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SPECIFICITY OF ENERGY TRANSFER TO PHOTOSYSTEM II BY IN VITRO REASSOCIATED HOMOLOGOUS AND HETEROLOGOUS MEMBRANE-BOUND PHYCOBILISOMES

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In a previous publication we have reported the in vitro reassociation of phycobiliproteins with thylakoids of *Fremyella diplosiphon* to form homologous, functional, membrane-bound phycobilisomes (Kirilovsky, D., Kessel, M. and Ohad, I (1983) Biochim. Biophys. Acta 724, 416–426). In the present work, using the same experimental system, we demonstrate the in vitro formation of heterologous, membrane-bound phycobilisomes. Analysis of phycobiliprotein association and binding curves disclosed two types of binding sites: specific sites which allow energy transfer to Photosystem II and non-specific sites which become occupied only after saturation of the Photosystem II specific sites. Binding to non-specific sites does not result in energy transfer. Both types of sites are present on cyanophyte thylakoids. Thylakoids of eukaryotic chloroplasts such as those of *Chlamydomonas reinhardtii* or *Euglena gracilis* can bind phycobiliproteins which reassociate to form intact membrane-bound phycobilisomes. However, only non-specific binding occurs in such heterologous systems. Limited proteolysis of membrane-bound phycobilisomes results in a rapid loss of the 94–95 kDa polypeptide assumed to be required for binding and energy transfer (Redlinger, T. and Gantt, E. (1982) Proc. Natl. Acad. Sci. USA 79, 5542–5546). Phycobilisomes lacking this polypeptide cannot bind to either specific or non-specific sites. Based on these results, we conclude that the 94–95 kDa polypeptide is required for the association of the phycobilisomes to both homologous and heterologous membranes; however, additional factors within the Photosystem II unit of cyanophytes are also required for establishing energy transfer.

Introduction

During recent years a wealth of information has been accumulated on the composition, structure and mode of assembly of phycobiliproteins forming the major light harvesting antenna of Photosystem II of cyanophyte thylakoids, the phycobilisomes [1–4]. The primary sequence of phycoerythrin, phycocyanin and allophycocyanin from various sources has been elucidated [5–8], and in vitro reconstitution of phycoerythrin, phycocyanin and allophycocyanin complexes as well as complete assembly of homologous and heterologous phycobilisomes has been achieved [9–14]. The results of such experiments have demonstrated that linkers or 'colorless' polypeptides are essential in the assembly of the phycobilisomes [11,13–17]. Also, there are indications that a 90–95

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; DCIP, 2,6-dichlorophenolindophenol; LDS, lithium dodecyl sulfate.

kDa polypeptide is required for binding and energy transfer from phycobilisomes to the membrane [18–20].

As opposed to the impressive progress made in the understanding of structure-function relationship in phycobilisome assembly, only limited information is available concerning the mechanism of phycobilisome binding to the membrane, the specificity of association with Photosystem II, and the identification of the polypeptide(s) involved in the specific binding and mechanism of energy transfer. A membrane-bound phycobilisome complex (MP) from *Anabaena variabilis* has been isolated and characterized by Katoh and Gantt [21]. Successful isolation, dissociation and in vitro reassociation of such a complex from *Fremyella diplosiphon* has recently been reported by Kirilovsky et al. [22]. In the present work the specificity of binding and energy transfer as well as the role of the 94 kDa polypeptide in this process was investigated, using homologous and heterologous complexes formed by in vitro reassociation of phycobiliproteins and thylakoids of different cyanophytes and of unicellular phycobilisome-less algae in which a chlorophyll *a*,*b*-protein complex serves as the main light-harvesting antenna.

Materials and Methods

Algae

Fremyella diplosiphon and *Anabaena variabilis* were grown at 26 °C in the mineral medium described by Hughes et al. [23], as modified by Allen [24]. The culture of these algae was as previously described [22]. For growth of *F. diplosiphon* green or red light was used, while *A. variabilis* was grown in white fluorescent light.

