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Amino acid sequences of α-allophycocyanin B from *Synechococcus* 6301 and *Mastigocladus laminosus*

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The primary structure of α-allophycocyanin B (α^{APB}) of *Synechococcus* 6301 was elucidated. Of the 162 amino acid residues in this polypeptide, 153 were placed by direct sequence determination and the nature of the remaining 9 residues deduced from the amino acid analysis of a peptide generated by the cleavage of α^{APB} with BNPS-skatole. The probable positions of these 9 residues were assigned by homology to other phycobiliproteins. α^{APB} showed the highest homology, 51%, to α^{AP}. Sequence comparisons suggest that tryptophan residues at positions 60 and 90 in α^{APB} might contribute to the red-shifted absorption and fluorescence emission maxima of α^{APB} relative to those of α^{AP}. N-terminal sequences of *Mastigocladus laminosus* α^{APB}, and *Synechococcus* 6301 α^{AP} and β^{AP} were also determined. The N-terminal 55 residues of *Synechococcus* 6301 β^{AP} isolated from the two complexes $(\alpha^{APB}\beta^{AP})_3$ and $(\alpha^{AP}\beta^{AP})_3$, respectively, were found to be identical.

α-Allophycocyanin B; Phycobiliprotein; Amino acid sequence; (*Synechococcus* 6301, *Mastigocladus laminosus*)

1. INTRODUCTION

α^{APB}-Allophycocyanin B (α^{APB}) is a phycobiliprotein subunit which carries one covalently bound phycocyanobilin [1]. It is located in the core of phycobilisomes where it acts as one of two different terminal energy acceptors [2]. It occurs in two copies per hemidiscoidal cyanobacterial phycobilisome within a complex containing allophycocyanin subunits and one linker polypeptide, $(\alpha^{APB}\alpha_2^{AP}\beta_3^{AP})L_C^{\sim 10}$ [3]. The fluorescence emission maximum of this complex is at 680 nm, in contrast to the 662 nm maximum of the complex $(\alpha^{AP} \triangleq \beta^{AP})_3L_C^{\sim 10}$, indicating that α^{APB} is responsible for the red shift of the fluorescence emission maximum. Such a red shift is not only observed in the complex, but also evident in the comparison of the free subunits: α^{APB} has an absorption maximum at 645 nm and a fluorescence emission maximum at 672 nm [1], while the corresponding maxima for α^{AP} lie at 615 and 642 nm, respectively. Since α^{APB} and α^{AP} each carry a phycocyanobilin chromophore, the different absorption and fluorescence properties of these two subunits must result from differences between their amino acid sequences. For this reason, we have investigated the amino acid sequence of α^{APB}. The amino acid sequences of α^{AP} from various organisms have been reported previously [5–8].

We present here an almost complete amino acid sequence of α^{APB}. The available information allows some inferences to be made about the origins of the red shift in the absorption and fluorescence emission spectra. The available se-

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Abbreviations: BNPS-skatole, 2-(2-nitrophenylsulfenyl)-3-methylbromoindolenine; phycobiliprotein subunits and linker polypeptides of phycobilisomes are abbreviated as described by Glazer [(1985) Annu. Rev. Biophys. Biophys. Chem. 14, 47–77]; AP, allophycocyanin

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quence will also be valuable in the identification of the gene encoding α^{APB}.

2. MATERIALS AND METHODS

2.1. *Materials*

Cellex-D and Bio-Gels were from Bio-Rad. Microgranular carboxymethylcellulose CM52 was obtained from Whatman, and DEAE-Sephadex A-50 from Pharmacia. BNPS-skatole and endoproteinase Lys-C were from Pierce and Boehringer Mannheim, respectively.

2.2. *Isolation of* Synechococcus *6301* α^{APB}

Allophycocyanin B was prepared as described [1,9]. The α^{APB} and α^{AP} subunits were then separated by ion-exchange chromatography on CM-cellulose in 8 M urea at pH 5 [1].

2.3. *Isolation of* Synechococcus *6301* α^{AP} *and* β^{AP}

These allophycocyanin subunits were separated by ion-exchange chromatography on DEAE-Sephadex A-50 in 8 M urea at pH 8 [10,11].

2.4. *Isolation of* Mastigocladus laminosus α^{APB}

The APC-I fraction of the Cellex-D column (see [12]) was further separated on Bio-Gel P-60 in 63 mM formic acid [12]. Fractions exhibiting a shoulder around 670 nm in the absorption spectrum were pooled and lyophilized. This material was purified further by ion-exchange chromatography on CM-cellulose in 8 M urea-20 mM ammonium acetate-1 mM 2-mercaptoethanol, pH 4.5. The column was developed with a linear gradient of 0–250 mM NaCl in the same solvent. α^{APB} eluted as a shoulder in the descending slope of the main peak formed by α^{AP}. It was identified by manual Edman degradation (Gln at position 5). α^{APB} was rechromatographed on a smaller column of the same type. By this method α^{APB} was enriched to a 1:2 mixture of α^{APB}:α^{AP}, which was analyzed by automated Edman degradation.

