# Kinetics of Allophycocyanin's Trimer–Monomer Equilibrium†

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ABSTRACT: Kinetic studies of the dissociation of allophycocyanin trimers to monomers have been performed by using stopped-flow techniques. The dissociation was monitored by two techniques: by light scattering to observe the molecular weight changes directly and by 650-nm absorbance to observe the linkage of quaternary structure to spectra. The light-scattering experiments showed a simple exponential decay of trimers to monomers with a dissociation constant of 0.23 $s^{-1}$. The absorption changes were complex, with two processes occurring. The faster absorption change appeared to be almost simultaneous with the molecular weight change (about 0.27 $s^{-1}$) and was perhaps totally coordinated with it. The slower absorption change (0.071 $s^{-1}$) was possibly a result of a conformational change in the chromophore arising during the conversion from newly dissociated monomers to equilibrium monomers.

Biliproteins are light-harvesting, photosynthetic pigments in blue-green algae, red algae, and cryptomonads. In blue-green and red algae, the biliproteins are organized into discrete multiprotein organelles, the phycobilisomes, on the exterior surface of the thylakoid membranes on the stromal side. The phycobilisomes of blue-green algae are hemidiscoidal: the core is primarily allophycocyanin and emanating from it are six rods that contain the other biliproteins. In the phycobilisomes, allophycocyanin occurs as trimers arrayed in stacks. Light is harvested primarily in the rods and then transferred from high to low energy, eventually reaching the core. There it goes from allophycocyanin to allophycocyanin 680 and then to chlorophyll in the thylakoid membranes [for reviews, see MacColl & Guard-Friar (1987) and Troxler (1986)].

The pivotal role of allophycocyanin as the recipient of excitation energy from C-phycocyanin (Gantt & Lipschultz, 1973) is a function of its 650-nm absorption and 660-nm fluorescence emission maxima. The spectroscopic characteristics are distinctive of allophycocyanin trimers ($\alpha_3\beta_3$), since the absorption and emission maxima monomers ($\alpha\beta$) are strongly blue-shifted (MacColl et al., 1980). The spectra reflect the presence of tetrapyrrole chromophores covalently attached to the apoproteins. There have been no kinetic studies on the assembly of any biliprotein aggregate. For allophycocyanin, the formation of trimers is, of course, particularly important.

In this study, we utilize the facts that allophycocyanin can be prepared in two homogeneous forms, trimer and monomer, and that the chaotropic salt sodium perchlorate quantitatively dissociates trimers to monomers (MacColl et al., 1981). Sedimentation equilibrium experiments on the model E analytical ultracentrifuge showed that in pH 7.0 sodium phosphate buffer allophycocyanin was a homogeneous trimer while at pH 7.0 and 1.0 M $NaClO_4$ it was a homogeneous monomer. The absorption and fluorescence spectra characteristic of monomers and trimers were obtained. The 650-nm absorption maximum of the trimers was entirely absent in the monomer spectrum. Stopped-flow kinetics in both the absorption and light-scattering modes were used to study this process for allophycocyanin isolated from the blue-green alga *Phormidium luridum*. In this way, the dissociation of the trimers (light scattering) could be compared to the changes in absorption which accompany the change in molecular weight.

## Experimental Procedures

Allophycocyanin was isolated from *P. luridum* and purified as described previously (MacColl et al., 1981). The final step in the purification was chromatography on hydroxylapatite (Bio-Gel-HT; Bio-Rad, Richmond, CA), adapted from the method of Zilinkas et al. (1978). The absorption spectra of samples in pH 7.0 phosphate buffer were recorded on a Cary 118 spectrophotometer (Applied Physics Corp., Monrovia, CA) before the kinetic studies. An extinction coefficient of 6.35 for a 1 mg/mL solution at 650 nm in a 1-cm cell was used to calculate the sample concentration.

To determine whether the 650-nm absorption band was completely lost upon dissociation, the following mixing experiment was performed on a Cary 118 spectrophotometer with two-compartment tandem cuvettes. One compartment in each reference and sample cell was filled with 2.0 M sodium perchlorate. The other compartment held an equal volume of pH 7.0 phosphate buffer (reference cells) or of allophycocyanin in the same buffer (sample cells). Absorption spectra were recorded from 600 to 670 nm before and after mixing.