Chlamydomonas reinhardtii y-1 cells were grown in semicontinuous cell culturing apparatus with acetate as a carbon source [25]. *Euglena gracilis* Z was grown as reported by Gurevitz et al. [26].

In vitro reassociation and trypsin treatment of phycobilisome-membrane complexes

Isolation of the phycobilisome-membrane complexes and reassociation experiments were carried out as described before [22]. Phycobilisome-membrane complexes in 0.3 M Na citrate and 0.5 M potassium phosphate buffer, pH 7.0 (0.2 mg chlorophyll/ml), were treated with bovine pancreas trypsin (type XI, 0.015 mg/ml) at 10°C. The reaction was stopped by the addition of 1 mM phenylmethylsulfonylfluoride (PMSF) and 0.06 mg/ml soybean trypsin inhibitor at different times.

Measurement of fluorescence emission spectra and photosynthetic activities

Room temperature spectra were recorded using a Perkin-Elmer spectrofluorimeter, model MPF4. The excitation was at 380 nm. Measurements of Photosystem II activity were carried out spectrophotometrically using diphenylcarbazide as an electron donor and 2,6-dichlorophenolindophenol (DCIP) as an electron acceptor [27].

Analysis of polypeptide pattern

Lithium dodecyl sulfate (LDS) polyacrylamide gel electrophoresis was carried out at 4°C, using 14% gel for isolated phycobilisomes, as described by Delepelaire and Chua [28].

Chlorophyll and phycobiliprotein concentrations were quantitated from absorption measurements according to the method of Bennett and Bogorad [29].

Results

Formation of hybrid membrane-bound phycobilisome complexes between phycobiliproteins and thylakoids of various cyanophytes

It has previously been reported that a mixture of phycobiliproteins obtained by dissociation of phycobilisomes in low salt concentrations can reassociate with isolated thylakoid membranes to form functional membrane-bound phycobilisomes [22]. Formation of hybrid complexes, using different phycobiliproteins and membranes from various cyanophyte species could also be obtained by the same procedure. The complexes formed by gradual increase in the salt concentration during 2.5 h of dialysis of appropriate mixtures of phycobiliproteins and membranes against 0.5 M phosphate buffer and 0.3 M sodium citrate, could be separated from the dialysis mixture by centrifugation on a linear sucrose gradient prepared in the same buffer. The sedimentation pattern of such hybrid complexes formed from *F. diplosiphon* or *A. variabilis* phycobiliproteins with membranes from

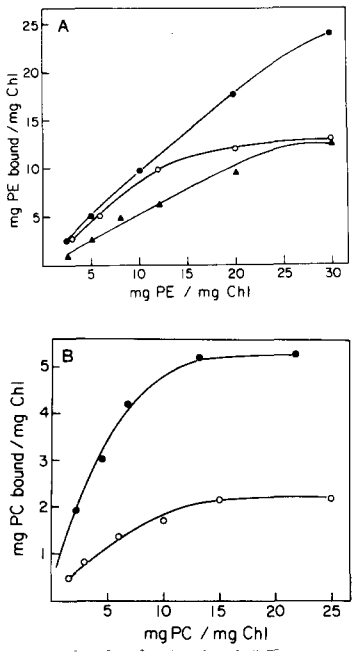

Fig. 1. Reassociation of phycobiliproteins and thylakoid membranes in homologous and heterologous systems as a function of their relative concentration in the reassociation mixture. Mixtures of phycobiliproteins and thylakoids, in which the phycobiliprotein concentration was kept constant and that of the membranes varied, were dialysed as described in Materials and Methods. The resulting phycobilisome-membrane complexes were isolated, and the ratio phycoerythrin/chlorophyll *a* or phycocyanin/chlorophyll *a* in the complexes was estimated. (A) *F. diplosiphon* phycobilisomes associated to *F. diplosiphon* membranes (○———○); *A. variabilis* membranes (●———●); and *C. reinhardtii* membranes (▲———▲). (Chlorophyll concentration was 0.02–0.3 mg chlorophyll *a*/ml, and phycoerythrin concentration was 0.6 mg/ml. PE, phycoerythrin. (B) *A. variabilis* phycobilisomes associated to *A. variabilis* membranes (●———●) and *F. diplosiphon* membranes (○———○). Membrane concentration was 0.012–0.6 mg chlorophyll/ml; phycobiliprotein concentration was 0.3 mg phycocyanin/ml.

