Amino acid sequences of allophycocyanin α- and β-subunits isolated from *Anabaena cylindrica*

Presence of an unknown derivative of aspartic acid in the β-subunit

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Allophycocyanin (APC) α- and β-subunits were isolated from *Anabaena cylindrica* and their amino acid sequences were determined. The α- and β-subunits consisted of 160 and 161 amino acid residues, respectively, and lacked tryptophan. The β-subunit contained an unknown derivative of aspartic acid. The sequences were compared with those of other allophycocyanins.

Allophycocyanin A. cylindrica *Amino acid sequence* *Aspartic acid derivative*

1. INTRODUCTION

Red algae and blue-green algae contain light-gathering protein complexes called phycobilisomes that absorb light of wavelength 500–650 nm. The solar energy absorbed is transferred to chlorophyll *a* with high efficiency through a regular array of various phycobiliproteins [1]. Allophycocyanin (APC) is a common component of all phycobilisomes and forms a core for the energy transfer from phycobilisomes to chlorophyll *a*. The molecule consists of 2 subunits, α and β, which form an $(\alpha,\beta)_3$ quaternary structure in phycobilisomes. Each subunit binds covalently to a phycocyanobilin (PCB) molecule through a cysteine residue of the protein [2,3]. The amino acid sequences of both subunits of APC have so far been determined from *Mastigocladus laminosus* [4] and *Cyanidium caldarium* [5], and only for the β-subunit of APC from *Anabaena variabilis* [6]. This paper describes the primary structure of both subunits of APC from *A. cylindrica*, a blue-green alga, and compares them with those of 3 other algal APC.

2. MATERIALS AND METHODS

All reagents and chemicals are described in [7]; *A. cylindrica* APC was prepared as in [8]. The purified APC was separated into α- and β-subunits by chromatography on a DEAE-cellulose column (2.5 × 35 cm) with a linear concentration gradient of ammonium acetate of 20–200 mM containing 8 M urea at pH 6.0. Each subunit was fragmented by CNBr treatment and the fragments chromatographed on an SP-Sephadex C-25 column (1.5 × 32 cm) with a linear concentration gradient of NaCl of 50–400 mM containing 8 M urea and 0.6% phosphoric acid. Enzymic digestions, separation of the digests, amino acid analysis, and N- and C-terminal sequence analysis were carried out essentially according to established methods, the references to these methods being cited in [7].

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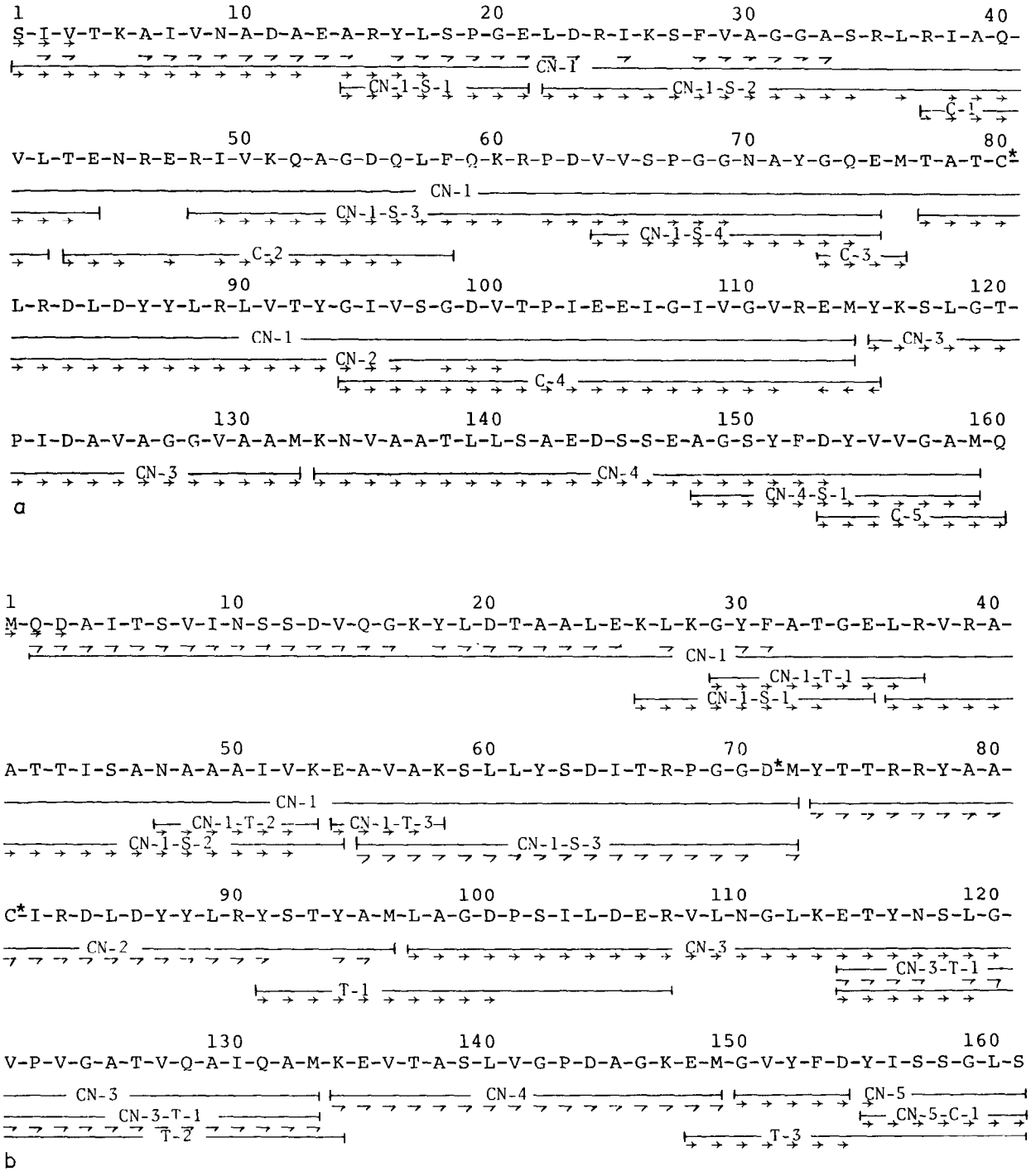

