HOMOLOGY OF AMINO-TERMINAL REGIONS OF C-PHYCOCYANINS FROM A PROKARYOTE AND A EUKARYOTE

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Received May 28,1974

SUMMARY: Comparison of the amino-terminal sequences of the α and β subunits of C-phycocyanin of a prokaryotic blue-green alga of Synechococcus sp. (Anacystis nidulans), determined in this laboratory, with the corresponding sequences for the subunits of C-phycocyanin of the eukaryotic red alga Cyanidium caldarium, recently presented by Troxler et al. (1974), Federation Proc. 33, 1258 Abs., shows extensive homology between these proteins. This confirms earlier conclusions on the evolutionary relationship of Cyanophyta and Rhodophyta based on immunological studies (Glazer et al. (1971) Proc. US Nat Acad. Sci. 68, 3005-3008).

The presence of photosynthetic accessory pigments, the phycobiliproteins (allophycocyanin, phycocyanin, phycoerythrin) in the prokaryotic Cyanophyta and the eukaryotic Rhodophyta has led to frequent suggestions of close evolutionary relationship between these two groups of organisms (see ref. 1 for a discussion and citations of the original literature). Recent investigations of the intracellular localization and of the molecular structure of the phycobiliproteins have lent powerful support to this belief. In the blue-green algae, the phycobiliproteins are located in discrete particles, the phycobilisomes associated with the surface of the photosynthetic lamellae (2). In red algal cells, they occupy an equivalent location within the chloroplast (3). In both blue-green and red algae, the monomer of each phycobiliprotein is made up of two distinct polypeptide chains, α and β, each carrying chromophore(s) (4-6). A particularly striking finding was the observation of extensive immunological cross-reactivity between corresponding blue-green and red algal phycobiliproteins (7,8). Further evidence of remarkable conservation of molecular structure was provided by the demonstration that the α and β subunits of phycocyanins derived from unrelated unicellular and filamentous blue-green algae could be combined to form stable hybrid molecules (9).

To provide additional evidence of the common evolutionary origin of blue-green and red algal phycobiliproteins, we have initiated the determination of the primary amino acid sequence of C-phycocyanin from a unicellular blue-green alga of Synechococcus sp. (Anacystis nidulans) with the aim of comparing it to the sequence of a phycocyanin of red algal origin. A partial comparison has now become possible with the recent publication of the amino-terminal sequences of the α and β subunits of the C-phycocyanin of the unicellular red alga Cyanidium caldarium (10). This comparison has revealed an impressive degree of homology between the sequences of the prokaryotic and eukaryotic proteins.

MATERIALS AND METHODS

Anacystis nidulans (Synechococcus sp., strain 6301) phycocyanin and its α and β subunits were purified as previously described (6,9).

Determination of amino-terminal sequences was carried out with the aid of the Beckman 890C Sequencer using the dimethylbenzylamine program of Hermondson et al. (11). Conversion of the phenylthiazolinones to the phenylthiohydantoins was performed by heating at 80° for 10 min in 1 N HCl. The PTH-amino acids were extracted with ethyl acetate and were identified, in each instance, by gas chromatography, by thin layer chromatography (12), and by amino acid analysis after regeneration in 6 N HCl at 150° for 22 hours. Aliquots of the aqueous phase from the ethyl acetate extraction were spotted on Whatman 3 MM paper and examined for the presence of arginine with the Sakaguchi reagent.

RESULTS AND DISCUSSION

The comparison of the amino-terminal sequences of the α and β subunits of the C-phycocyanins of Anacystis nidulans and Cyanidium caldarium is shown in Table I. Identical residues are present in 22 of 32 positions in the amino-terminal sequences. Substitutions representing single base changes in the codon account for a further five positions. The data leave little doubt that the genes coding for the α and β subunits of C-phycocyanin in these two organisms arise from the same ancestral gene. Parenthetically, the taxonomic position of

TABLE I

Comparison of the Amino-terminal Sequences of the α- and β-Subunits of the C-Phycocyanins of *Anacystis nidulans* and *Cyanidium caldarium*[a]

Phycocyanin α-subunit	5 10 15
Anacystis nidulans	Ser-LYS-THR-PRO-Leu( )GLU-ALA-Val-ALA-ALA-ALA-ASX-( )( )GLY-
Cyanidium caldarium[b]	Met-LYS-THR-PRO-Ile-Thr-GLU-ALA-Ile-ALA-ALA-ALA-ASN-(Ala)-Arg-GLY-
Phycocyanin β-subunit	5 10 15
Anacystis nidulans	Thr-Phe-Asp-ALA-PHE-Thr-LYS-VAL-VAL-ALA-Gln-ALA-Asp-ALA-ARG-GLY-GLU-PHE-Leu-
Cyanidium caldarium[b]	Met-Leu-Asn-ALA-PHE-Ala-LYS-VAL(VAL)ALA-Ala-ALA-Asn-ALA-ARG-GLY-GLU-PHE-Lys-

[a] Identical residues are given in capitals; substitutions involving a single base change in the codon are underlined.

[b] Data from Troxler *et al.* (10).

TABLE II

Amino-terminal Residues Determined by Edman Degradation of C-Phycocyanin of Oscillatoria agardhii

(Data of Torjesen and Sletten (14))

The yield of each amino acid at each position was expressed in percentage of the total amount of the amino acids recovered at each position. The recovery was determined by comparing the total amount of the amino acid recovered at each step with the amount of protein taken as starting material.

Position	1	2	3	4	5	6	7
Yield (%)	Met 100	Phe 48 Lys 52	Asp Thr	Ala 46 Pro 54	Phe 50 Leu 50	Ser	Glu 42 Lys 58
Recovery (%)	83	48		37	33		26

Position	8	9	10	11	12	13
Yield (%)	Ala 46 Val 54	Val 67	Ala Ser	Gln Ser	Ala 60	Asp 48
Recovery (%)	27	25			23	18

the eukaryote Cyanidium caldarium as a red alga appears to be secure (13). Troxler et al. (10) have pointed out that a 60% homology exists within the first 27 residues of the α and β chains of Cyanidium C-phycocyanin. Similar, though less marked, evidence of evolutionary relationship may be discerned by comparison of the subunit sequences of the blue-green algal protein.

Information on the amino acid sequence of the α and β subunits of C-phycocyanins of filamentous blue green algae is lacking. However, Torjesen and Sletten (14) have published the quantitative results of the Edman degradation of the C-phycocyanin of the filamentous blue-green alga Oscillatoria agardhii. A summary of their results is given in Table II. The data of Torjesen and Sletten (14) is compatible with the following amino-terminal sequences for the phycocyanin of Oscillatoria agardhii, aligned by homology with Anacystis phycocyanin (residues in identical positions in the two proteins are capitalized):

α subunit:

Met-LYS-THR-PRO-LEU-Ser-GLU-ALA-VAL-(Ser)-(Ser)-ALA-ASP-

β subunit:

Met-PHE-ASP-ALA-PHE-Ser-LYS-VAL-VAL-ALA-GLN-ALA-ASP-

Although data on the separate subunits would be preferable, near-identity of sequence in the amino-terminal regions of the corresponding subunits of the two cyanophytan proteins is suggested by this preliminary comparison.

More extensive sequence information on blue-green and red algal phycocyanins may indicate which representatives of the former diverse group of organisms were related to the closest evolutionary precursors of the red algae, or of the red algal chloroplast.

Acknowledgments: This investigation has been supported in part by Grant GM 11061 and Training Grant GM 00364 from the National Institute of General Medical Sciences, United States Public Health Service.

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