used by others. Another important difference is that these workers find the permeability changes to be *permanent* (all of the trapped substance is released) whereas we find the permeability change to be *transient*.

In conclusion, our results clearly show that light induces a transient change in the permeability of the disk membrane and thereby causes a definite release of part of the trapped calcium from the interior of the disk vesicles. The amount of calcium released is determined primarily by the amount of rhodopsin bleached and by the amount of the calcium within the disk vesicles at the time of bleaching. Similar releases were obtained for substances other than calcium, indicating that the permeability change is relatively nonspecific.

Acknowledgments

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Energy Transfer among the Chromophores in Phycocyanins Measured by Picosecond Kinetics†

T. Kobayashi, E. O. Degenkolb,‡ R. Bersohn,§ P. M. Rentzepis,* R. MacColl, and D. S. Berns

ABSTRACT: Energy-transfer processes in the algal light-harvesting proteins, the phycocyanins, have been studied by means of picosecond absorption spectroscopy. After excitation at 530 nm, the absorption at several wavelengths in the range 480–669 nm decayed with a short time constant (picosecond) and a long time constant (>1 ns). For *C*-phycocyanin, energy transfer from the β to the α subunits is interpreted as being a likely candidate for the short time constant; the long time constant probably is the excitation lifetime of the chromophore on the α subunits. The time constants for energy transfer in monomers, trimers, and hexamers of *C*-phycocyanin extracted from a blue-green alga, *Phormidium luridum*, were measured as ~85, ~56, and ~32 ps, respectively. The corresponding time constant in the cryptomonad phycocyanin 645 from *Chroomonas* species was found to be less than 8 ps.

Algae are assigned according to their color and morphology to 15 classes, e.g., cyanophyta (blue-green algae), rhodophyta (red algae), cryptophyta (cryptomonads), chlorophyta (green algae), etc. The first three classes are of special interest because in addition to chlorophylls and carotenoids, they contain an array of accessory pigments called phycobiliproteins whose function is to absorb visible light and transfer energy stepwise until it eventually reaches chlorophyll *a*. The accessory pigments, phycocyanins, allophycocyanins, and phycoerythrins, consist of tetrapyrrole chromophores covalently linked to a protein (Troxler, 1975; Bogorad, 1975). Direct observation of the energy-transfer process between two or more different kinds of phycobiliproteins on the time scale of several tens of hundreds of picoseconds has been studied recently by Porter et al. (1978) for intact algae and also for the isolated light-harvesting complex by Searle et al. (1978). Fluorescence picosecond spectroscopy was applied to the energy transfer between different kinds of phycobiliprotein in these studies.

† From Bell Laboratories, Murray Hill, New Jersey 07974 (T.K., E.O.D., R.B., and P.M.R.), and Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201 (R.M. and D.S.B.). *Received February 16, 1979.*
‡ Present address: GTE Laboratories, Consumer Products Division, Waltham, MA 02154.
§ Present address: Department of Chemistry, Columbia University, New York, NY 10027.

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However, the direct observation of the energy-transfer process among chromophores in the same phycobiliprotein has not been studied previously.

The focus of the present paper is on the phycocyanins. In particular, two phycocyanins are compared. One extracted from a blue-green alga is called *C*-phycocyanin (Troxler, 1975; Bogorad, 1975), and the other extracted from a cryptomonad is called phycocyanin 645 (MacColl et al., 1973). The location of the phycocyanin in the cell differs in the genetically more advanced eucaryotic cryptomonad from the location in the procaryotic blue-green algae (Gantt, 1975; Gantt et al., 1971). Moreover, the distributions of biliproteins and chlorophylls are very different in the two classes of algae, suggesting a very different electronic energy-transfer pathway (MacColl & Berns, 1978).

C-Phycocyanin has two subunits, an α chain (M_r 16 300) (Glazer et al., 1973; Bennett & Bogorad, 1971) and a β chain (M_r 17 600). The protein aggregates strongly, being found usually as a hexamer ($\alpha_6\beta_6$) but also as a trimer ($\alpha_3\beta_3$) and a monomer ($\alpha\beta$) depending on the medium. Phycocyanin 645 also has two subunits, α (M_r 9500) and β (M_r 17 700), but seems to occur only as the dimer ($\alpha_2\beta_2$) (MacColl et al., 1973).

