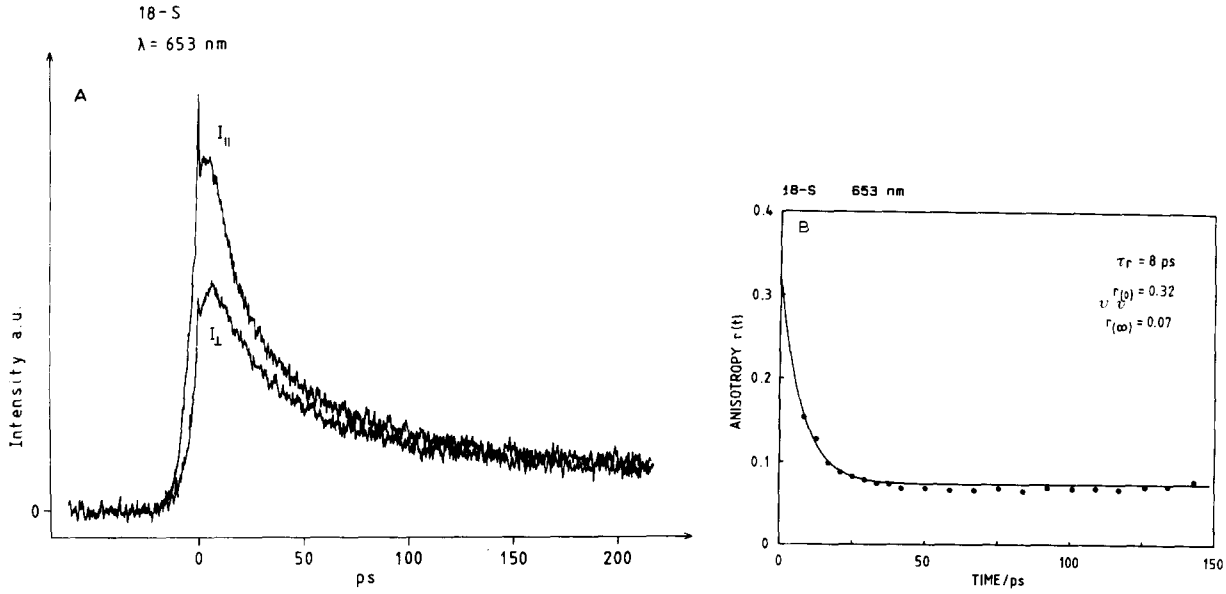

Fig. 6. Pump-probe on 18-S measurements with parallel and perpendicular polarization of the excitation light at 653 nm. (B) A fit of anisotropy calculated from the data of (A) to the sum of an exponential function and a constant term.

TABLE II

LIFETIMES (τ_i, ps) AND RELATIVE AMPLITUDES (A_i, %) OF THE FLUORESCENCE OF 18-S PARTICLES AS A FUNCTION OF WAVELENGTH

The data were calculated using a global analysis (see text for further information). The errors for the short lifetimes are ± 5 ps, those of the long timetimes ± 5%.

λ_{exc} (nm)	λ_{em} (nm)	A_1	A_2	A_3	A_4	τ_1	τ_2	τ_3	τ_4
580	595	68	28	2					
580 [a]	640	−1.0	0.49	0.06	0.25				
600	615	48	38	6	8	22	60	1045	1810
600 [a]	640	−1.0	3.9	0.9	4.3				
620 [a]	640	−1.0	1.2	0.25	1.3				
655 [a]	675	−1.0	−0.18	0.12	2.9				

[a] Absolute amplitudes normalized to a value of −1.0 for the negative term; a negative amplitude denotes a rise term.

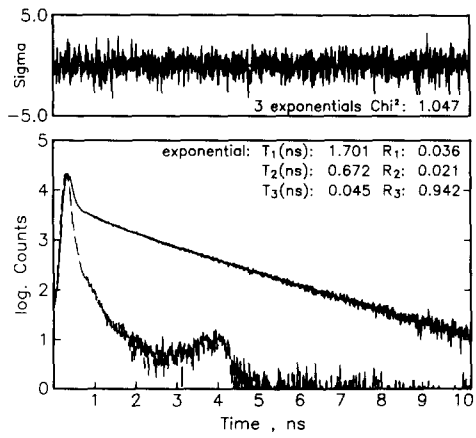

Fig. 7. Single photon counting experiment on 18-S particles with excitation at 580 nm and emission at 595 nm.

