REASSEMBLY OF PHYCOBILISOMES FROM ALLOPHYCOCYANIN AND A PHYCOCYANIN–PHYCOERYTHRIN COMPLEX

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1. Introduction

Phycobilisomes are supramolecular aggregates of phycobiliproteins which function as the major light harvesting antennae in blue–green (cyanobacteria) and red algae [1]. Isolation of intact phycobilisomes has now been accomplished from many algae [1–6]. The major criterion for the functional integrity of these organelles is the demonstration that they exhibit highly efficient transfer of excitation energy from phycoerythrin (PE) to phycocyanin (PC) and finally to allophycocyanin (APC) [7,8]. A structural model for the phycobilisomes of *Porphyridium cruentum* was first proposed in [7] on the basis of kinetics of pigments released, concomitant energy transfer uncoupling, and electron microscopy studies. Accordingly, phycobilisomes consisted of an allophycocyanin core, surrounded by phycocyanin, and phycoerythrin (when present) on the periphery [3,4].

Phycobilisomes of both blue–green and red algae contain a small number of polypeptides, other than the well characterized α and β of the phycobiliproteins, which account for ~10–15% of the phycobilisome's total protein [5,6,9,10]. It has been suggested that some of these polypeptides (group II) are involved in the assembly and positioning of the phycobiliproteins within the phycobilisomes [5].

Ever since phycobilisomes were first isolated, a major goal has been to reconstitute them in vitro from their phycobiliprotein components. Although purified phycobiliproteins can readily aggregate into specific PE, PC, or APC crystals, they do not form mixtures similar to the aggregation states in phycobilisomes. The missing components are probably special binding proteins, as first suggested in [5].

Phycobilisomes under controlled conditions are known to dissociate into free phycobiliproteins, as well as specific phycobiliprotein complexes [2,11,12]. Binding between APC and PC appears to be weaker than that between PE and PC. This has been exploited in the present study in phycobilisomes from the blue–green alga (cyanobacterium) *Nostoc* sp. An APC fraction was separated from a PE–PC complex in a way that made it possible to accomplish the first in vitro reconstitution of phycobilisomes.

Abbreviations: PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin; C, phycobiliproteins of cyanophytan origin with specific spectral characteristics; F, fluorescence intensity

2. Materials and methods

Phycobilisomes were isolated [2] from 10–14 day old *Nostoc* sp. cultures grown in white light. Phycobilisomes were taken directly from a sucrose gradient and concentrated overnight in a dialysis bag against Sephadex G-200 powder. This reduced the volume to ~1/3rd of the original. The concentrated phycobilisomes (A_{572} 40) were dissociated by dialysis for 1–18 h against various ionic conditions (table 1). NaCl (0.1 M) was included in the dissociation buffer to enhance the stability of APC. Separation of the dissociated phycobilisomes, obtained by the above procedure, was accomplished by layering a 2 ml sample (diluted with dialysis buffer to A_{572} 12) on a sucrose step gradient buffered with 0.4 M K-phosphate (pH 7.0). The sucrose step gradient was otherwise identical to that reported for phycobilisome isolation [2]. The gradients were centrifuged for 4 h in a Beckman 42.1 rotor at 42 000 rev./min (136 000 × g) at 20°C.

Reconstitution experiments were done by combining the pigmented zones from the gradient above. The mixtures were dialyzed overnight against 2 M sucrose in 0.75 K-phosphate buffer (pH 7.0) which reduced the volume to 1/5th of the original. This was followed by dialysis against 0.75 M K-phosphate buffer (2 changes over 2 h) to reduce the sucrose concentration prior to layering and centrifugation on a typical phycobilisome isolation gradient buffered with 0.75 M K-phosphate (pH 7.0) [2]. Fluorescence was recorded on an Aminco-Bowman spectrofluorimeter equipped with an automatic corrected spectrum attachment [13]. For fluorescence measurements, phycobilisomes after various treatments, were diluted into 0.75 M K-phosphate (pH 7.0) containing 1 M sucrose with a final A_{572} 0.05 and were excited at 545 nm. Absorption spectra were measured on a Cary model 17 spectrophotometer. All operations were carried out at 24°C.

3. Results

3.1. *Effects on phycobilisome stability*

Intact phycobilisomes characteristically have a high fluorescence intensity at 680 nm that arises from long-emitting APC [14]. When the phycobiliproteins, within the phycobilisomes, become energetically uncoupled it is reflected by the appearance of fluorescence of the individual phycobiliproteins or complexes. The C–PE fluorescence maximum occurs at 583 nm and that of complexed C–PC at 655 nm. Fluorescence ratios were used to monitor the relative intactness of phycobilisomes in various states. The greater the F680/F655 ratio, and smaller the F583/F655 ratio, the more intact were the phycobilisomes. Typical values of intact PBS at 24°C were F680/F655 = 1.7 ± 0.02 and F583/F655 = 0.05 ± 0.02 (table 1). The most stable condition was in 0.75 M K-phosphate buffer (pH 7.0) in the presence of 1 M sucrose. Lowering the phycobilisome concentration resulted in uncoupling and a faster rate of dissociation under various conditions (table 1, cf. e,f). The same effect was obtained by lowering the ionic strength (table 1, c–e) which is known to dissociate phycobilisomes with time [2].