An Aminco-Morrow stopped-flow apparatus (American Instruments, Silver Spring, MD) equipped with a DASAR (data acquisition, storage, and retrieval) system (American Instruments) was used to study the dissociation kinetics of allophycocyanin. In this system, the solutions are stored in two syringes, which simultaneously drive the solutions through a mixing chamber and into an observation cell. The dead time for the solution to flow from the mixing compartment to the observation chamber is about 3 ms. Thus, the reaction can be studied 3 ms after it has begun. The protein sample was mixed with equal volume of 2.0 M sodium perchlorate in pH 7.0 phosphate buffer, and the change in intensity of the 650-nm band was monitored by transmission, while the molecular weight changes were monitored in the 90° light-scattering mode also using 650-nm light. Data were stored in memory and could be displayed on the oscilloscope after the reaction was complete. The data were also recorded on a cassette in an electronic data terminal (Silent 700 ASR; Texas Instruments, Houston, TX) for further calculations on a Vax computer.

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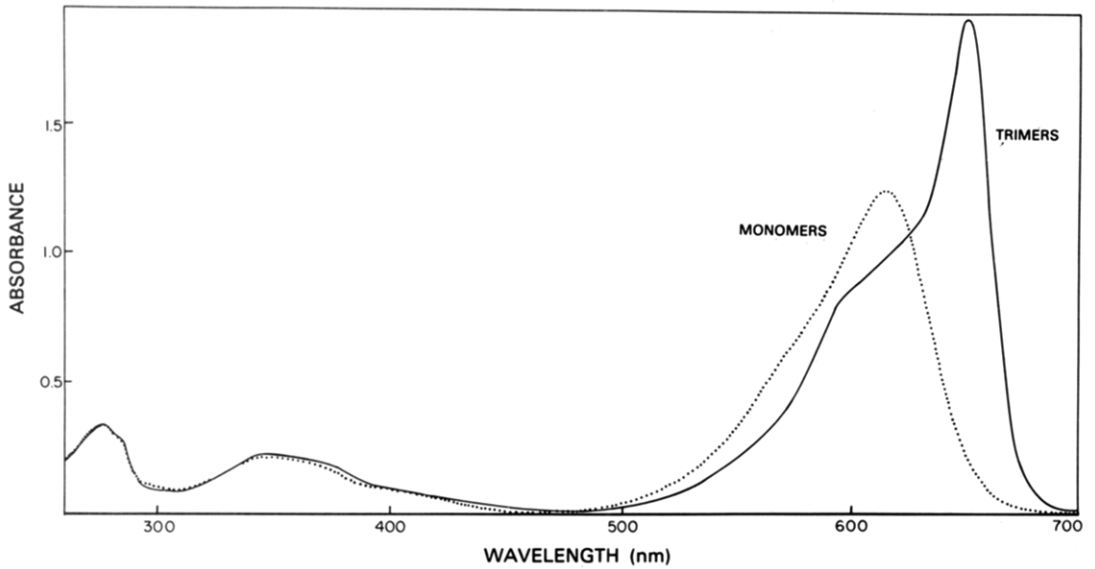


FIGURE 1: Absorption spectra of allophycocyanin trimers and monomers. The solvent was pH 7.0, 1.0 M sodium perchlorate for monomers and pH 7.0, 0.1 ionic strength, sodium phosphate for trimers. Spectra were recorded at room temperature. Protein concentrations were equal for both spectra.

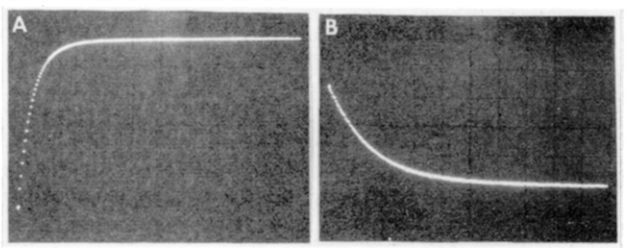


FIGURE 2: Photographs of oscilloscope tracings for the conversion of allophycocyanin trimers to monomers, monitored by (B) light scattering and (A) change in transmission at 650 nm. Initial protein concentrations were 0.1272 mg/mL in both cases, and the final concentration was 0.0636 mg/mL. The initial solvent was pH 7.0, 0.1 ionic strength, sodium phosphate buffer. The final solvent was pH 7.0 and 1.0 M sodium perchlorate. Experiments were performed at room temperatures (23 °C). For the light-scattering experiment, the data were taken at 0.2 s/point, and the transmission data were recorded at 0.5 s/point.