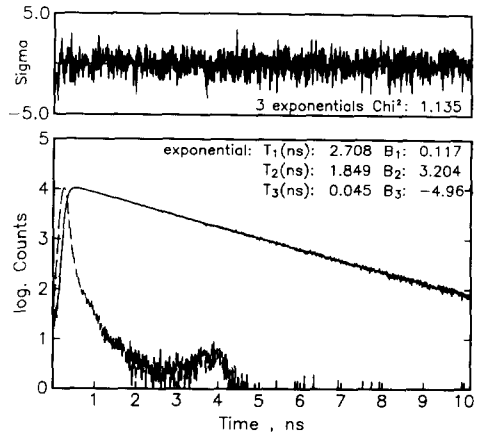

Fig. 8. Time-resolved fluorescence at 675 nm of 18-S particles excited at 580 nm.

into two components with the lifetimes 22 and 60 ps, respectively. These can be seen as decays or risetimes, depending on the detection and excitation wavelength. Results of this analysis are given in Table II. These lifetimes obtained from the global analysis are, within the experimental errors, in good agreement with the picosecond absorption data.

Discussion

Phycocyanin energy transfer kinetics

The monomeric unit of C-phycocyanin is composed of two subunits, α and β, containing one and two C-phycocyanin chromophores, respectively. In *Mastigocladus laminosus*, where the most detailed spectral study has been made so far, the absorption maximum of the α chromophore is at about 618 nm [26], while the β subunit absorbs at 606 nm [26]. The shape and absorption maximum of the individual chromophores in the β-subunit are not unambiguously known at present, but it is likely that β-155 absorbs at about 594 nm, while β-84 absorbs at about 625 nm [26]. Scheer et al. [27] have obtained similar results (see Refs. 29 and 30 for notations). The C-phycocyanin trimer with the 27 kDa linker polypeptide is situated closest to the core in phycobilisome of *Synechococcus* 6301 and has an absorption maximum at about 640 nm [19].

The chromophores of the phycobiliproteins are (following the original suggestion of Dale and Teale [28]) usually divided into sensitizing (s) and fluorescing (f) chromophores. It has been suggested that four the C-phycocyanin β-subunit s = β-155 and f = β-84 [26,27]. The allophycocyanin trimer has maximum absorption at 650 nm [12], while the other trimer forming the core of the 18-S particle, which contains the 75 kDa polypeptide, absorbs at 655 nm [12]. Both trimer spectra have broad shoulders around 600 nm.

The absorption recovery experiments on 18-S particles at 600 nm (Fig. 2A) show a double-exponential decay with the lifetimes 15 and 54 ps. The fast lifetime is clearly longer than the fastest lifetime of about 10 ps observed in phycobilisomes of *Synechococcus* 6301 and the mutant AN 112 at similar wavelengths [4,5]. This result implies that the energy transfer, s $\rightarrow$ f, in the trimeric C-phycocyanin units in the 18-S particle is slower than in the hexameric C-phycocyanin units of the phycobilisomes. This finding reflects the larger number of efficient acceptors available for an s-chromophore in a hexameric aggregate as compared to a trimeric one. In a recent time-resolved fluorescence study of different aggregates of C-phycocyanin from *Synechococcus* 6301 it was shown that the lifetime of the fast energy transfer decreased from 35 ps in the trimer to 10 ps in the hexamer [8]. It has also recently been shown [16,17] that there are two fast energy transfer processes in trimers of *M. laminosus*, with lifetimes of 22–27 and 100–120 ps, respectively. If the lifetimes are similar in C-phycocyanin trimers of *Synechococcus* 6301, as indicated by the work of Holzwarth et al. [8], it is clear that the 17 ps energy transfer process in 18-S must also involve energy transfer processes to chromophores in the core. These compete efficiently with the energy transfer processes within a trimer. Consequently, in such a case there is time only for a partial depolarization to occur within a C-phycocyanin trimer of 18-S before the excitation energy is transferred to the core. The large anisotropy ($r(\infty) = 0.28$) at $t > 50$ ps observed at about 600 nm in the 18-S particles see Fig. 3 and Table I) is a strong support for such an interpretation. In the phycobilisomes which have long lifetimes this value is considerable lower, i.e., $r(\infty) =$ 0.08 [4,5]. Recent time-resolved and steady-state polarization studies on C-phycocyanin trimers of *M. laminosus* also gave anisotropies in the range 0.07–0.14 [17,26]. In monomers of C-phycocyanin, on the other hand, we have found [17] that $r(\infty)$ $= 0.29$, which is surprisingly similar to the 18-S anisotropy. As mentioned above, a likely explanation for the relatively high anisotropy in 18-S particles as compared to C-phycocyanin aggregates is a competing transfer from some of the excited C-phycocyanin chromophores to the core before a complete redistribution of the excitation energy betwen all of the C-phycocyanin chromophores within a trimer is achieved.

