but not in uninfected or HSV-infected fibroblasts. Probes made from HSV genome (J. Nelson, unpublished results) hybridized to HSV-infected fibroblasts but did not bind HCMV-infected fibroblasts.

23. Seropositivity was determined by an enzyme-linked immunosorbent assay (ELISA), with desiccated infected fibroblasts used as the antigen (*30*). Serum that gave twice the response (as measured in optical density units) on HCMV-infected versus uninfected fibroblasts was defined as seropositive for CMV. T-cell proliferation to HCMV (2 background) after 5 days of culture correlated with results from the ELISA.
24. In situ hybridization was done according to modified methods of Brahic and Haase (*31*), Angerer (*32*), and Brigatti (*33*). Human peripheral blood mononuclear cells were obtained from normal seropositive and seronegative donors and isolated on Ficoll-Hypaque gradients. Cells were washed three times in phosphate-buffered saline (PBS), adjusted to 10×10^6 cell/ml and plated on glass slides coated with 0.01 percent polylysine. Air-dried cells on slides were fixed for 20 minutes with periodatelysine paraformaldehyde (PLP) (*34*) and for 15 minutes in 95 percent ethanol, and then were stored at 4°C. Briefly, fixed cells were hydrated through washing with graded ratios of ethanol to water, treated with 0.2*M* HCl (10 minutes), washed and permeabilized with 1 percent Triton X-100 for 1.5 minutes, postfixed with PLP for 4 minutes, washed in PBS-glycine (2 g/liter), then in PBS, and dehydrated to 100 percent ethanol. The hybridization mix consisted of 50 percent deionized formamide, 5× Denhardt's solution, 5× hybridization salts (0.9*M* NaCl, 50 m*M* NaH_2PO_4, and 5 m*M* EDTA), 10 percent Dextran sulfate, heparin (30 U/ml), salmon sperm DNA (500 μg/ml), and BHK cell RNA (250 μg/ml). ^{35}S-labeled probes were added to a concentration of 0.25 to 0.5 μg/ml (about 1×10^8 cpm/μg); the entire mixture was boiled for 2 minutes, then chilled on ice; and dithiothreitol was added to a concentration of 10 m*M*. The solution was placed on the cells, covered with sheets of Gel-bond (FMC Corporation, Rockland, Maine), and sealed with rubber cement. Hybridization was carried out for 18 hours at 37°C in a humidified chamber. Cover slips were then removed and slides were washed with 2× SSC (0.3*M* sodium chloride, 0.03*M* sodium citrate) for 30 minutes at 21°C, 30 minutes with 0.1× SSC at 21°C, 10 minutes with 0.1× SSC at 60°C, 20 minutes with 0.1× SSC at 37°C, and 5 minutes with 2× SSC, then dehydrated through ethanol and dried. Slides were dipped in Kodak NTB-2 emulsion, exposed for 3 to 4 days at 4°C, then developed with Kodak D19 and standard fixer.
25. Immunofluorescence for FACS sorting of T-cell populations was done as follows. Washed PBM's (2×10^7) were incubated for 40 minutes on ice in either biotinylated antibody to Leu3 (OKT4) or Leu2 (OKT8) diluted 1:10. After two washings in cold PBS, cells were incubated for 30 minutes on ice with fluorescein isothiocyanate-coupled Avidin at a dilution of 1:50 and washed and resuspended in 3 ml of PBS for sorting. All reagents were obtained from Beckton-Dickinson. Windows on the FACS (*35*) were set to select only for the very brightly staining cells.
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36. This is publication 3876-IMM from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037. Supported in part by PHS grants AI-07007, AI-21640, and CA-35048; by American Cancer Society Junior Faculty Award JFRA-115 (to J.A.N.); NIH postdoctoral fellowship 5T32GM07437 (to R.D.S.). We thank Peggy Farness and Stephen Francis for their cooperation, Clayton Wiley and Jim McDougall for consultation, and Gay Wilkins for secretarial assistance.