A. variabilis or *F. diplosiphon*, respectively, showed the presence of a major band at apparent buoyant densities corresponding to between 28 and 35% sucrose, containing all the mebranes and at least 85% of the phycobiliproteins initially present in the reassociation mixture. The residual phycobiliproteins remained on top of the gradient.

Examination of room temperature fluorescence emission spectra of hybrid complexes showed the usual emission pattern characterized by peaks at 660 and 680 nm, as expected for native membrane-bound phycobilisomes. No emission of free or dissociated phycobiliproteins was detected.

Titration curves in which the amount of bound phycobiliproteins is measured as a function of the relative concentration of phycobiliproteins and membranes in the reassociation mixture, are shown in Fig. 1. In these experiments the phycobiliprotein concentration (measured as mg phycoerythrin or phycocyanin/ml) was kept constant, while that of the membranes (measured as mg chlorophyll *a*/ml) was varied. The results show that membranes of *A. variabilis* bind larger amounts of phycobiliproteins than those of *F. diplosiphon*, regardless of the source of phycobiliproteins. However, the total amount of phycobiliproteins bound/mg chlorophyll *a* was slightly higher when phycobilisomes containing phycoerythrin were used.

The ratio phycoerythrin to allophycocyanin in the reassociation mixtures containing *F. diplosiphon* phycobiliproteins was about 3 : 1. However, this ratio was found to be 5 or more in the bound reassociated complexes formed in reassociated mixtures containing limiting amounts of membranes (i.e., high ratio phycobiliprotein/membrane). The ratio phycocyanin to allophycocyanin in the reassociation mixtures containing *A. variabilis* phycobiliproteins was 2 : 1. The reassociated phycobilisomes formed in the presence of limiting amounts of membranes exhibited a higher ratio of phycocyanin to allophycocyanin (3 : 1). These results indicate that under conditions in which the membranes are the limiting factor, excess amounts of phycoerythrin or phycocyanin are attached to the phycobilisome-membrane complex (Fig. 1).

The stability of the reassociated complexes was also tested by measurements of their dissociation as a function of reducing the salt concentration (Fig. 2). The dissociation pattern of native complexes of *A. variabilis* or *F. diplosiphon* are shown in Fig. 2A. The dissociation pattern of the reassociated homologous complex of *A. variabilis* is similar to that of the native one (Fig. 2B). The dissociation pattern of *F. diplosiphon* complexes indicates that both the native (Fig. 2A) and the reassociated complexes (Fig. 2B) are less stable than those of *A. variabilis*. In reassociated complexes the stability appears to be dictated by the properties of the

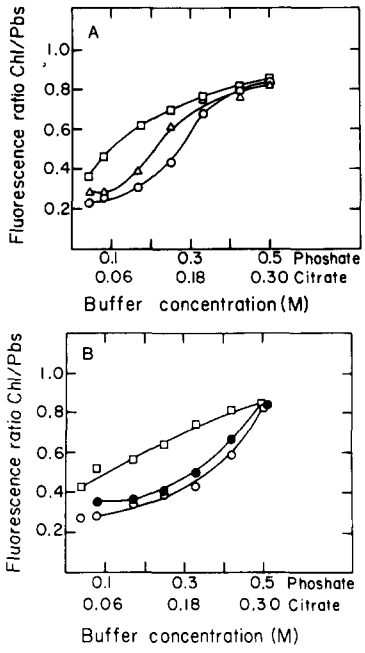

Fig. 2. Dissociation of native and in vitro reassociated homologous or heterologous phycobilisome-membrane complexes as a function of phosphate citrate buffer concentration. The different complexes were incubated in various concentrations of buffer for 15 min, and the ratio fluorescence 680/660 nm (chlorophyll/allophycocyanin) at room temperature was recorded. (A) Native complex of *A. variabilis* (□———□), *F. diplosiphon* grown in green light (○———○) and grown in red light (△———△). (B) Reassociated complexes with membranes and phycobilisomes of *A. variabilis* (□———□) membranes and phycobiliproteins of *F. diplosiphon* containing phycoerythrin (●———●) and *A. variabilis* membranes associated with phycobilisomes of *F. diplosiphon* (○———○).