A clue to what chromophores might take part in this 17 ps energy transfer is obtained by studying the amplitude variation of this signal as a function of excitation wavelength. We note that the amplitude of the 17 ps component decreases dramatically as we go from 600 to 590 nm (cf.

Figs. 2 and 4). This might indicate that it is not the β-155 chromophores that contribute to the fast transfer to the core, but rather the β-84 and/or the α-84 chromophores which absorb at longer wavelengths, i.e., at about 620 nm.

We finally would like to discuss the kinetic data on the basis of the probable structure of 18-S [12,13] (for a schematic illustration see Fig. 9) and the structural data available from X-ray crystallography [29,30]. A likely molecular model for our results is the following. The β-155 chromophores absorbing at 594 nm [26] are preferentially excited at 590 nm. Since the β-155 chromophores are situated on the periphery of the trimer [29,30], the energy transfer from β-155 to the allophycocyanin core is most probably mediated by the β-84 and/or α-84 chromophores. Therefore, excitation of β-155 is expected to give rise to a longer energy transfer time to the core than direct excitation of α-84 or β-84, if these chromophores couple more strongly to the core as suggested by the anisotropy data. Our data thus indicate that the α-84 and/or β-84 chromophores are in close contact with the core, in contrast to the β-155 chromophore. As mentioned above, experiments on free C-phycocyanin trimers have shown that the shortest energy transfer lifetime is 22–27 ps [16,17]. The fast component in the transfer to the core of 15–20 ps thus competes efficiently with this process. From structural data [29,30], it also follows that β-84, with its central position in the trimer, is the most likely candidate to mediate energy transfer to the core (see Fig. 9).

Energy transfer within the allophycocyanin core

The fluorescence spectrum of 18-S particles (Fig. 1), with a maximum at 680 nm independent of the excitation wavelength, is very similar to those of intact phycobilisomes of *Synechococcus* 6301 and AN 112 [4,5]. This shows that the energy transfer from C-phycocyanin and allophycocyanin to the final emitter is very efficient also in the 18-S particles, and that the final emitter might be the same in the two systems.

In earlier studies on phycobilisomes of *Synechococcus* 6301 and AN 112, Gillbro et al. [4,5] found that the kinetics of energy transfer within the allophycocyanin core was the same in both systems. This result was expected, since the cores

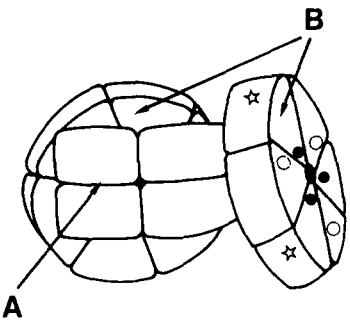

Fig. 9. A schematic drawing of an 18-S particle (after Glazer et al. [12,13]). The core fragment consisting of two allophycocyanin trimers (A) and the C-phycocyanin trimers (B) are indicated. The positions of the C-phycocyanin chromophores are just approximate. α-84 ○, β-84 ●, β-155 *.

are identical in these two phycobilisomes [17]. In 18-S, however, only a fragment, i.e., two trimeric discs, of the phycobilisome core remains. This fragment also contains the coloured 75 kDa linker polypeptide but not the allophycocyanin-B chromophore [12].

When the allophycocyanin-chromophores are excited close to their absorption maxima at about 650 nm we observe complex absorption recovery kinetics (see Fig. 5 and Table I). This can be resolved into three components with the lifetimes 15 ps, 65 ps and 1.6 ns, respectively. We interpreted these components as follows. The fast phase is believed to be due to a transfer between the allophycocyanin trimers, the intermediate lifetime is attributed to the overall energy transfer from allophycocyanin to the 75 kDa polypeptide and the long lifetime is due to the fluorescence decay of the excited 75 kDa chromophore. The fast phase is also accompanied by a relaxation of the anisotropy to a constant value of about 0.09 at $t > 50$ ps. The relaxation of the anisotropy seems to be faster, i.e., about 10 ps, than the isotropic signal ($\tau \approx 15$ ps). We believe that this difference is larger than the error limits of the experiments. This indicates that there is a fast transfer among allophycocyanin chromophores with identical spectra but with different orientations. A transfer between such chromophores will contribute only to the anisotropy relaxation. In a recent study, Yeh et al. [32] measured the steady-state polarization on allophycocyanin trimers of *Anabaena variabilis*. They found a polarization value (p) of