5 July 1985; accepted 17 October 1985

Kinetics of Energy Flow in the Phycobilisome Core

Abstract. *Light energy absorbed by the 576 bilin chromophores in the six rods of the phycobilisome of the cyanobacterium* Synechocystis *6701 is funneled into a 1.5 × 10^6 dalton core. The 72 bilins of the core function as a single unit with respect to the rate-limiting processes for energy flow within these particles.*

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In cyanobacteria and red algae, light spanning nearly the entire visible spectrum (450 to 650 nm) is used efficiently in photosynthesis. This light harvesting is performed by a family of intensely colored proteins, biliproteins, organized within macromolecular complexes called phycobilisomes (*1*). Detailed information is available on the structure of two cyanobacterial phycobilisomes (*2–5*). A schematic representation of the phycobilisome of *Synechocystis* 6701 is shown in Fig. 1. These phycobilisomes have molecular weights of $\sim 7 \times 10^6$ daltons and contain ~625 bilin chromophores. Energy absorbed by any one of these chromophores is transferred to the terminal acceptors within the phycobilisome (Fig. 1) with an efficiency >97 percent with respect to the fluorescence of isolated phycobiliproteins (*6*).

In an earlier study (*6*), we examined the kinetics of energy flow within the *Synechocystis* 6701 phycobilisome and showed that disk-to-disk transfer was the rate-limiting step for energy flow. Under the assumption that the phycoerythrin-to-phycoerythrin and phycoerythrin-to-phycocyanin transfer times are equal, the average disk-to-disk transfer time in wild-type phycobilisomes (Fig. 1A) was calculated to be 24 ± 4 psec (mean ± standard error of the mean). For 620-nm excitation of phycocyanin in mutant strain CM25 phycobilisomes (Fig. 1B), the rise time of terminal acceptor emission at 680 nm was 25 ± 4 psec (*6*). This rise time includes delays arising from disk-to-disk transfer from phycocyanin to phycocyanin and from phycocyanin to allophycocyanin, as well as from any energy transfer within the core. We predict on the basis of our previous results (*6*) that, if the phycocyanin-to-phycocyanin energy transfer time in the rods and the phycocyanin-to-allophycocyanin transfer time between the rods and the core are similar to the average phycoerythrin-to-phycoerythrin and phycoerythrin-to-phycocyanin disk-to-disk transfer time calculated in our previous study, then the transfer of energy from allophycocyanin to the terminal acceptors within the core must take place in <10 psec (*7*). This is a remarkably rapid transfer rate since the core contains 68 allophycocyanin chromophores and only four terminal acceptor chromophores, two on α^{APB} polypeptides and two on L_{CM}^{99} polypeptides (*3*).

We describe here experiments that test our prediction. This test was made possible by the isolation of highly purified phycobilisome cores (*8*) from a recently described mutant of *Synechocystis* 6701, strain UV16 (*5*). The purified cores were examined by electron microscopy and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as well as by steady-state absorption and emission spectroscopy. Electron microscopy of the purified core preparation (Fig. 2) showed only particles virtually devoid of rod components with the morphology of typical *Synechocystis* 6701 wild-type phycobilisome cores (*4, 5, 9*).

A comparison of the polypeptide patterns of wild-type and strain CM25 phycobilisomes, and of purified cores of strain UV16, was obtained by means of SDS-PAGE. Densitometric quantitation of such gels showed that the purified cores contained all of the core polypeptides present in wild-type phycobilisomes, except that the level of L_{RC}^{27} (see Fig. 1A) was ~20 percent lower in the purified cores than in the wild-type or CM25 particles. Since a considerable portion of this polypeptide lies outside the core and is normally protected by the proximal phycocyanin, it is possible that the decrease in the amount of this polypeptide is due to proteolytic cleavage in strain UV16, either within the cells or during preparation.

The absorption spectrum of the purified cores has a ratio of the absorbance at 650 nm to the absorbance at 620 nm of 2.16 (*8*). This value is consistent with the presence of no more than the equivalent of one trimer of phycocyanin in these particles (*10*). The maxima of the steady-state emission spectra of purified cores and of CM25 phycobilisomes, obtained with 580-nm excitation, were coincident.

As in our earlier study (*6*), we used a tunable picosecond, optical parametric source (*11*) to excite the samples. The resulting emission was time-resolved with an ultrafast streak camera (*6*). We isolated the emission from the terminal acceptors by detecting only the emission that was passed by a 10-nm (full-width at half-maximum) interference filter centered at 680 nm. This wavelength region corresponds to the peak of the emission from the terminal acceptors.