Electron micrographic studies by several groups (Luftig & Haselkorn, 1967; Padan et al., 1967; Berns & Edwards, 1965) showed that *C*-phycocyanin aggregates to a hexamer which has a regular hexagonal structure. Fluorescence depolarization studies of *C*-phycocyanin (Goedheer, 1956; Goedheer & Birnie, 1965) offer an interesting insight on the advantage of the hexagonal configuration of *C*-phycocyanin for energy transfer. The depolarization of phycocyanin fluorescence is most efficient at pH values of 4.7–6.0, where the hexamer is the predominant structure. The almost complete depolarization of fluorescence must be a result of an internal inductive energy transfer since rotatory Brownian motion of such a large protein molecule would not allow complete fluorescence depolarization. The hexamer structure certainly would favor internal energy transfer between subunits and cause subsequent fluorescence from the almost randomly oriented chromophores. At pH 7.0 the depolarization decreases because the trimer becomes more predominant at this pH. An array of hexamers appears therefore to be a structure providing for efficient energy transfer to chlorophyll.

The fluorescence properties of biliproteins have been studied carefully by Teale & Dale (1970) and Dale & Teale (1970), who found that the polarization of the biliprotein fluorescence is complex with the polarization increasing at longer wavelengths. Because for a single fluorescing entity no change in polarization should occur throughout the first excited state, they proposed that two types of chromophores are present. These were designated "sensitizing" and "fluorescing". In the protein the s chromophores transfer excitation energy with high efficiency to the fluorescing f chromophores which adsorb at slightly longer wavelengths than the s chromophores. Presumably, the isolated s chromophore could fluorescence, but in the complete protein the rate of its energy loss by transfer is much greater than by radiation. In a nice confirmation of the Teale & Dale model, Glazer et al. (1973) found, after separating α and β subunits of *C*-phycocyanin, that the spectrum of the lighter α subunit was red-shifted from that of the heavier β subunit. Consequently the α and β subunits are assigned as the carriers of the f and s chromophores, respectively. Moreover, Glazer & Fang (1973) showed that two s chromophores are covalently attached to the β subunit and one f to α. Experiments on cryptomonad phycocyanin (MacColl et al., 1976) again showed that the α subunit for detergent-denatured solutions had its absorption spectrum red-shifted when compared to the β subunit.

Experimental Section

Picosecond Absorption Spectroscopy System. A single amplified picosecond pulse selected from a train of pulses generated by a mode-locked Nd^{3+}/glass laser was used for generating the excitation pulse at 530 nm in a KDP crystal and the probing picosecond continuum in a 10-cm water or D_2O cell. The amplification was achieved by a Nd^{3+}/YAG rod which decreased the bandwidth from ~100 to 3 cm^{-1} without increasing the pulse width of 6 ps (Huppert & Rentzepis, 1978). A benefit of this amplifying method is that the conversion efficiency from fundamental to the second harmonic was quite improved by the narrowing of the spectral width of the fundamental pulse. The beam diameter was ~3 mm. The time resolution was obtained by using three kinds of echelons. One has 14 segments separated by 6 ps, covering a 78-ps time region. Another has 11 segments separated by 20 ps, spanning a time region of 174 ps. A third one has 14 steps, the first four separated by 6 ps, two by 12 ps, two by 2 ps, two by 50 ps, two by 100 ps, and the last one by 6 ps. The total time span of this echelon is 394 ps. In each case great care was taken to make certain that a single pulse without any satellite pulses was used.

The determination of the time dependence of absorbance changes in transient and ground states was done by a dual-beam spectrometer described originally by Netzel & Rentzepis (1974). For a detailed description, see Rentzepis (1978). The fluorescence dependence on the excitation pulse energy was measured by using the same laser system and vidicon detector as in the absorption experiments. The excitation light was discriminated against by means of the appropriate filters, and the intensity of the excitation beam was changed by calibrated neutral density filters and measured by a calibrated energy meter.

Materials. *C*-Phycocyanin from the blue-green alga *Phormidium luridum* and phycocyanin 645 from cryptomonad *Chroomonas* species were purified and stored by procedures described earlier (MacColl et al., 1971, 1973). The criteria of purity were $A_{620}/A_{280} > 4.0$ for *C*-phycocyanin and $A_{645}/A_{280} > 5.5$ for phycocyanin 645. Sodium dodecyl sulfate gel electrophoresis experiments show proteins with these ratios to be homogeneous.