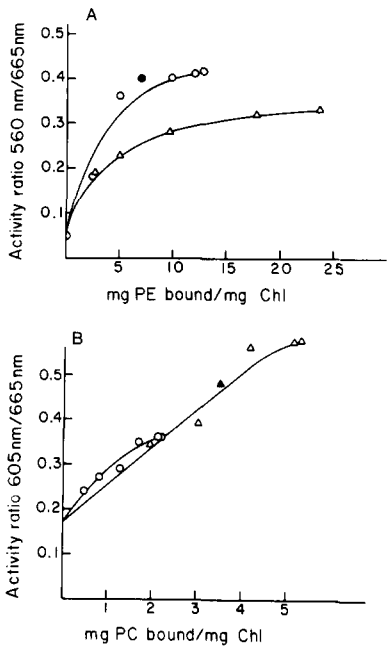

Fig. 3. Energy transfer from phycobilisome to Photosystem II in reassociated homologous and heterologous systems. (A) Phycobiliproteins of *F. diplosiphon* (grown in gree light) reassociated with *F. diplosiphon* (○———○) or *A. variabilis* (△———△) thylakoids; (●) native complex of *F. diplosiphon*. (B) Phycobiliproteins of *A. variabilis* reassociated to *A. variabilis* (△———△) or *F. diplosiphon* thylakoids (○———○); native complex of *A. variabilis* (▲).

phycobiliproteins and not by those of the membranes.

Energy transfer in homologous and heterologous complexes

In order to test the ability of reassociated heterologous complexes containing phycoerythrin to transfer energy to Photosystem II, the ratio of Photosystem II activity elicited by phycoerythrin absorbed light (560 nm) to that elicited by chlorophyll *a* absorbed light (665 nm) was measured [22]. The results show that energy transfer to photosystem II from *F. diplosiphon* phycobilisomes reassociated with *A. variabilis* membranes is lower than that obtained for homologous complexes (Fig. 3A).

A plateau is reached in energy transfer when the ratio of bound phycoerythrin to chlorophyll *a* is about 10 mg/mg in both complexes (Fig. 3A).

When *A. variabilis* phycobiliproteins were reassociated to *F. diplosiphon* membranes, energy transfer was similar to that obtained in the homologous complex containing *A. variabilis* phycobiliproteins and membranes at equal ratios of phycoerythrin bound/chlorophyll *a* (Fig. 3B). However, in the range of phycobiliprotein concentration used, no plateau of energy transfer was reached for complexes containing *A. variabilis* phycobiliproteins. Energy transfer increased with the amount of phycobiliproteins used, almost to the point of saturation of phycobiliprotein binding, indicating that in these complexes there is less non-specific binding as compared to the situation obtained when *F. diplosiphon* phycobiliproteins were used (compare Fig. 3A with 3B).

Energy transfer from phycobiliproteins of *F.*

TABLE I

ENERGY TRANSFER FROM *FREMYELLA DIPLOSIPHON* PHYCOBILISOMES TO PHOTOSYSTEM II IN HETEROLOGOUS REASSOCIATED COMPLEXES

PE, phycoerythrin; Chl, chlorophyll; MP, membrane-bound phycobilisomes.