3.2. *Fractionation of dissociated phycobilisomes*

We had found that a PE–PC complex could be generated from dissociated phycobilisomes by following the condition (e) in table 1. Separation of dissociated phycobilisomes was accomplished on a 0.4 M K-phosphate sucrose gradient and resulted in two fractions shown in fig.1 and 2A. A top blue band contained all the recoverable APC, ~5% of the total PE, and lacked PC. The APC and PE were neither aggregated nor energetically coupled as shown by their presence near the top of the gradient and by their independent fluorescence peaks (fig.1B). Free PC, if present in the top fraction, would have been revealed by fluorescence at ~645 nm (with excitation at 600 nm). A faster migrating purple band consisted of the PE–PC complex (fig.1C,D; fig.2A), containing 95% of PE and all of the PC. It represents an energetically functional complex. The major emission at 655 nm originates from PC,

Table 1
Effect of ionic strength, time and protein concentration on the dissociation of phycobilisomes

Buffer (pH 7.0)	A_{572}/ml	Incubation time (h)	F583/F655	F680/F655
(a) 0.75 M K-phosphate 1 M sucrose	12	18	0.05	1.7
(b) 0.75 M K-phosphate	12	18	0.08	1.0
(c) 0.40 M K-phosphate	12	18	0.08	0.56
(d) 0.25 M K-phosphate	12	1	0.05	0.51
(e) 0.1 M K-phosphate 0.1 M NaCl	12	2	0.12	0.50
(f) 0.1 M K-phosphate 0.1 M NaCl	3	2	0.41	0.41

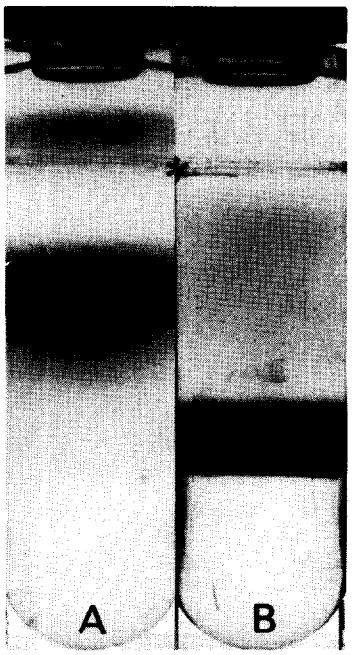

Fig.1. Fractions of dissociated phycobilisomes separated on a sucrose gradient (see fig.2A). The absorbance (A) and fluorescence (B) from the top fraction show that it contains mostly free allophycocyanin (F665 nm) and some free phycoerythrin (F583 nm). The lower fraction shows the absorbance (C) of phycoerythrin and phycocyanin, with the fluorescence (F655 nm) (D) arising from complexed phycocyanin. Samples for fluorescence had an absorbance of 0.05 at the respective maxima and were excited at 545 nm (——) and at 600 nm (. . .).

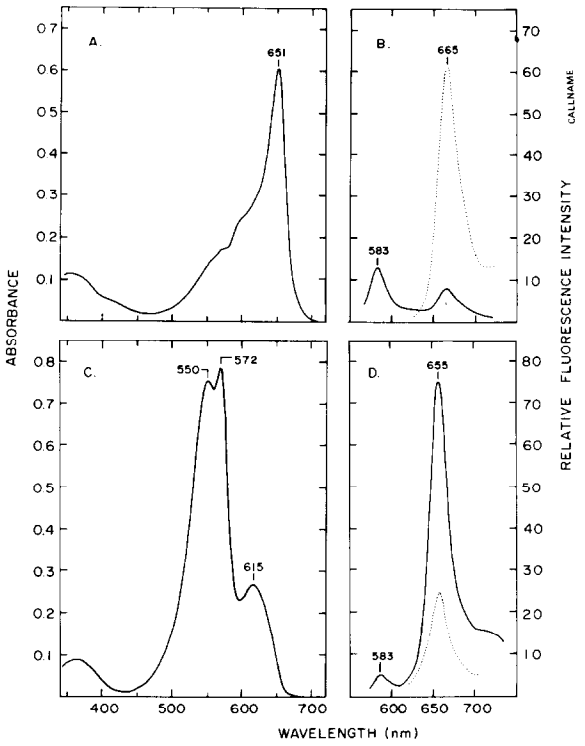

Fig.2. (A) Bands from dissociated phycobilisomes on a sucrose gradient (0.4 M K-phosphate pH 7.0). Top band: allophycocyanin and small amount of phycoerythrin. Lower band: phycoerythrin–phycocyanin complex. (B) The dark bottom band contained phycobilisomes reconstituted by combining the fractions in A. Unrecombined phycobiliproteins remained on top and middle of gradient. Asterik marks cast line on Beckman centrifuge tube.

even though PE is the main pigment component (cf. fig.1C,D). Furthermore, excitation of only PC (at 600 nm) did not increase the fluorescence intensity. Separation and in vitro recombination of this complex has also been accomplished and will be described separately (in preparation).

