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Communication

N-Terminus Conservation in the Terminal Pigment of Phycobilisomes from a Prokaryotic and Eukaryotic Alga[1]

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ABSTRACT

High molecular weight polypeptides from phycobilisomes, believed to be involved in facilitating the energy flow from phycobilisomes to thylakoids, are conserved in the prokaryote *Nostoc* sp. and the eukaryote *Porphyridium cruentum*. Partial N-terminal sequence analysis of the phycobilisome-polypeptides of *Nostoc* (94 kilodalton) and *Porphyridium* (92 kilodalton) revealed 55% identity in the first 20 residues, but no significant homology with sequences of other phycobiliproteins or phycobilisome-linkers. Polypeptides (94 and 92 kilodalton) from *Nostoc* thylakoids free of phycobilisomes, previously presumed to be involved in the phycobilisome-thylakoid linkage (M Mimuro, CA Lipschultz, E Gantt 1986 Biochim Biophys Acta 852: 126) exhibit the same immunocrossreactivity but are different from the 94 kilodalton-phycobilisome polypeptide by having blocked N-termini and a different amino acid composition.

Specific high mol wt polypeptides appear to serve as conduits in energy transfer from phycobilisomes to thylakoids. Only those isolated from phycobilisomes have been partially characterized until now. They range in mol wt from about 75 to 120 kD as measured by their migration upon polyacrylamide gel electrophoresis (4, 5, 14). Their location in the core of the phycobilisome has been clearly established in several species (6, 17). These polypeptides are blue in color, and have long been regarded as representing the terminal pigment of the phycobilisome because their fluorescence maxima are longer (>660 nm) than that of allophycocyanin (4, 6, 9, 12). An absorbance maximum at 665 nm and an emission maximum at 683 nm are characteristic of the 94 kD polypeptide isolated from *Nostoc* phycobilisomes (10). Allophycocyanin-B, with a much lower mol wt, also has long wavelength spectral properties which are similar to, but not identical with those of the high mol wt polypeptides (8). The analysis from *Nostoc* suggests that energy transfer from the phycobilisome to photosystem II through the 94 kD polypeptide is considered to be more likely than through allophycocyanin-B (10).

The presence of high mol wt polypeptides in thylakoids with a migration pattern on SDS-PAGE similar to the polypeptides in phycobilisomes, led to the suggestion that they are involved as a phycobilisome-thylakoid 'anchor' (15). This was further strengthened when it was shown that antibodies, specific for the 94 kD phycobilisome-polypeptide (*Nostoc*), immunoprecipitated high mol wt polypeptides from Triton-solubilized thylakoids (free of phycobilisomes) (10). This assumption is now being reconsidered in view of new amino acid analysis data presented here.

Conservation in the protein structure of high mol wt polypeptides across phylogenetic lines has been inferred from immunocross-reactivity studies, because high mol wt polpeptides (*e.g.* 75–120 kD) in phycobilisomes isolated from many red algae and cyanobacteria were shown to cross-react with antisera to the 94 kD polypeptide of *Nostoc* (4, 18). In this contribution we present the first partial N-terminal sequences from a prokaryotic and eukaryotic alga, which show a high degree of homology in the sequence of terminal phycobilisome pigments of *Porphyridium* and *Nostoc*.

MATERIALS AND METHODS

Thylakoid membranes were prepared at 4°C from cells of *Nostoc* passed twice through a French pressure cell (10,000 p.s.i.) in 10 mM HEPES/0.3 M sucrose/10 mMEDTA (pH 7.5). Thylakoid membranes remained in the supernatant following a 10,000*g* centrifugation (10 min) to remove cell debris. The thylakoids were concentrated by adding $MgCl_2$ to a final concentration of 15 mM. They were subsequently pelleted (27,000*g*, 20 min), and twice more resuspended in the buffer plus $MgCl_2$ with a glass homogenizer and pelleted, until finally being concentrated between layers of 0.75 and 2.0 M sucrose in the buffer above (95,000*g*, 1 h). Phycobiliproteins were no longer detectable by absorbance spectroscopy after these treatments. Additional rinses did not affect the 92 to 94 kD polypeptide concentration in the thylakoid preparations, nor did rinsing with 0.1% Triton X-100/50 mMNa-phosphate buffer/1 mMEDTA (pH 7.0).

The rinsed thylakoid samples were fully solubilized at 80°C (5 min) in 2% LiDS (20:1 detergent to Chl) and 15 mM dithiothreitol. They were analyzed on 9 to 15% Laemmli gels containing 0.1% LiDS and stained with Coomassie blue for scanning. For isolation of the 92 to 94 kD polpeptides 6% Laemmli gels were used since greater separation was obtained. These bands were excised and the electrophoresis was routinely repeated, but other polypeptide bands were not detectable at any gel concentration. For amino acid analysis the 94 and 92 kD thylakoid polypeptides were treated similar to the 94 kD phycobilisome polypeptide of *Nostoc* sp.