Lyophilized *C*-phycocyanin was dissolved in buffer solutions (0.1 ionic strength) of pH 3.9 (acetate), pH 5.5 (acetate), and pH 8.0 (phosphate) to obtain predominantly monomer, hexamer, and trimer forms of biliprotein, respectively. *C*-Phycocyanin exists as $(\alpha\beta)_n$ units with some common values of n being 1, 3, and 6. The percentage of each varies as a function of algal source, protein concentration, sample history, temperature, and solvent. Thus, analytical ultracentrifugation experiments (Model E), following previous protocols (MacColl et al., 1971), were performed with conditions and samples identical with those used in the picosecond experiments. The results were as follows: pH 3.9, 100% monomer; pH 8.0, ~85% trimer (remainder hexamer); and pH 5.5, ~88% hexamer (remainder trimer and monomer).

Solutions of phycocyanin 645 were prepared in pH 6.0, 0.1 ionic strength, sodium phosphate buffer, which gives a single dimer structure (MacColl et al., 1973). Ultracentrifuge experiments show that this protein is a single species (4.5 S) over a wide protein concentration range at pH 6.0.

Results

Dependence of Transient Absorption and Ground-State Depletion Kinetics on Excitation Energy. The absorbance

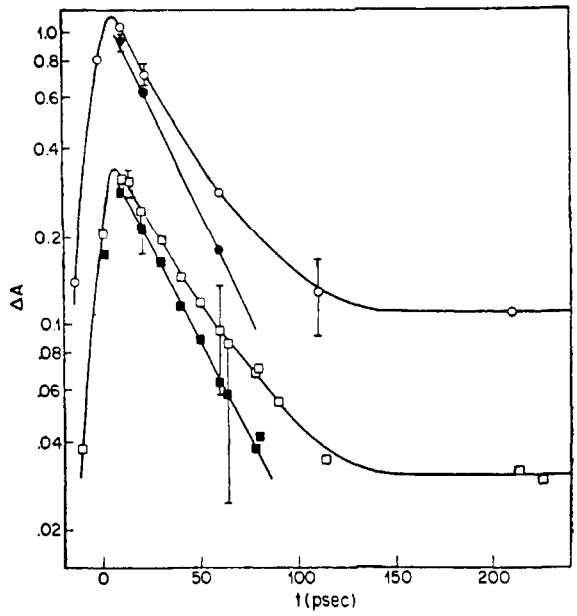

FIGURE 1: Absorbance change, ΔA, at 669 nm vs. time observed for *C*-phycocyanin in pH 5.5 buffer (hexamer). (○ and □) Time dependence of absorbance change obtained by an interrogation pulse with polarization parallel to that of the excitation pulse with the energy of 2 and 0.2 mJ, respectively. (● and ■) ΔA vs. time for the short component. These values were obtained by subtracting the ΔA value of the long lifetime component from the observed ΔA.

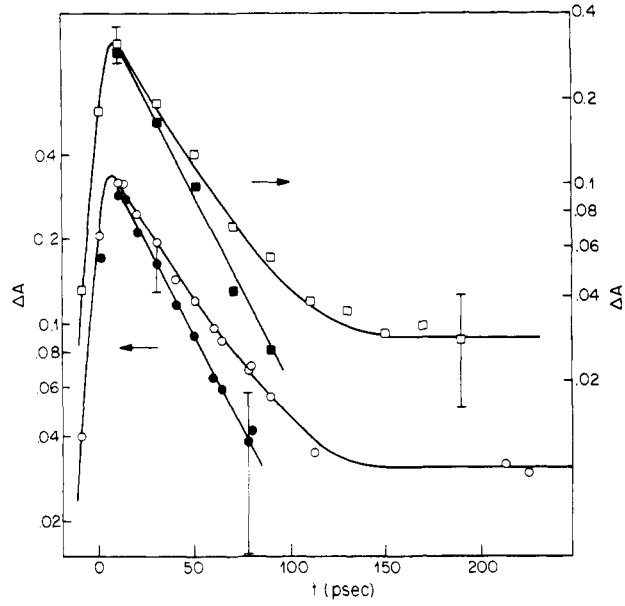

FIGURE 2: Absorbance change, ΔA, at 669 nm vs. time observed for *C*-phycocyanin in pH 5.5 buffer (hexamer). (○ and □) ΔA vs. time obtained with interrogation light parallel and perpendicular, respectively, to the 2-mJ excitation pulse. (● and ■) Same meaning as in Figure 1.