3.3. *Reconstitution of phycobilisomes*

Favorable conditions were explored for reconstitution of phycobilisomes from the separated fractions (table 2). The most favorable reassociation occurred in 0.75 M K-phosphate with 2 M sucrose. Of the phycobiliproteins, ⩾70% recombined into functional phycobilisomes under this condition. These phycobilisomes had an absorption identical to the original ones (not shown). Likewise, the fluorescence of reassociated and original phycobilisomes was highly similar (table 2a,b). The reassociated phycobilisomes migrated into the interface between the 1 M and 2 M sucrose layers, from which normal phycobilisomes are routinely recovered (fig.2B). However, a minor second band with a slightly slower sedimentation velocity, but identical in its spectral properties to original phycobilisomes, was also observed. Perhaps the sedimentational velocity of native phycobilisomes was higher due to secondary aggregation, as has been suggested to occur when Triton X-100 is used [9]. Secondary aggregation probably did not occur to the same extent in the reconstituted phycobilisomes.

The initial conditions of phycobilisomes dissociation were critical in getting reassociation. Reconstitution of phycobilisomes was possible from fractions at different degrees of dissociation (table 1c–f). Even when a large proportion of the PE had dissociated from phycobilisomes as in table 1f, recombination into phycobilisomes was very high. However,

Table 2
Favorable and unfavorable conditions for the recovery of reassociated phycobilisomes

Buffer (pH 7.0)	A_{572}/ml	Incubation time (h)	F583/F655	F680/F655
(a) Control:				
0.75 M KPO_4				
1 M sucrose	12	0	0.05	1.7
(b) Dissociation condition:				
0.1 M KPO_4				
0.1 M NaCl	12	2	0.12	0.50
Reassociation condition:				
0.75 M KPO_4				
2 M sucrose	12	18	0.04	1.5
(c) Dissociation condition:				
0.1 M KPO_4				
0.1 M NaCl	12	18	0.14	0.28
Reassociation condition:				
0.75 M KPO_4				
2 M sucrose	12	18	0.13	0.31
(d) Dissociation condition:				
0.1 M KPO_4				
0.1 M NaCl	12	2	0.12	0.50
Reassociation condition:				
0.75 M KPO_4	12	18	0.10	0.47

fractions obtained from prolonged dialysis (18 h) (table 2c) were not recombinable. The fluorescence maximum of unrecombinable APC, after prolonged dialysis, shifted from 665–654 nm with a 50% decrease in the intensity of fluorescence maximum at 680 nm indicating a substantial decrease in energy transfer from short (~660 nm) to long (~680 nm)-emitting APC forms [14,15]. No changes were observed in the spectral properties of the PE–PC complex after prolonged dissociation in the low ionic strength as described above.

4. Discussion

This study showed that under limited dissociating conditions, it was possible to get a specific dissociation and subsequent reassociation between APC and a PE–PC complex. The fluorescence spectrum of unrecombinable APC showed almost no energy transfer from short- to long-emitting APC forms, and a shift of fluorescence maximum from 665–654 nm. This is similar to changes observed in isolated PC forms [16] and the PE–PC complex from *Porphyridium sordidum* where initial longer-wavelength absorbing (627–637 nm) and -emitting (650–653 nm) forms gradually revert to shorter-wavelength absorbing (615–623 nm) and -emitting (640–643 nm) forms which are then unrecombinable (in preparation). Several explanations can be forwarded for the loss of recombinability of phycobilisomes under unfavorable conditions:

(1) A small irreversible conformational change occurs in APC that does not allow energy transfer between short- and long-emitting APC forms, and which may be a pre-condition for establishing attachment and energy-transfer from the PE–PC complex to APC fraction.
(2) A loss of a labile component(s) from the APC fraction that is (are) responsible for binding APC forms together, and which may be required for the further attachment of the PE–PC complex.

Several polypeptides, in addition to the common α and β polypeptides of phycobiliproteins, have been observed in *Nostoc* phycobilisomes, in purified APC forms, and in the PE–PC complex (unpublished). Similar polypeptides in several red and blue–green algae have been suggested to take part in the assembly and positioning of the phycobiliproteins within the

phycobilisome [5]. It is probable that both the conformation of the APC forms (possibly in a complex) and additional polypeptides are required for phycobilisome reassociation. In conclusion, phycobilisome assembly was accomplished in vitro. This opens up the possibility of preparing phycobilisomes lacking one or more specific polypeptides and exploring their role in the phycobiliprotein complexes and energy transfer.

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