High mol wt polypeptides were prepared from isolated phycobilisomes of *Nostoc* sp. and *Porphyridium cruentum*, and separated by SDS (or LiDS) gel electrophoresis (10, 12). The gel bands were excised and the electrophoresis step repeated. The

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polypeptides were electroeluted from the mashed gel, and exhaustively dialyzed (4°C, 5 changes, 2 L, 2 d) resulting in low concentrations of phosphate buffer (pH 7.0) and detergent. For amino acid analysis and sequencing the final buffer concentration was 0.004% LiDS and 2 mM K-phosphate for *Nostoc*, and 0.002% SDS and 0.5 mM Na-phosphate for *P. cruentum*. Polypeptides thus isolated are designated by species, source, and apparent mol wt on PAGE: N-PBS-94 was derived from *Nostoc* phycobilisomes with mol wt of 94 kD; N-T-94 and N-T-92 from *Nostoc* thylakoids with mol wt of 94 and 92 kD, respectively; and PC-PBS-92 from *Porphyridium cruentum* phycobilisomes with mol wt of 92 kD.

For crossed immunolectrophoresis the procedure of Plumley and Schmidt (11) was adapted.

For amino acid analysis, and amino acid sequencing by gas phase analysis, we acknowledge the service of A. Smith.[4]

RESULTS AND DISCUSSION

A similarity in the protein structure of the terminal pigment of phycobilisomes from different organisms was previously suggested from immunoreactivity studies. It had been shown that in 15 cyanobacterial and 10 red algal species a high mol wt component was recognized by antibodies to a 94 kD polypeptide of *Nostoc* sp. isolated from phycobilisomes (4, 18). A comparison of the primary structure of high mol wt polypeptides from phycobilisomes of the prokaryote *Nostoc* sp. and the eukaryote *Porphyridium cruentum* was therefore carried out.

In Table I is shown the sequence of the first 20 amino acid residues of the *Nostoc* (N-PBS-94) and *Porphyridium* (PC-PBS-92) polypeptides. Eleven of the 20 residues are identical, with 5 more differing by a single base change in the codon. The sequences, although accounting for only a few percent of the total protein, are remarkable in several respects. First, the degree of homology is high between the analogous proteins of a prokaryote and a eukaryote. In addition, they lack any similarity to the N-terminal sequences of phycobilisome-rod-linker polypeptides, or any other of the many phycobiliproteins whose sequence has been determined (1–3, 7, 16, 19). Finally, conservation of sequence homology in other phycobiliproteins tends to be at the C-terminus rather than at the N-terminus (19).

An earlier suggestion that the high mol wt polypeptide may be a protein partially located in the phycobilisome and partially in the thylakoid was based on immunoprecipitation studies in *Nostoc* (5, 10). It had been found that polyclonal antibodies, apparently monospecific for N-PBS-94, immunoprecipitated high mol wt polypeptides not only from phycobilisomes, but also from thylakoids from which phycobiliproteins had been removed by exhaustive rinsing (10). The single polypeptide from phycobilisomes (N-PBS-94), and the two polypeptides from thylakoids (N-T-94, N-T-92), were further investigated in *Nostoc*. The N-PBS-94 represents about 1.5% of the protein in isolated phycobilisomes, and the N-T-94 and N-T-92 together (in a ratio of about 3:2, respectively) represent about 6% of the Coomassie blue stainable protein in rinsed thylakoids (5).

Each of the three polypeptides, N-PBS-94, N-T-94, and N-T-92, gave an immune reaction with the same antibody (Fig. 1). From this, it can be assumed that they have similar epitopes. This result also shows that N-T-92 was not a nonspecific co-isolating protein, as might have been assumed for the previous immunoprecipitation results (*cf.* 10, Fig. 5). Whereas it is possible that the antigen from isolated phycobilisomes was contaminated with the 92 or 94 kD thylakoid-polypeptide, our evidence does not support this. On Ouchterlony double diffusion plates (not shown) there was a single line of identity, without spurring, against N-PBS-94 (antigen), phycobilisomes, and thylakoids.