about 0.08 (or $r = 0.06$) independent of excitation wavelength. This is in good agreement with the constant value of the anisotropy obtained by us after the initial fast relaxation. Comparing the 18-S results with data on AN 112 phycobilisomes [5] we see that the results are similar for the fast process. This implies that the double disc of 18-S is a functional unit with respect to the fast energy transfer process.

The kinetic component of 65 ± 10 ps is, within the error limits, similar to the corresponding component in AN 112 phycobilisomes which was found to be 51 ± 5 ps in absorption and 50 ± 9 in emission. We assign this component to the transfer of excitation energy from the allophycocyanin antenna to the final emitter. If this is correct, it appears that this transfer rate is not limited by the size of the allophycocyanin core, but rather by a rate-determining step between some allophycocyanin chromophore and the 75 kDa polypeptide. Judging from the similarity of the lifetimes found in phycobilisomes and 18-S for this energy transfer, it is reasonable to suggest that also in phycobilisomes the rate-limiting transfer is between allophycocyanin and the 75 kDa polypeptide. The fluorescence data also are in agreement with this interpretation, since the risetime of the 680 nm emission upon excitation at about 660 nm was about the same for AN 112 and 18-S [5], i.e., about 50 ps. Also the global analysis performed on several of the fluorescence traces (see Table II) shows that there occurs a 60 ps component also in the emission risetime of 18-S particles at 675 nm.

The long lifetime seen in both the absorption recovery and the fluorescence measurements is assigned to the decay of the final emitter 75k. The most accurate value for this lifetime of 1.85 ± 0.10 ns is obtained in fluorescence (Fig. 8). This similarity of this lifetime to that found in intact phycobilisome [4-6] provides strong support, although perhaps not conclusive evidence for the idea, that the 75 kDa polypeptide is the final emitter also in intact phycobilisomes.

Finally, we wish to comment on the low amplitude fluorescence component, which has a lifetime of about 0.65 ns at short wavelengths and increases to about 1.5 ns at longer emission wavelengths. In the the global analysis a value of about 1.0 ns appears for this component. The most likely explanation is that the 18-S preparations contain a small amount of three C-polycyanin and/or allophycocyanin trimers or monomers. It is known that such aggregates have fluorescence lifetimes in the range 0.6–2.7 ns [8,16,17].

Conclusions

We have shown by fluorescence and absorption recovery experiments that the 18-S particle of AN 112 is a functional entity of the phycobilisome in terms of the energy transfer kinetics. By comparing the transfer kinetic in 18-S particles with intact phycobilisomes of *Synechococcus* 6301 and AN 112 several conclusions regarding the paths of energy transfer can be drawn.

Our data shown that the rate-limiting step in the energy transfer from the rods to the core occurs between the C-phycocyanin trimer next to the core and the allophycocyanin chromophores, in agreement with recent model calculations [9]. A tentative interpretation assigns the 15–20 ps lifetime to the energy transfer to the core from directly excited β-84 or α-84 chromophores. For structural reasons, β-84 should be situated close to the binding site (or surface) connecting the rods with the core. The 50–60 ps lifetime is tentatively attributed to the transfer from the distant β-155 chromophore to the core, most probably mediated by other C-phycocyanin chromophores. Part of this component might also derive from an equilibrated energy distribution between the rods and the core, which then decays simultaneously through energy transfer to the final 75k emitter. Such a fast equilibration of excitation energy between different connected pigment pools has recently been observed in purple bacterial systems [31]. Since in 18-S we do not observe the 10 ps lifetime of AN 112 phycobilisomes, it is concluded that hexameric C-phycocyanin units are required for this fast energy transfer component. This is in full agreement with studies of the kinetics within isolated C-phycocyanin aggregates [8,16,17]. The energy transfer within the core fragment of 18-S particles and the intact core in phycobilisomes follows similar paths. This shows that also for allophycocyanin the smallest functional units consists of two coupled trimers. It is also strongly suggested that the coloured 75k linker polypeptide

is the final emitter also in intact phycobilisomes of *Synechococcus* 6301 and AN 112.

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