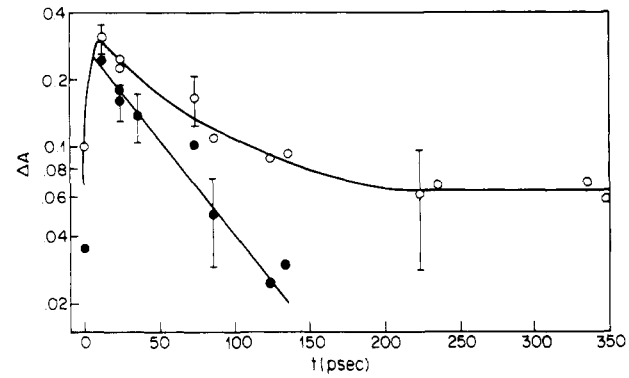

FIGURE 3: (○) Absorbance change, ΔA, at 669 nm vs. time observed for *C*-phycocyanin in pH 8.0 buffer (trimer). (●) Same meaning as ○ in Figure 1.

changes after excitation with a 6-ps, 530-nm pulse were monitored as a function of time at 669 and 510 nm for *C*-phycocyanin in pH 5.5 (hexamer), pH 8.0 (trimer), and pH 3.9 (monomer) buffers. The results are shown in Figures 1–4. The decay kinetics of the 510- and 669-nm bands were monitored with light polarized parallel and perpendicular to the excitation beam. In addition, the effect of pulse intensity was examined by varying the excitation energy from 2 to 0.2 mJ for *C*-phycocyanin and phycocyanin 645.

The decay curve of the transient observed for *C*-phycocyanin in the pH 5.5 buffer (hexamer) solution with the 669-nm monitoring light polarized parallel to the excitation light and a pulse energy of 2 mJ is shown in Figure 1. The time dependence of ΔA with excitation energy was measured for 2, 1, 0.4, and 0.2 mJ. In Figure 1, only the results obtained for 2- and 0.2-mJ excitation energy are displayed, from which it is evident that the decay kinetics are not affected within this excitation energy range.

Examination of the decay curves shows that ΔA does not relax in a monophasic form but rather in a biphasic form under both 2- and 0.2-mJ excitation. Similar behavior was found for 1- and 0.4-mJ excitation. Analysis of the ΔA decay curves shows that the two decay time constants of the transient observed at 669 nm are 31 ± 2 ps and >1 ns for 2-mJ excitation and 34 ± 2 ps and >1 ns for 0.2-mJ excitation. The decay time constants of the short component at 1- and 0.4-mJ excitation were 35 ± 3 and 31 ± 3 ps, respectively. Within the excitation energy range from 0.2 to 2 mJ, we did not observe any changes in the decay kinetics of either component with pulse energy. This observation is interpreted to mean that there is no detectable change in the decay kinetics of transient of *C*-phycocyanin in pH 5.5 (hexamer) even if the excitation energy is changed from 0.2 to 2 mJ.

The decay kinetics of *C*-phycocyanin in pH 5.5 buffer (hexamer) monitored by 669-nm light polarized perpendicularly to the 530-nm, 0.2-mJ excitation light are shown in Figure 2. The formation of the 669-nm transient is shorter than 8 ps, while the decay curve consists of two decay lifetimes with time constants of 34 ± 2 ps and >1 ns for parallel interrogation and 32 ± 2 ps and >1 ns for perpendicular interrogation. The ratios of the long to the short component of the decay curve were found to be the same for a parallel and a perpendicular monitoring beam. This observation indicates that there is no detectable difference in the decay kinetics of the transient observed at 669 nm between parallel and perpendicular monitoring for *C*-phycocyanin in the pH 5.5 buffer solution.

The decay kinetics of the transient absorption at 510 nm for *C*-phycocyanin in pH 5.5 buffer (hexamer) were observed. It was found that the biphasic decay behavior, the decay constants, and the absence of an effect of excitation energy and polarization on the decay kinetics were completely similar to the observation at 669 nm.