Fig.1. Summaries of the sequence studies of *A. cylindrica* allophycocyanin: (a) α-subunit and (b) β-subunit. (⟶, ⟶, ⟵) Solid-phase and manual Edman degradation, and carboxypeptidase Y and/or A digestion, respectively. (CN-, S-, T-, C-) CNBr, staphylococcal V8 protease, tryptic, and chymotryptic peptides, respectively. C*, cysteine binding PCB; D*, an unknown amino acid, probably a derivative of aspartic acid.

Peptide binding PCB was subjected to Edman degradation and the PCB-binding residue thus released was dissolved in ethanol to confirm the bound pigment by spectral measurement showing the typical absorbance at 600 nm. The cysteine residue binding to the pigment was identified on an amino acid analyzer after hydrolysis with 6 N HCl containing 0.21 M dimethyl sulfoxide [9].

An unknown amino acid was identified by amino acid analysis and field-desorption mass spectrometry [10] of the purified peptides.

3. RESULTS AND DISCUSSION

About 175 mg *A. cylindrica* APC was obtained from 90 g wet cells. The A_{650}/A_{280} ratio was about 3.8. The α- and β-subunits were well separated by DEAE-cellulose column chromatography and sufficiently pure for the sequence study.

The N-terminal sequences of both subunits were determined up to the 33rd and 31st steps, respectively, by both manual and automated Edman degradation. The C-terminal sequences of the α- and β-subunits were determined to be -Met-Gln by carboxypeptidase A digestion and -(Gly,Leu)Ser by carboxypeptidase Y digestion, respectively.

The α-subunit was cleaved by CNBr to yield peptides CN-1 to -4 after SP-Sephadex C-25 column chromatography and these were separately digested with staphylococcal protease. Peptides CN-1-S-1 to -4, and CN-4-S-1 were obtained in pure form by high-performance liquid chromatography (HPLC). Another portion of the α-subunit was digested with chymotrypsin and 7 pure peptides, C-1 to -7, were obtained by HPLC. These peptides were sequenced by Edman degradation and carboxypeptidase Y digestion. A summary of the sequence study of the α-subunit is given in fig.1a.

The β-subunit was cleaved by CNBr to yield peptides CN-1 to -5 after separation by SP-Sephadex C-25 column chromatography. Peptide CN-1 was digested with staphylococcal protease and trypsin, and the digests were separated by HPLC to yield peptides CN-T-1 to -3, and CN-1-S-1 to -3. Peptide CN-5 was digested with chymotrypsin and HPLC yielded peptide CN-5-C-1. Another portion of the β-subunit was digested with trypsin and peptides T-1 and T-2 were purified by HPLC. All these peptides were sequenced by Edman degradation and carboxypeptidase Y digestion. The results are summarized in fig.1b. The total numbers of residues in the α- and β-subunits were 160 and 161, respectively, and both lacked tryptophan.

When peptide CN-2 of the α-subunit was sequenced by Edman degradation, a PTH-amino acid binding PCB was detected at the 4th step. The residue was found to be cysteine after HCl hydrolysis [9]. This was located at the 80th position. Similarly, PCB of the β-subunit was bound to Cys-81.

Peptide CN-1-S-3 (β-subunit) contained an unknown amino acid. At the 17th step of Edman degradation a PTH derivative was eluted between PTH-serine and -homoserine in our HPLC elution system. Amino acid analysis of this peptide gave 2 molecules of aspartic acid. A chymotryptic peptide, residues 63–73, containing an unknown amino acid was separately isolated and subjected to field-desorption mass spectrometry giving a mass number of 1239 which is 29 larger than that expected from the composition if the residue in question had been aspartic acid. Although we could not study this compound further, we concluded that it was a derivative of aspartic acid. Addition of methanolamine to the aspartyl carboxyl group would account for the extra mass, but we have no evidence to confirm this at the moment. Sidler et al. [11] have recently found γ-hydroxylysine in *Cryptomonas* phycoerythrin-545, but our compound seemed to be different from theirs.