Experimental system		Activity (μmol DCIP reduced/mg Chl per h)		Activity ratio 560 nm/665 nm	mg PE bound/mg Chl *a*
		Excitation 560 nm	Excitation ≥ 665 nm		
A. variabilis	membranes	7.1	119	0.06	–
	MP	33.7	134.8	0.25	6
Pseudosynechococcus [a]	membranes	10.1	72.2	0.14	–
	MP	23.6	84.4	0.28	5.2
Pseudanabaena [a]	membranes	5.35	71.4	0.075	–
	MP	16.5	82.7	0.2	8
Phycobilisomeless mutant of	membranes	8.14	90.5	0.09	–
F. diplosiphon	MP	21.2	92.3	0.23	8
C. reinhardtii	membranes	35	145.8	0.24	–
	MP	29.9	130	0.23	6
E. gracilis	membranes	12.2	81.3	0.15	–
	MP	11.5	82.2	0.14	5

[a] These cyanophytes were isolated from the Dead Sea by Dr. A. Oren.

diplosiphon to Photosystem II in the heterologous complexes occurred also when the membranes used were obtained from a phycobilisome-less mutant of *F. diplosiphon* as well as from *Pseudanabaena* and *Pseudosynechococcus* (Table I).

Binding of phycobiliproteins to membranes from green algae

In order to test the ability of phycobilisomes to transfer energy to photosynthetic membranes which do not contain phycobilisomes in situ, experiments were carried out in which *F. diplosiphon* phycobiliproteins were reassociated to *C. reinhardtii* and *E. gracilis* thylakoids. Membrane-bound phycobilisomes were obtained, as ascertained by examination of their sedimentation pattern in sucrose gradients which could resolve dissociated phycobiliproteins from free phycobilisome membranes and membrane-bound phycobilisomes (Fig. 4, see also Ref. 22). Room-temperature fluorescence-emission spectrum (Fig. 5) showed the presence of the phycobilisomes' characteristic 660 nm emission peak. Binding of phycobiliproteins to *C. reinhardtii* thylakoids as a function of their relative concentration is shown in Fig. 1. As opposed to the ability of reassociated phycobiliproteins of heterologous complexes to transfer energy to membranes obtained from cyanophytes, no energy transfer to Photosystem II was observed in heterologous complexes contain-

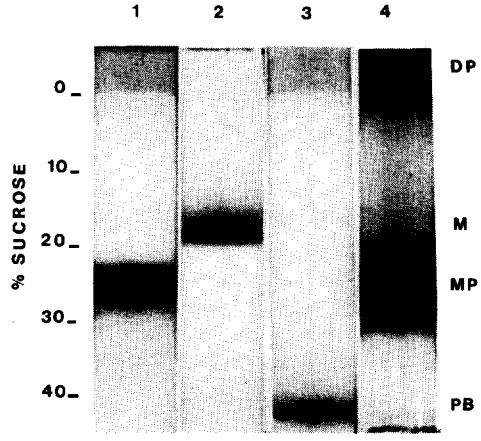

Fig. 4. Analysis by sucrose density gradient centrifugation of various preparations of in vitro associated phycobiliproteins and membranes. *F. diplosiphon* phycobilisomes associated with (1) *C. reinhardtii* or (4) *F. diplosiphon* membranes; (2) *C. reinhardtii* membranes; (3) reassociated phycobilisomes of *F. diplosiphon*. DP, dissociated phycobilisomes; M, membranes; MP, phycobilisome-membrane complex, PB, intact phycobilisomes.

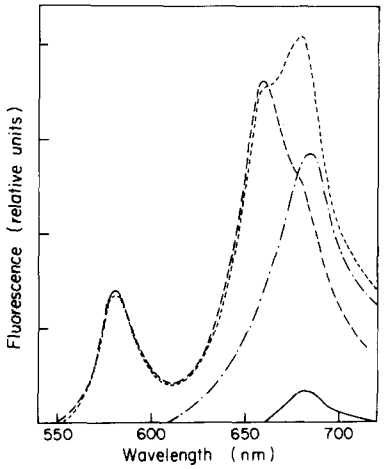

Fig. 5. Fluorescence emission spectra of *F. diplosiphon* membranes (———) of *C. reinhardtii* membranes (·–·–·), and of *F. diplosiphon* phycobiliproteins associated to *F. diplosiphon* membranes (— — —) or *C. reinhardtii* membranes (------). The chlorophyll concentration was 10 μg/ml and the excitation was 380 nm.