The time dependence of the absorbance changes observed with interrogation light at 669 nm polarized parallel to the 530-nm excitation beam with 0.2-mJ energy for *C*-phycocyanin in pH 8.0 buffer (trimer) is shown in Figure 3. The decay curve is also found to be composed of two components, one having a short lifetime of 56 ± 6 ps and another with a lifetime longer than 1 ns. There was no detectable difference in the decay kinetics of the trimer transient when the excitation

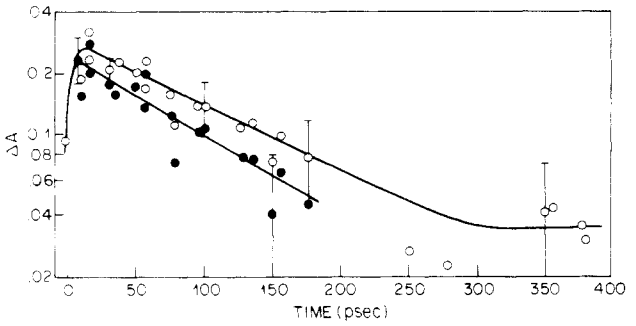

FIGURE 4: (O) ΔA at 669 nm vs. time observed for *C*-phycocyanin in pH 3.9 buffer (monomer). (●) Same meaning as in Figure 1.

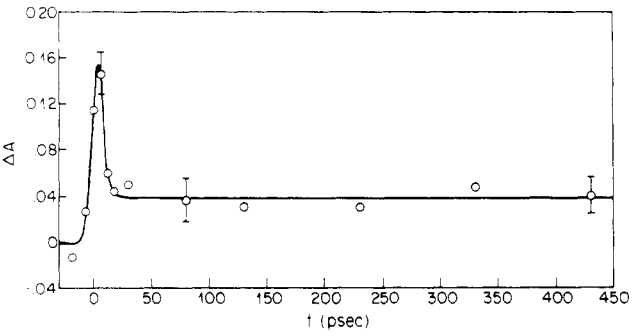

FIGURE 5: Absorbance change, ΔA, at 669 nm vs. time observed for phycocyanin 645 in pH 6.0 buffer (dimer).

energy was varied between 2 and 0.2 mJ, as in the case of the hexamer transient. Neither did we observe any large differences in decay kinetics when the interrogating beam was polarized parallel or perpendicular to the excitation light pulse. The decay kinetics observed at 510 nm also exhibit the same decay time constants and independence of excitation energy and polarization as described above for 669 nm for *C*-phycocyanin in pH 8.0 buffer (trimer).

The change in absorbance, ΔA, as a function of time for *C*-phycocyanin in pH 3.9 buffer (monomer) at 669 nm with an interrogation light beam polarized parallel to the 530-nm, 2-mJ excitation light is shown in Figure 4. The decay curve has two components, as with the hexamer and trimer, a long-lived one of more than 1 ns and a short-lived one of 62 ± 10 ps at high-energy excitation (2 mJ) and 85 ± 7 ps at low-energy excitation (0.2 mJ). Experimentation with the polarization of the interrogating light beam perpendicular to that of the excitation light light beam gave the same results as in the case of parallel polarizations shown in Figure 4. The effect of excitation energy on the decay kinetics at 510 nm of the monomeric form of *C*-phycocyanin was not studied because of its low solubility and low absorption at this wavelength (OD was 0.16 at 530 nm in the 2-mm cell). No effect is expected, however, because none was found for the trimer (pH 8.0) or the hexamer (pH 5.5) of *C*-phycocyanin.

ΔA vs. time for the dimer of phycocyanin 645 in pH 6.0 buffer interrogated at 510 nm and with polarization parallel to that of the 530-nm, 0.2-mJ excitation light beam is shown in Figure 5. This figure is also composed of a short and long component, corresponding to less than 8 ps and more and 1 ns, respectively, as was the case of *C*-phycocyanin. As in the other cases, we did not observe any substantial changes in the time dependence when the energy of excitation was varied between 2 and 0.2 mJ. Neither was there a detectable difference in the decay kinetics between parallel and perpendicular polarized interrogation light. Similar results with regard to energy and polarization dependence were found for other absorption wavelengths, including 669 and 480 nm. In addition, we investigated the kinetics of the bleaching and recovery of the ground state of phycocyanin 645 by monitoring ΔA as a function of time at 550 and 555 nm. The recovery of the ground-state bleaching was found to be composed of two processes, a fast one with a time constant of <8 ps and a slow one with a time constant of >1 ns.