Figure 3A (thin trace) shows the time-resolved emission at 680 nm from purified UV16 cores after excitation with 620-nm pulses. On the basis of the known chromophore counts and the molar absorption spectra of the complexes that make up the purified cores (*10*), >95 percent of the absorbance at 620 nm is due to allophycocyanin. Direct absorption by the terminal acceptor complexes accounts for the balance. The time-resolved emission shown in Fig. 3A (thin trace) represents the sum of the signal from 3005 individual laser shots. The smooth curve through the data is a computer-generated curve obtained through an iterative, convolute-and-compare fitting technique (*12*). For the purified UV16 cores, an average, best-fit, single-exponential rise time of 6.6 ± 3.6 psec is obtained. For the excitation pulse widths (9.7 ± 0.5 psec) used in this study, the picosecond apparatus had an instrument-limited rise time of ~6 psec; thus energy transfer within the cores occurs on a time scale faster than can be resolved with the present instrumentation. This assertion is confirmed by the time-resolved emission profile of 680-nm emission from a solution of nile blue laser dye in methanol (Fig. 3B). The rise time of the emission from such a solution is expected to be <<1 psec (*13*). The nonzero rise time of the emission from the nile blue solution measured here (average, best-fit, single-exponential rise time of 6.1 ± 3.2 psec) is thus indicative of the maximum temporal resolution available with the present apparatus.

Also shown in Fig. 3A (thick trace) is the time-resolved, 680-nm emission profile obtained from mutant strain CM25 phycobilisomes upon 620-nm excitation. On the basis of the known chromophore distribution and molar absorbance spectra (*3*), we calculated that irradiation of CM25 phycobilisomes with 620-nm light would result in an initial distribution of excitations that is localized predominantly in the rod substructures, with 83 percent of the light absorbed by phycocyanin complexes in the rods and 17 percent absorbed by allophycocyanin complexes in the core. The average, best-fit, single-exponential rise time for the CM25 emission profile is 25 ± 4 psec, markedly slower than the rise time of the UV16 emission profile (Fig. 3A).