## RESULTS

The absorption spectra of allophycocyanin are shown in Figure 1. The ratio $A_{620}/A_{max}$ was calculated to be 0.62 for trimers, which is in good agreement with the data reported by Zilinskas et al. (1978). Disappearance of the 650-nm band is accompanied by dissociation of trimer. The mechanism of the dissociation of trimer (T) into monomer (M) is governed by the rate constant $k_{31}$:

$$T \xrightarrow{k_{31}} M \tag{1}$$

The disappearance of trimer can be written as

$$dC_T(t)/dt = -k_{31}[C_T(0) - x] \tag{2}$$

where $C_T(0)$ is the trimer concentration before mixing, $C_T(t)$ is the actual concentration, and $x$ is the concentration change at any time $t$. Integration of eq 2 with the boundary condition, i.e., at $t = 0$, $x = 0$, gives first-order kinetics as

$$\ln\left[\frac{C_T(t)}{C_T(0)}\right] = -k_{31}t \tag{3}$$

By defining the transmittance signal at time $t$ as $h(t)$ and the signal at time $t = 0$ as $h(0)$, eq 3 becomes

$$\ln\left\{\log\left[\frac{h(0)}{h(t)}\right]\right\} = -k_{31}t \tag{4}$$

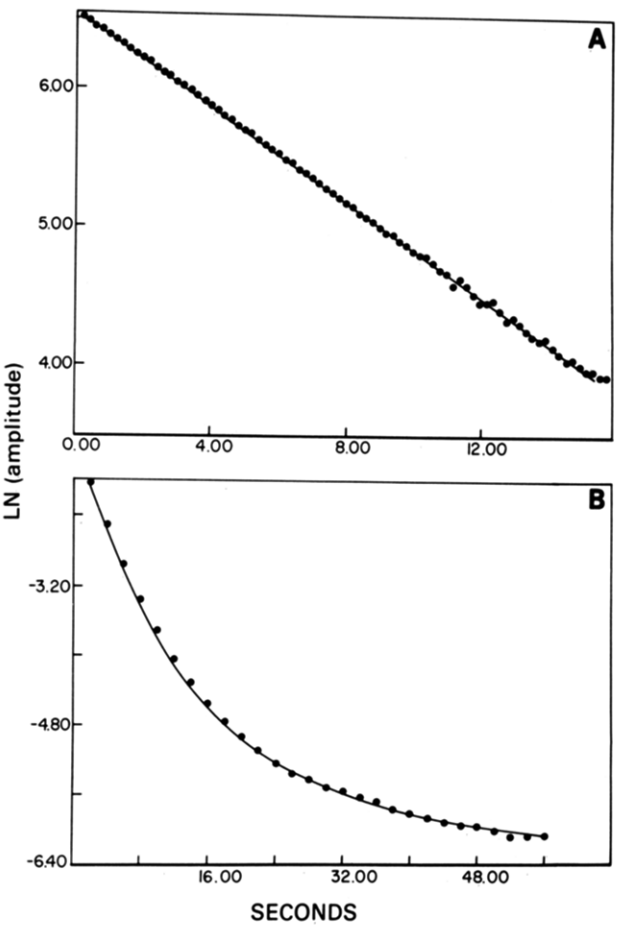


FIGURE 3: Change in light scattering (A) and transmission (B) at 650 nm as a function of time for the conversion of allophycocyanin trimers to monomers. Protein concentration and solvents are identical with those in Figure 2.

According to eq 4, one can plot ln {log $[h(0)/h(t)]$} vs. time $t$, and the slope is equal to $-k_{31}$.

Typical reaction traces are shown in Figure 2 for the light-scattering and transmittance modes. Figure 3 gives the logarithm plots of eq 4 at one concentration for the light-scattering data. The plot is quite linear. The values of $k_{31}$ calculated from four concentrations are listed in Table I.