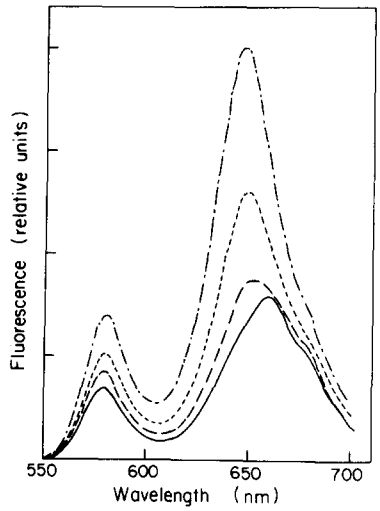

Fig. 6. Fluorescence emission spectra of phycobilisomes released from membrane-bound complexes following trypsin treatment of native complexes. The released phycobilisomes were separated from the rest of the complex by centrifugation (80000 × *g*, 10 min at 5°C) after 3 min (— — —), 15 min, (------) and 120 min (·–·–·) of trypsin incubation. (———) Fluorescence emission spectrum of reassociated phycobilisomes

ing *C. reinhardtii* or *E. gracilis* membranes (Table I).

Effect of trypsinization on binding of phycobiliproteins to thylakoid membranes

Trypsin treatment of native membrane-bound phycobilisomes resulted in their gradual dissociation from the membranes and release of phycobilisomes in which the characteristic fluorescence emission band at 680 nm is reduced, while the 650 nm emission band is increased (Fig. 6).

Dissociated phycobiliproteins obtained from a membrane-bound phycobilisome complex treated with trypsin for increasing periods of time, gradually lost their ability to reassociate and form func-

TABLE II

DISSOCIATION AND REASSOCIATION OF MEMBRANE-BOUND PHYCOBILISOMES FOLLOWING TRYPSIN TREATMENT

Native *Fremyella diplosiphon* membrane-bound phycobilisome complexes were treated with trypsin, as indicated. At various times, aliquots were taken and the residual membrane-bound phycobilisomes were isolated by centrifugation (A); the residual trypsin treated samples were dissociated and reassociated as described in Materials and Methods, and the reassociated membrane-bound phycobilisomes were isolated by centrifugation (B). PS II, Photosystem II; PE, phycoerythrin; Chl, chlorophyll.

Time of trypsin incubation (min)	A Native phycobilisome-membrane complex		B Reassociated phycobilisome-membrane complexes after trypsin treatment	
	PE bound/Chl *a* (mg/mg)	PS II activity ratio: 560/665	PE bound/Chl *a* (mg/mg)	PS II activity ratio: 560/665
0	8.2	0.59	7.2	0.47
15	3.2	0.41	2.7	0.27
30	3.1	0.36	2.8	0.29
120	2.3	0.32	2.6	0.26

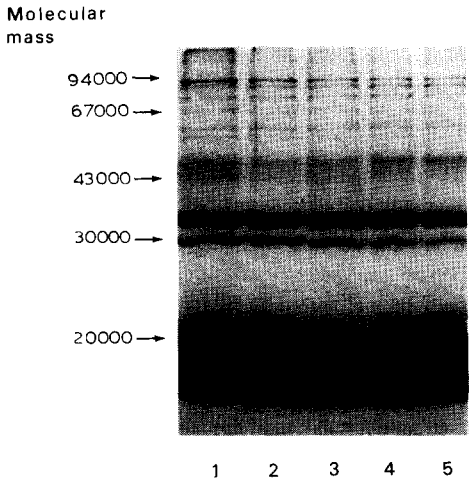

Fig. 7. Polypeptide pattern on lithium dodecyl sulfate polyacrylamide gel electrophoresis of isolated phycobilisomes from phycobilisome-membrane complexes after 0 (1), 3 (2), 6 (3), 15 (4) and 30 min (5) of trypsin incubation. Experimental conditions were as in Table II.