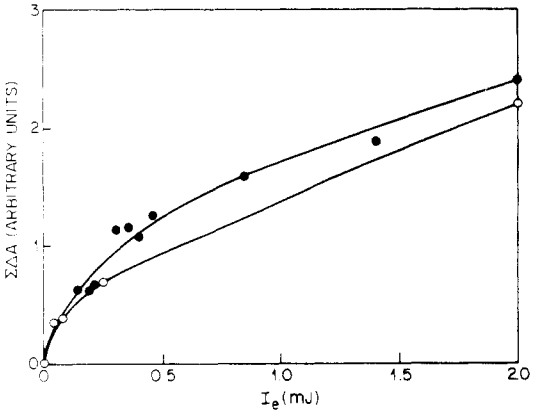

FIGURE 6: ΔA at 669 nm vs. excitation energy, I_e, for *C*-phycocyanin in pH 5.5 buffer (hexamer) at 669 nm (O) and for phycocyanin 645 in pH 6.0 buffer (dimer) at 510 nm (●).

The experimental results are summarized as follows. Excitation at 530 nm causes a transient absorption band at 669 and 510 nm for *C*-phycocyanin in buffer of pH 5.5 (hexamer), 8.0 (trimer), and 3.9 (monomer) and for phycocyanin 645 in pH 6.0 buffer (dimer). The changes in optical density at 550 and 555 nm are negative (bleaching) for phycocyanin 645 in pH 6.0 buffer (dimer). All the decay or recovery curves observed for transient absorption or bleaching were composed of a fast and a slow component. The time constants of the fast process were 31 ± 6 ps for the hexamer (pH 5.5), 56 ± 5 ps for the trimer (pH 8.0), and 85 ± 7 ps for the monomer (pH 3.9) of *C*-phycocyanin and shorter than 8 ps for the dimer of phycocyanin 645 in pH 6.0 buffer. The time constants of the slow processes were all found to be longer than 1 ns.

For examination of the effect of pulse energy on ΔA, the integrated absorbance changes from the time zero echelon segment to the last echelon were plotted as a function of energy. The results for *C*-phycocyanin in pH 5.5 buffer (hexamer) at 669 nm and phycocyanin 645 in pH 6.0 buffer (dimer) plotted in Figure 6 show a typical saturation curve. Similar saturation curves were observed for *C*-phycocyanin in pH 3.9 (monomer) and pH 8.0 (trimer) buffer. Since our results show that the decay kinetics are not affected by the pulse energy, the saturation effect must be caused by successive excitation from the transient to higher excited states or due to ground-state depletion of *C*-phycocyanin or phycocyanin 645. The relative total fluorescence of monomer, trimer, and hexamer of *C*-phycocyanin is plotted against laser output from 0.01 to 5 mJ (Figure 7). The linear response for all three aggregates suggests that no measurable artifacts are being produced from the interaction of the laser beam with the protein.

Discussion

The fast component of the decay of ΔA for all of the systems studied may be explained by two very different mechanisms. One explanation is that the fast decay is due to superradiance or concentration quenching caused by the high concentration of excited species; the other explanation is that the fast decay is caused by energy transfer from the s to the f chromophores or possibly among a group of s (or f) chromophores. Superradiance or concentration quenching is made less plausible by

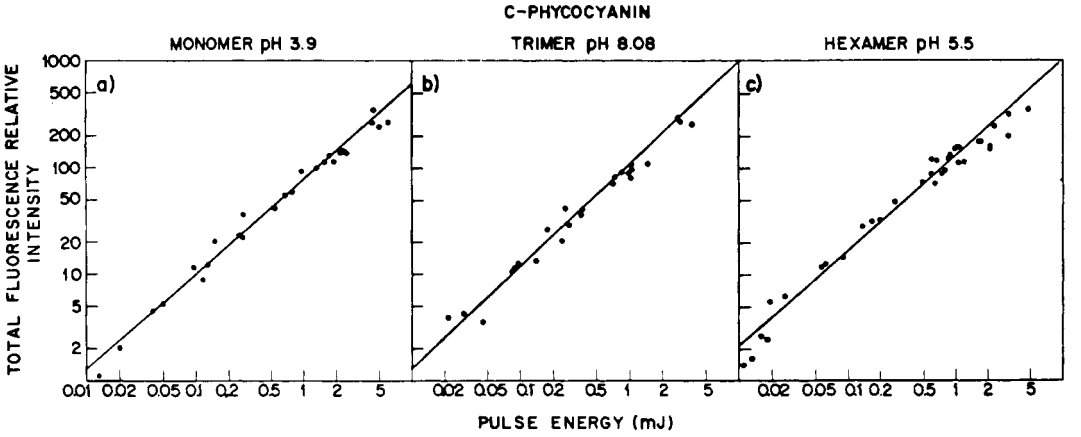

FIGURE 7: Relative intensity of the total fluorescence band of *C*-phycocyanin as a function of excited pulse energy. (a) Monomer; (b) trimer; and (c) hexamer.

the observations that the decay kinetics of the trimer and hexamer are practically independent of excitation energy in the range 0.2–2 mJ while the monomer decay time constant increased from 62 ± 10 ps at 2 mJ to 85 ± 7 ps at 0.2 mJ. The saturation effect shown in Figure 6 may be the result of either successive excitation of the transient of *C*-phycocyanin or phycocyanin 645 or depletion of the ground-state population. Likewise, the fluorescence results (Figure 7) tend to eliminate the superradiance and concentration quenching possibilities.