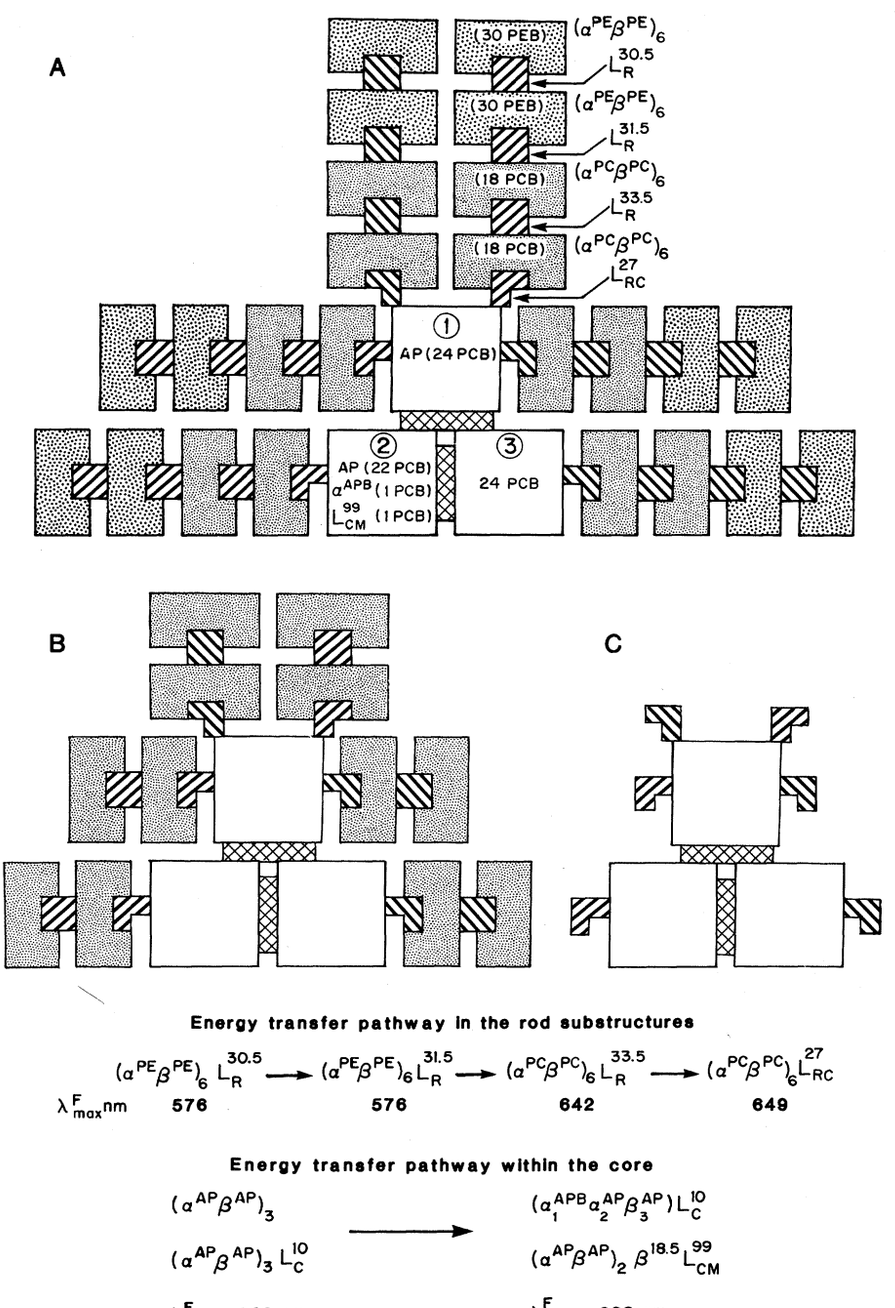

Fig. 1. Schematic representation of the structure of the wild-type phycobilisome from the cyanobacterium *Synechocystis* 6701 (A) and of incomplete phycobilisomes from the mutant strains CM25 (B) and UV16 (C). Abbreviations: PEB, phycoerythrobilin; PCB, phycocyanobilin (the number of bilins present in each domain of the structure is shown in parentheses); α^{PE} and β^{PE}, α and β subunits of phycoerythrin; α^{PC} and β^{PC}, α and β subunits of phycocyanin; AP, allophycocyanin; α^{APB}, α subunit of allophycocyanin B; L denotes a linker polypeptide, and the subscript indicates its location (R, rod; RC, rod-core junction; C, core; CM, core-membrane junction); the superscript is its molecular weight × 10^{-3} (*1, 3*); λ^F_{max}, wavelength of the emission maximum.

These results provide new insights into the details of energy transfer in phycobilisomes. The cores can be viewed as consisting of two distinct energy transfer domains; the upper core cylinder in Fig. 1 consists solely of allophycocyanin complexes, whereas each of the two lower, identical core cylinders contains one terminal acceptor chromophore per set of five allophycocyanin-type chromophores [that is, each pair of trimeric disks in these cylinders contains either an α^{APB} or an L^{99}_{CM} polypeptide terminal acceptor (*3*)]. Given the nearly equal allophycocyanin chromophore counts in each of the three core cylinders, approximately one-third of the 620-nm excitation will be absorbed in each cylinder. Since all this excitation eventually arrives at the terminal acceptor, there must be at least two intracore transfer steps for energy that flows through the upper core cylinder.

It is clear that energy transfer from both the upper cylinder and the basal cylinders must be very rapid to account for the experimentally measured rise time of the 680-nm terminal acceptor emission from the UV16 cores. At present, only the combination of these two processes can be experimentally observed. However, a kinetic model (*7*) can be used to set limits on the possible values for the rates of the individual energy transfer steps. In particular, if the rate of energy transfer between the upper cylinder and a basal cylinder is assumed to be instantaneous, then the longest possible transfer time within the basal cylinder is <6 psec. Similarly, if the transfer time within the basal cylinders is assumed to be instantaneous, the longest possible transfer time out of the upper core cylinder is <11 psec. Earlier measurements suggest that the latter case is the most likely. The steady-state emission spectra of core complexes containing terminal acceptors are dominated by the 680-nm emission of the terminal acceptors, indicating very rapid energy transfer (*2*). Perhaps more important, transfer from the upper cylinder to the basal cylinder is similar to an interdisk transfer, whereas transfer within each basal cylinder is an intradisk transfer. We have demonstrated that intradisk transfers in R-phycoerythrin and allophycocyanin occur in <8 psec (*6*). It is therefore likely that energy transfer from the upper core cylinder to a basal cylinder is the rate-limiting step for energy flow within the phycobilisome core, and that the transfer time for this process is <11 psec.

There is a major difference between the organization of the core and rod substructures. In the core, the disk elements of the three contiguous cylinders are in edge-to-edge contact; in the rods, the disks are in face-to-face contact. The finding that interdisk transfer in the core is more rapid than in the rods leads to the conclusion that the arrangement of donor and acceptor chromophores in the core is more favorable for energy transfer than that in the rods. Indeed, these results show that the 72 bilin chromophores of the 1.5×10^6-dalton core function as a single unit with respect to the rate-limiting processes for energy flow in phycobilisomes.