The structures of *A. cylindrica* APC subunits were compared with those of *C. caldarium* [5], *M. laminosus* [4] and *A. variabilis* [6] in fig.2. There are identities of 65–81% and 73–89% among the α_I and β-subunits, respectively (table 1), indicating that there has been more constraint of mutability in the β-subunit than in the α-subunit during the evolution of these subunits. The identity between α- and β-subunits in the same organism was only about 30%, indicating that the gene for APC duplicated prior to speciation, as found in hemoglobin [12] and ferredoxin [13].

High homologies were found particularly around the PCB-binding regions (fig.2). A method for predicting the secondary structures [14] of these regions suggested that they were arranged in antiparallel β-sheets. The β-subunit contained a high level of tyrosine and arginine in this region.

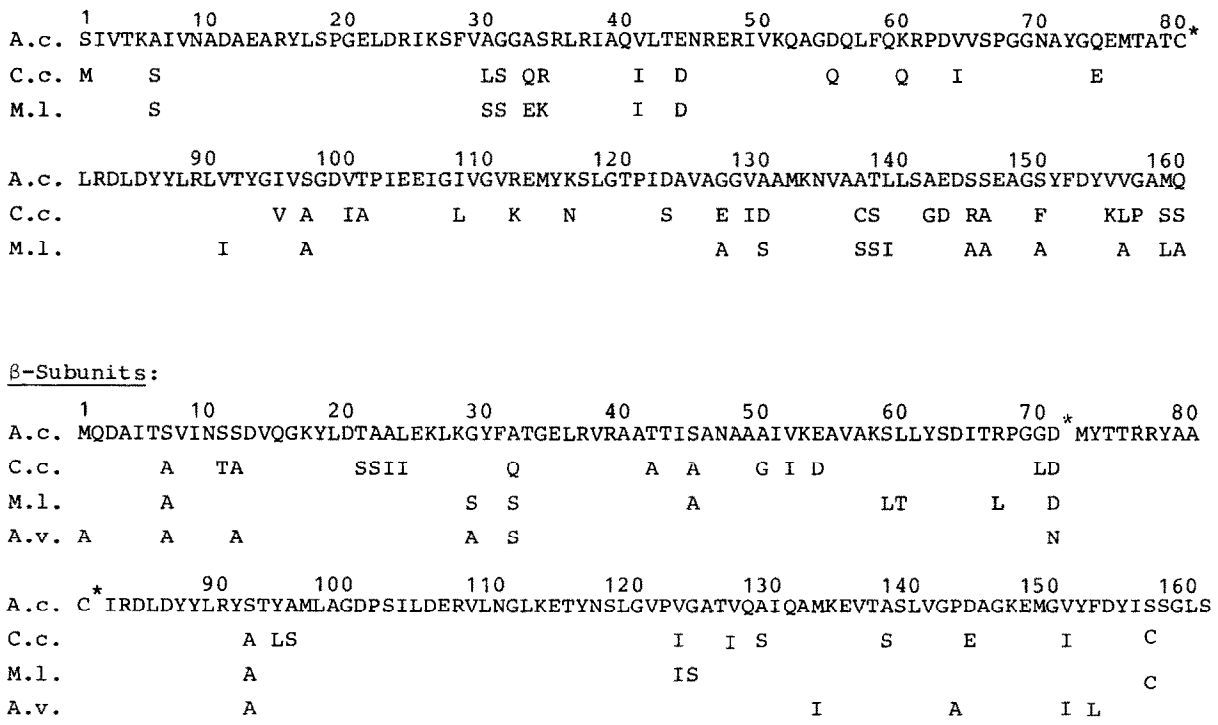

Fig.2. Comparison of amino acid sequences of α- and β-subunits of APC from various organisms. A.c., C.c., M.l., A.v., *A. cylindrica, C. caldarium, M. laminosus* and *A. variabilis*, respectively. Amino acids shown below the sequences of *A. cylindrica* APC subunits are those of other APC subunits found to be different.

Table 1

Matrices of amino acid differences among the α- and β-subunits of APC from 4 algae

α-Subunits				β-Subunits				
	A.c.	C.c.	M.l.		A.c.	C.c.	M.l.	A.v.
A.c.	0	35	20	A.c.	0	25	12	11
C.c.	35	0	30	C.c.	25	0	24	26
M.l.	20	30	0	M.l.	12	24	0	15
				A.v.	11	26	15	0

The matrices were derived on the basis of the alignments given in fig.2. A.c., *Anabaena cylindrica*; C.c., *Cyanidium caldarium*; M.l., *Mastigocladus laminosus*; A.v., *Anabaena variabilis*

These structural characteristics of APC must contribute to the unique spectral properties of this molecule.

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