Table I: Dissociation Constants of Allophycocyanin

| final protein concn (mg/mL) | $k_{31}$ ($s^{-1}$), light scattering | initial slope ($s^{-1}$), absorption |
|---|---|---|
| 0.0355 | 0.237 | 0.284 |
| | 0.236 | 0.280 |
| | 0.233 | 0.266 |
| 0.0482 | 0.230 | 0.288 |
| | 0.230 | 0.283 |
| | 0.230 | 0.276 |
| | 0.232 | 0.273 |
| 0.0636 | 0.231 | 0.263 |
| | 0.224 | 0.264 |
| | 0.240 | 0.277 |
| | 0.230 | 0.266 |
| | 0.230 | 0.265 |
| 0.077 | 0.240 | 0.280 |
| | 0.234 | 0.267 |
| | 0.230 | 0.268 |
| | 0.230 | 0.267 |

When the same protocols were applied to the conversion of allophycocyanin trimers to monomers in the absorption mode at the same protein concentration, the plot was clearly nonlinear (Figure 3). The absorbance data (Table I) were analyzed by an algorithm for the least-squares estimation of nonlinear parameters (Marquardt, 1963). The data were readily resolved by a two-exponential fit. The average slopes for the four concentrations were $0.27 \pm 0.01$ and $0.071 \pm 0.021$ $s^{-1}$. The initial (faster) decay in the 650-nm absorbance was the major of the two decays. The ratio of the amplitudes ($P_1/P_3$) in the general equation used to fit the data, $y = P_1e^{-P_2x} + P_3e^{-P_4x}$, was about 8, where $P_2$ was the initial slope. The standard deviation for a $P_2$ (initial slope) for any particular experiment was approximately 1%.

## Discussion

The allophycocyanin monomer is a complex of two subunits ($\alpha$ and $\beta$) with similar molecular weights (Brown et al., 1975). Each monomer has two phycocyanobilin chromophores (Glazer & Fang, 1973). The complete amino acid sequences of allophycocyanin from two sources (Sidler et al., 1981; Offner et al., 1983) show 160 residues for the $\alpha$ subunit and 161 residues for the $\beta$ subunit. A chromophore is attached to a cysteine at residue 80 of the $\alpha$ subunit and at residue 81 of the $\beta$ subunit.

The monomer's lack of a 650-nm absorption band may be caused by either or both of two possibilities: (i) in trimers, a strongly coupled exciton may exist between pairs of chromophores on contiguous monomers (MacColl et al., 1980, 1981; Csatorday et al., 1984); (ii) the chromophores on trimers and monomers may have different conformations (Murakami et al., 1981; Mimuro et al., 1982; Sugimoto et al., 1984). It is difficult to completely endorse either of these possibilities alone.

The CD studies by Csatorday et al. (1984) removed the strongest argument against the exciton-splitting model by demonstrating, through deconvolution of the CD spectrum, that the characteristic exciton CD shape occurs for trimers, although it was masked in the experimental spectrum. Our kinetic results (Figures 2 and 3) further support this model. In the proposed exciton interaction between chromophores on adjacent monomers, when trimers dissociate into monomers, the reputed pair of chromophores must also be separated. In fact, the dissociation must occur at exactly the same rate as the disappearance of 650-nm absorption. Our results suggest that this necessary condition for the exciton-coupling model may be met. The light-scattering kinetics show a single event occurring at 0.23 $s^{-1}$, and the absorption kinetics show a rapid component of change at 0.27 $s^{-1}$.

The two-step kinetics of the 650-nm absorption band upon trimer dissociation are very interesting. Events in the modulation of the absorption spectra are clearly occurring slower than the dissociation to monomers. This observation suggests that the newly formed monomers are spectroscopically different than the final state of the monomers:

$$\alpha_3\beta_3 \rightarrow 3[\alpha\beta] \rightarrow 3\alpha\beta \quad (5)$$

Furthermore, some of the spectroscopic differences between trimers and monomers appear to be caused by conformational changes in the chromophores that occur after the dissociation to monomers. Scheer and Kufer (1977) have shown that the conformation of chromophores is more extended on a native biliprotein (C-phycocyanin) than on the denatured proteins. Our kinetic experiments extend this concept to allophycocyanin and are the first direct measure of a particular stage of the conformational process. The trimeric aggregation state impresses a particular conformation on its chromophores, which in monomers slowly relax to a new conformation.

Even if the strongly coupled exciton model is correct for allophycocyanin's 650-nm absorption maximum, these conformational changes—as suggested by Murakami et al. (1981), Mimuro et al. (1982), and Sugimoto et al. (1984)—are also important in the modulation of chromophores by trimers. The noncyclic nature of these tetrapyrroles allows great flexibility in establishing multiple conformations through protein–chromophore interaction.

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