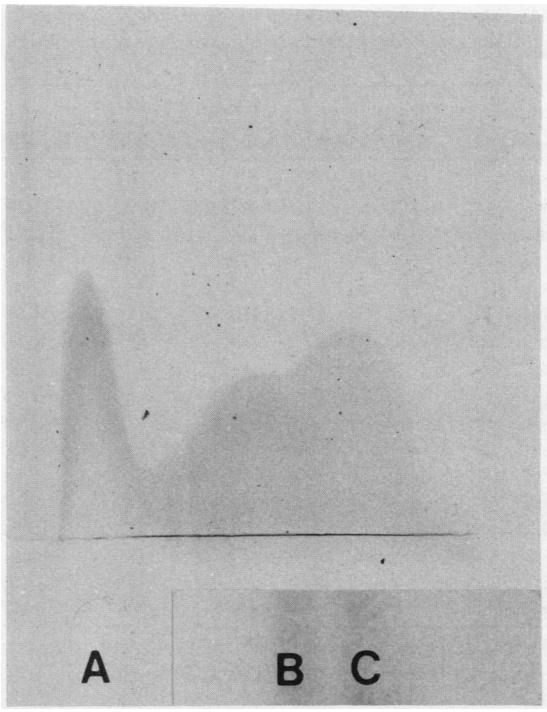

FIG. 1. Crossed immunoelectrophoresis pattern of high mol wt polypeptides of *Nostoc* thylakoids reacted with antisera to 94 kD-*Nostoc*-phycobilisome polypeptide, stained with Coomassie blue. Sample placement: A, N-PBS-94 polypeptide applied in solution; B, N-T-94 polypeptide; C, N-T-92 polypeptide applied in strip of gel.

In attempting to ascertain the N-terminal sequence of the three polypeptides we found that for N-T-94 and N-T-92 this terminus was blocked, unlike that of N-PBS-94 which had been purified from phycobilisomes by the same method as that used to purify the thylakoid polypeptides. Also, a comparison of the amino acid composition (Table II) revealed significant differences between the polypeptides from phycobilisomes and thylakoids. The thylakoid polypeptides, with a greater proportion of negatively charged residues, appear to be more acidic and probably more hydrophobic (higher glycine). Only the thylakoid polypeptides contained methionine, and they had a greater histidine content as well. Like the *Nostoc* phycobilisome polypeptide, that from *Porphyridium* phycobilisomes (PC-PBS-92), also had a low histidine content, an absence of methionine, and relatively greater

[4] We acknowledge the service of the Protein Structure Research Laboratory of the University of California, Davis.

Table I. *Amino Acid Sequence at the NH_2 Terminus of the High Mol Wt Polypetides from Phycobilisomes of Nostoc sp. and Porphyridium cruentum*

Residues in parentheses represent one determination, others are from two determinations.

	1	5	10	15	20
Nostoc:	(S)-V-K-A-S	-G-G-S-S-	V-A-(R)-P-Q-	L-Y -Q-(G)-L-(	A)-V-
Porphyridium:	V-() -K-A-S	-G-G-S-P-	V-V- K -P-Q-	L-Y-(K)-()-	A-(S)-

Table II. *Amino Acid Composition of High Mol Wt Polypeptides from Porphyridium cruentum Phycobilisomes and Nostoc sp. Phycobilisomes and Thylakoids*

Amino Acids	Residues[a]/1000			
	N-PBS-94	N-T-94	N-T-92	PC-PBS-92
Asp	82	73	76	107
Ser	85	102	109	87
Thr	55	36	36	42
Glu	144	116	120	119
Pro	48	33	31	42
Gly	94	270	278	99
Ala	91	80	75	70
Val	60	51	49	48
Cys	3	7	5	6
Met	0	4	3	0
Ile	49	36	36	67
Leu	71	58	57	88
Tyr	44	29	24	42
Phe	52	29	27	59
Lys	48	29	27	61
His	5	11	11	6
Arg	69	36	36	57

[a] The residues/1000 residues indicate the average of at least two amino acid analyses of 24 h hydrolysates of each polypeptide.

proportions of arginine, phenylalanine, lysine, tyrosine, and proline.

The difference in the amino acid composition does not support our previous assumption that the 94 kD polypeptides from phycobilisome and from thylakoids are the same protein in *Nostoc*, the immnounocross-reactivity results notwithstanding. It appears highly probable that in *Nostoc* the high mol wt phycobilisome-polypeptide is distinct from the thylakoid-polypeptides, but that they all share common epitopes. It is also possible that the thylakoid components are glycosylated, and perhaps thereby blocked. A high mol wt polypeptide, blocked at the N-terminus, was recently reported in *Anacystis* (13). Whether this is analogous to either the phycobilisome or thylakoid polypeptides of *Nostoc* cannot be determined because the isolation conditions did not involve separation of phycobilisomes and thylakoids.

Assuming that the high mol wt polypeptides in thylakoids (N-T-94 and N-T-92) are distinct from that of the phycobilisome (N-PBS-94), they may have significant functions in the phycobilisome-thylakoid attachment. It is possible that the thylakoid polypeptides constitute a complex involved in the proper attachment of the phycobilisome and the proper chromophore orientation of the phycocyanobilins and the Chl. In this regard, it is interesting to note the higher histidine content in the thylakoid polypeptides as possible binding sites of Chl. Experiments are in progress to look for Chl-binding high mol wt polypeptides from *Nostoc* thylakoids.

It will also be interesting to ascertain whether these polypeptides are encoded in different genes or are differently processed proteins of the same gene product.

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