The fact that the decay kinetics do not depend on wavelength both for *C*-phycocyanin and phycocyanin 645 means that the transient absorptions observed at 669 nm and at 510 nm are due to the same species. The fast and slow components in the decay kinetics imply the existence of two intermediate species. Consistency with all of our experimental results is obtained by assigning the rapidly decaying species to the s sensitizing chromophore and the slowly decaying species to the f fluorescing chromophore. Thus, the faster rate is the rate of energy transfer from the s to the f chromophore [or among a population of s (or f) chromophores]; the slower rate is the rate of decay of the f chromophore, which should be identical with the fluorescence decay rate.

A key observation is that the decay kinetics do not depend on the orientation of polarization of the interrogating light with respect to that of the 530-nm exciting light. There are two possible explanations—the energy transfer process also randomizes the oscillators or the oscillators are randomized on a time scale shorter than the energy-transfer process. The latter is extremely improbable because (a) the energy of fluorescence is only slightly less than the energy of absorption, implying that the absorption in question on each chromophore is to be the lowest singlet state, (b) it is very difficult for a large chromophore covalently bound to a protein to reorient on the 10^{-11}–10^{-10}-s time scale, and (c) in the case of the $\alpha\beta$ monomer of *C*-phycocyanin there is incomplete but substantial fluorescence polarization in spite of an initial energy transfer. Thus, we believe that the process of energy-transfer sites also broadens the angular distribution of excited dipoles. The fluorescence lifetime of *C*-phycocyanin (source unspecified) has been found to be 1.14 ± 0.01 ns (Merkele et al., 1969), when extracted from *Schizotrix calicola* 2.25 ± 0.2 ns, and from *Anacystis nidulans* 1.8 ± 0.2 ns (Teale & Dale, 1970). These data justify associating the slower decay component with a lifetime of >1 ns with the fluorescence lifetime.

By assumption that our fast transfer times are caused by s to f transition, the following calculations are of interest. If the reciprocals of the short time constants are identical with the energy-transfer rates, then we can derive some information on the related position of s and f sites on different subunits. The Förster theory of inductive energy transfer defines a parameter R_0 such that for an electronic donor and acceptor separated by a distance R the rate of transfer of energy k_{TR} is given by

$$k_{TR} = k_0(R_0/R)^6 \quad (1)$$

where k_0 is the rate of decay in the absence of energy transfer. We have assumed that the lifetime of the excited s chromophore in the absence of f acceptors would be similar to that of the acceptor, i.e., ~2 ns or $k_0 = 5 \times 10^8$ s^{-1}.

The time constants for energy transfer in *C*-phycocyanin monomer ($\alpha\beta$), trimer ($\alpha_3\beta_3$), and hexamer ($\alpha_6\beta_6$) were 85, 56, and 32 ps, respectively. Assuming in eq 1 that $k_0 = 5 \times 10^8$ s^{-1}, one finds that for the monomer $R = 0.59R_0 \simeq 39$–42 Å. If we assume for the trimer an equilateral triangular configuration $(\alpha\beta)_3$ in which the monomer structure remains unchanged, the enhanced transfer rate is due to energy transfer from s chromophores in one $\alpha\beta$ unit to f chromophores in other $\alpha\beta$ units. If there is just one such additional acceptor for each s site, then the rate of transfer to it is given by $(10^{12}/56) - (10^{12}/85) - (6.1 \times 10^9)$ s^{-1} and the distance of this acceptor from the s site is $R = 0.66R_0 = 44$–48 Å. In the hexamer a similar calculation, again assuming an invariant structure for the $\alpha\beta$ subunit, yields $R = 0.54R_0 = 36$–39 Å. The electron microscopic results referred to show that the diameter of the globular $\alpha\beta$ units is ~40 Å (Berns & Edwards, 1965). Thus, none of the interchromophoric distances seem unreasonable.