tional membrane-bound phycobilisomes. The residual energy-transfer activity of these trypsin-treated complexes was proportional to the fraction of reassociated phycobilisomes in both native and reassociated complexes (Table II). An examination of the polypeptide pattern of reisolated phycobiliproteins obtained from trypsin-treated complexes

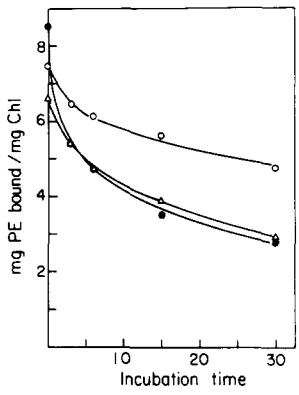

Fig. 8. Effect of trypsin treatment on membrane-bound phycobilisome complexes. Dissociation of the native *F. diplosiphon* phycobilisome-membrane complex as a function of trypsin incubation (●———●); reassociation of the phycobiliprotein obtained from trypsin-treated *F. diplosiphon* complexes for various time periods to *F. diplosiphon* (△———△) or to *C. reinhardtii* (○———○) membranes.

showed an initial loss of the 94–95 kDa polypeptide, followed by a gradual degradation of the 27 kDa polypeptide and the appearance of lower apparent molecular weight degradation products (Fig. 7). Trypsin treatment of isolated thylakoids did not prevent their ability to bind phycobiliproteins and form energy-transferring membrane-bound phycobilisomes. No significant changes in the thylakoid polypeptide pattern of trypsin-treated membranes were observed (data not shown).

Gradual loss of the 94–95 kDa polypeptide due to progressive trypsinization prevented reassociation of the phycobiliproteins to both cyanophytes and *C. reinhardtii* membranes (Fig. 8).

Discussion

In a previous work we have demonstrated that dissociated phycobiliproteins can be reassociated in vitro, in the presence of isolated homologous thylakoids, to form functional membrane-bound phycobilisomes [22]. The reassociated complex was similar to the native complex from which the phycobiliproteins and membranes were derived in terms of its spectroscopic properties, specificity of energy transfer to Photosystem II, polypeptide composition and structure, as observed by electron microscopy. Analysis of reassociation and binding of phycobiliproteins and membranes as a function of their relative concentration revealed two types of phycobilisome binding i.e., binding to sites specific for Photosystem II resulting in functional association, and non-specific binding whereby phycobilisomes can attach to the membranes but do not trasfer energy either to Photosystem II or I [22].

These results prompted us to asses whether specific binding could also be obtained in heterologous systems in which the phycobiliproteins and thylakoids used would be obtained from different cyanophytes. The data obtained here demonstrate that assembly and specific binding of phycobilisomes to Photosystem II can also occur in heterologous systems, exhibiting properties quite similar to those of native or homologous systems, as described before [22].

The density of the specific Photosystem II binding sites varies in different membranes. Thus, *A.*

variabilis thylakoids bind more functional phycobilisomes, both homologous and heterologous, than *F. diplosiphon* thylakoids. The amount of phycobilisomes bound is also affected by the presence of phycoerythrin which seems to increase the amount of non-specific binding. This seems to be due to the hydrophobicity of phycoerythrin [20] which might interact by hydrophobic non-specific bonds, either with the hydrophobic phase of the membrane or with other membrane-bound phycobilisomes.

The stability of the phycobilisome-membrane association in low salt concentration is somewhat lower in reassociated complexes as compared with native ones. The dissociation pattern of heterologous complexes as a function of lowering the salt concentration seems to be influenced primarily by the phycobilisome's origin rather than by that of the binding membrane. However, at the usual phosphate-citrate buffer concentration of 0.5 and 0.3 M, respectively [22], both the homologous and heterologous complexes are stable and transfer energy to Photosystem II in a way comparable to that of the native homologous complexes.