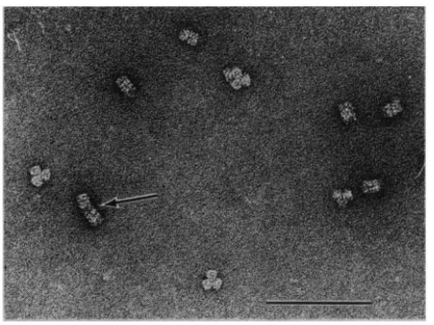

Fig. 2. Electron micrograph of purified cores from *Synechocystis* 6701 mutant strain UV16. The core particles are seen both in face view and edge view. In face view, all three core elements are clearly discernible. The arrow points to two core particles in end-to-end contact with each other seen in edge view; uranyl formate negative stain; bar length, 100 nm.

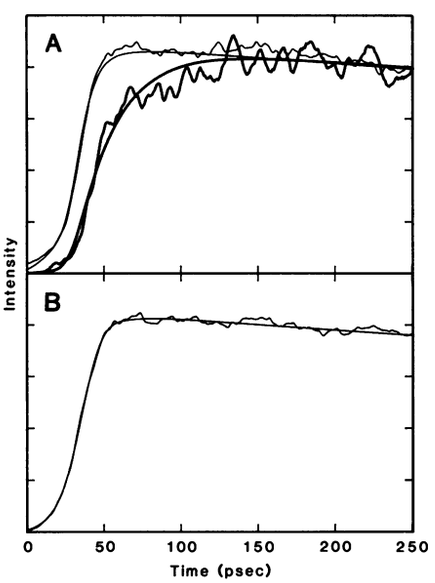

Fig. 3. Time-resolved emission profiles. (A) Temporal profiles of 680-nm emission from UV16 cores (thin trace) and CM25 phycobilisomes (thick trace). The smooth curves are computer-generated fits to the data. (B) Temporal profile of 680-nm emission from nile blue in methanol. The excitation wavelength in all cases was 620 nm.

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8. Cells of strain UV16, cultured in medium BG11 [R. Y. Stanier, R. Kunisawa, M. Mandel, G. Cohen-Bazire, *Bacteriol. Rev.* **35**, 171 (1971)], were thoroughly suspended in 0.65*M* NaH_2PO_4-K_2HPO_4 buffer, 1 m*M* disodium EDTA, and 1 m*M* phenylmethanesulfonyl fluoride at *p*H 7.0 (referred to below as the "*p*H 7 buffer") at 0.1 g (wet weight) per milliliter and were passed twice through a French pressure cell at 14,000 pounds per square inch, all at 4°C. Triton X-100 was added to the broken cell suspension to 2 percent (weight/volume); the suspension was stirred intermittently at 23°C for 30 minutes and then centrifuged at 27,000*g* at 18°C for 30 minutes. Aliquots (1.5 ml) of the supernatant were layered onto 12-ml linear gradients of sucrose (0.25*M* to 1.25*M*) in the *p*H 7 buffer, and the gradients were centrifuged in a Sorvall TST41.14 rotor at 86,200*g* at 18°C for 18 hours. An aquamarine band that sedimented into the 0.75*M* sucrose zone was collected. This fraction showed a 650-nm/620-nm absorbance ratio of 1.3. After dilution of this fraction with an equal volume of the *p*H 7 buffer, 1.5-ml aliquots were applied to step gradients, each made up of 2.5, 2.5, and 5.0 ml of 1.0, 0.75, and 0.50*M* sucrose, respectively, in the *p*H 7 buffer, and centrifuged as described above. A well-defined band of purified cores was collected from the 0.50*M* to 0.75*M* sucrose interface. The allophycocyanin concentration of the purified core fraction was 0.07 ± 0.02 mg/ml, and the 650-nm/620-nm absorbance ratio was 2.16 ± 0.10 (mean ± standard error of the mean) (average of five preparations).
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14. Work in the Department of Microbiology and Immunology and in the Department of Molecular Biology was supported by NSF grants PCM 82-08158 and PCM 84-01519, respectively. Work in the Laboratory of Chemical Biodynamics and the Department of Chemistry was supported by the Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the Department of Energy under contract DE-AC03-76SF00098. S.W.Y. is a Procter and Gamble fellow. J.H.C. is an Alfred P. Sloan research fellow and a Henry and Camille Dreyfus teacher-scholar. We are grateful to L. K. Anderson and F. A. Eiserling for providing *Synechocystis* 6701 mutant UV16.

18 April 1985; accepted 28 August 1985