Acknowledgments

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Immunochemical Analysis of Rabbit Antihuman Fibrinopeptide B Antibodies†

George D. Wilner,* David W. Thomas, Hymie L. Nossel, Paul F. Robbins, and M. Susan Mudd

ABSTRACT: The serologic immune response to human fibrinopeptide B (hFPB), a unique thrombin proteolytic product of the fibrinogen Bβ chain (Bβ1–14), was studied as a basis for developing immune probes for sequential regions in the fibrinogen molecule. Outbred rabbits hyperimmunized with hFPB analogue–bovine albumin conjugates produced antisera specific for native hFPB, as measured by radioimmunoassay using [^{125}I]Tyr-hFPB analogue as a tracer. By use of this assay system, these sera were found capable of distinguishing free hFPB from the peptide bound to its parent fibrinogen molecule. Immunoreactive sites seen by these sera were characterized in terms of displacement of bound radiolabeled tracer by synthetic homologues of the hFPB sequence. In four immune rabbit sera, displacement of binding comparable on a molar basis using hFPB as inhibitor was obtained with fragments Bβ3–14, Bβ5–14, and Bβ7–14 but not with fragment Bβ9–14. In an additional three sera, inhibition comparable to that of intact hFPB occurred only with fragments Bβ3–14 and Bβ5–14. In five of the seven sera tested, Arg^{14} seemed to contribute critically to hFPB antigenicity. Isoelectric focusing experiments showed that the immune response of each sera was limited to 8–10 discrete bands, indicating that these functional restrictions in site specificity were associated with a limited structural heterogeneity. Our data suggest that the region comprising the COOH-terminal 8–10 residues of hFPB is immunologically hindered by its attachment to the Bβ chain of its parent fibrinogen molecule. These findings are analogous to those of earlier studies on the immunochemistry of human fibrinopeptide A (hFPA) wherein immunogenic determinants in hFPA which were characteristic of the free peptide in solution could be localized to the COOH-terminal decapeptide sequence [Wilner, G. D., Nossel, H. L., Canfield, R. E., & Butler, V. P., Jr. (1976) *Biochemistry 15*, 1209]. Taken together, these results suggest that antisera which are specific for free fibrinopeptides in solution may be directed against conformationally ordered regions shown to be present in the COOH-terminal halves of these peptides [Huseby, R. M. (1973) *Physiol. Chem. Phys. 5*, 1]. It is concluded that antibodies of limited heterogeneity may be produced by immunization with peptide haptens possessing limited numbers of determinants and that antibodies so produced are potentially useful as sequence-specific immune probes.

The transformation of soluble fibrinogen into insoluble fibrin is preceded by the highly selective, limited proteolysis of two pairs of small activation peptides from the NH_2 terminal of the Aα and Bβ chains of fibrinogen (Blombäck, 1967; Blombäck et al., 1966). These activation peptides, termed fibrinopeptide A (hFPA)[1] and fibrinopeptide B (hFPB) after their respective chains of origin, consist of the NH_2-terminal 15 residues of the human Aα chain and the NH_2-terminal 14 residues from the human Bβ chain of fibrinogen (Blombäck, 1967). The primary structure of these peptides is shown in Figure 1. These activation peptides are thought to function as enzymatically labile protecting groups, covering specific polymerization sites located in the NH_2-terminal (E) region of the fibrinogen molecule (Blombäck et al., 1978). Our major interest in fibrinopeptides has been in their measurement as specific indices of thrombin action in vivo (Wilner, 1978). Sensitive and specific radioimmunoassays have been developed by ourselves and others for the quantitation of fibrinopeptides

† From the Department of Pathology and Laboratory Medicine, The Jewish Hospital, St. Louis, Missouri, the Departments of Pathology and Microbiology–Immunobiology, Washington University School of Medicine, St. Louis, Missouri 63110, and the Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York. *Received May 29, 1979.* Supported by Grants HL-22642 and HL-15486 from the National Heart, Lung, and Blood Institute, National Institutes of Health, and U.S. Public Health Service Grant AI-14226 from the National Institute of Allergy and Infectious Diseases.

* Present address: Division of Laboratory Medicine, The Jewish Hospital of St. Louis, St. Louis, MO 63110. Recipient of Research Career Development Award HL-70447.

[1] Abbreviations used: hFPA, human fibrinopeptide A; hFPB, human fibrinopeptide B; Dnp, dinitrophenyl; Boc, N^{α}-*tert*-butoxycarbonyl.

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