As previously demonstrated for the homologous complexes [22], non-specific binding also occurs in heterologous complexes after saturation of the specific binding sites. However, when the thylakoids used for the reassociation experiments are derived from organisms in which a chlorophyll *a,b*-protein complex serves as the major light-harvesting antenna of Photosystem II, only non-specific binding can be detected.

It has been reported earlier that binding of phycobilisomes to Photosystem II and energy transfer require the presence of a 94–95 kDa polypeptide [17–20], which is a constituent of the phycobilisome and might appear in the thylakoids as well [19]. The results presented here clearly demonstrate that while the presence of this polypeptide is required for the binding of phycobilisome to the membranes, it is not sufficient for the formation of the specific link and energy transfer to Photosystem II. Thus, mild trypsin treatment which causes a rapid degradation of this polypeptide in phycobilisome-membrane complexes, releases intact phycobilisomes (see also Ref. 31). Phycobilisomes in which the 94–95 kDa polypeptide has been degraded by proteolysis cannot bind again to intact thylakoids of either homologous or heterologous cyanophytes or thylakoids from algae such as *C. reinhardtii* in which only non-specific binding occurs.

It should be noted that energy transfer to Photosystem II of the latter membranes does not occur, even if this polypeptide is intact and present in the normal amount in the reassociated phycobilisome. Based on these results, we conclude that the formation of a Photosystem II specific binding site for energy transfer requires the presence of additional polypeptide(s) characteristic of the Photosystem II complex of thylakoids from organisms which usually contain phycobilisomes and which are yet to be identified. The fact that mild trypsin treatment of the phycobilisome-membrane complex sufficient to degrade the 94–95 kDa polypeptide does not affect the membrane, suggests that such components, if present, are not available to proteolysis under these experimental conditions. The possibility should also be considered that the presence of the light harvesting chlorophyll *a,b*-protein complex in green algal thylakoids, whose partially surface-exposed polypeptides are hydrophilic and charged [32,33], might prevent the hydrophobic interaction between the 94–95 kDa polypeptide of the phycobilisome and the core antenna reaction center II complex, which is buried in the membrane [34]. If this were the case, energy transfer might occur from phycobilisomes to isolated photosystem II particles lacking the light-harvesting complex. Experiments designed to test this possibility are now in progress.

An additional aspect of the results presented in this work is related to the composition of the reassociated phycobilisomes formed at various relative concentration ratios of phycobiliproteins and thylakoids. In reassociation conditions in which the thylakoids were present at limiting concentrations relative to phycobiliproteins, a higher ratio of phycoerythrin or phycocyanin to allophycocyanin was found in the reassociated complex than in the original phycobiliprotein mixture used for reassociation. Assuming that the amount of 94–95 kDa polypeptide and allophycocyanin bound, which form the core of phycobilisomes, is limited by the binding sites on the membrane, one would expect that in mixtures containing excess amounts of phycobiliproteins relative to the membranes,

phycobilisomes will be formed with larger amounts of phycocyanin or phycoerythrin, thus resulting in higher ratios of phycoerythrin or phycocyanin to chlorophyll *a* than in the native complex. Addition of phycoerythrin alone to already formed phycobilisomes containing only phycocyanin and allophycocyanin might occur during the early phase of the chromatic adaptation process in *F. diplosiphon*. It was reported before that a significant increase in the ratio phycoerythrin/phycocyanin could be detected within 5–7 h following transfer of cells grown in red light to green light [35]. Under these conditions, no detectable increase could be observed in the total content of phycocyanin or chlorophyll *a* of the cultures. Furthermore, the newly formed phycoerythrin was integrated into functional phycobilisomes, as ascertained by measurements of fluorescence excitation-emission spectra (Kirilowsky, D. and Ohad, I., unpublished data). Hence, the in vitro reassociation experimental system desribed here might mimic to some extent the process of in vivo assembly of phycobilisomes. Whether the 'large' phycobilisomes containing excess phycocyanin or phycoerythrin are evenly distributed between specific and non-specific binding sites, or are bound only to one of these two types of sites, cannot yet be decided on the basis of the data available so far. Experiments aimed towards elucidating these questions are now under way.

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