2.5. *Cleavage of* Synechococcus *6301* α^{APB} *with BNPS-skatole*

Carbamoylated α^{APB} [12] (5 mg) was dissolved in 5 ml of 60% acetic acid containing 20 mg phenol and 5 mg tyrosine. BNPS-skatole (31 mg) was added and the reaction conducted under freon at 40°C for 40 h. Undissolved BNPS-skatole was removed by centrifugation and the peptide-containing supernatant solution desalted on Bio-Gel P2 (⩽400 mesh) and separated on Bio-Gel P-30 (⩽400 mesh) in 50% formic acid.

2.6. *Cleavage with endoproteinase Lys-C*

Uncleaved material recovered from the BNPS-skatole reaction mixture was digested with endoproteinase Lys-C as in [13]. The resulting peptides were desalted on Bio-Gel P-2 and separated on Bio-Gel P-10 (⩽400 mesh) in 50% formic acid.

2.7. *Analytical methods*

Automated and manual Edman degradation and amino acid analysis were performed as described in [12].

3. RESULTS AND DISCUSSION

The amino acid sequence established for α^{APB} by the studies described below is shown in fig.1. The N-terminal sequence of the intact polypeptide was established by sequential Edman degradation up to Gln^{76}. Since amino acid analysis showed the presence of two Trp, cleavage of α^{APB} at these residues with BNPS-skatole was attempted. Peptides generated by this cleavage were separated into four peaks on Bio-Gel P-30 in 50% formic acid (not shown). Peak 1 was blue and contained uncleaved material which was further digested with endoproteinase Lys-C. Peak 2 was blue and contained the peptide Thr^{3}-Trp^{90}, which was sequenced up to Asp^{46}. Peak 3 was colourless and represented the C-terminal peptide starting with Tyr^{91}. This peptide was sequenced up to Gly^{172}. In peak 4 another chromopeptide, Gln^{61}-Trp^{90}, was identified by amino acid analysis. This peptide had a blocked N-terminus, probably due to the cyclization of the N-terminal Gln to pyrrolidonecarboxylic acid under the acid-cleavage and gel-filtration conditions [14]. The residues of this peptide which were not placed by sequence determination were positioned by analogy with the corresponding residues in other phycobiliproteins from *M. laminosus*, such as α^{AP}, β^{AP} [5] and $\beta^{16.2}$ [15].

Three peptides from the endoproteinase Lys-C digestion of peak 1 were isolated and sequenced. Thr^{33}-Lys^{49} was sequenced up to Ser^{47} and the C-terminal peptide starting with Glu^{138} was sequenced up to Glu^{174}. By homology to other

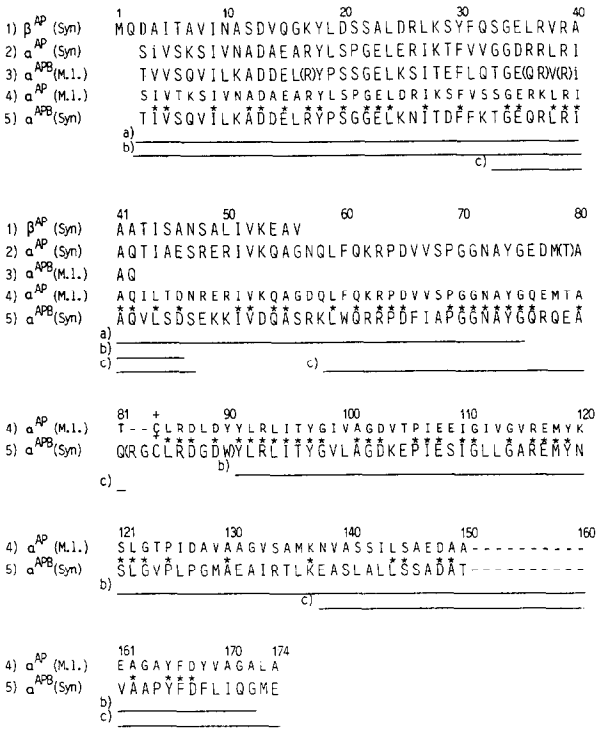

Fig.1. Amino acid sequence of α^{APB} from *Synechococcus* 6301 (Syn, see line 5) compared to the complete amino acid sequence of α^{AP} from *Mastigocladus laminosus* (M.l., line 4) and to the N-terminal sequences of α^{APB} from M.l. (line 3) and α^{AP} (line 2) and β^{AP} (line 1) from Syn. Homologous residues in α^{APB} from Syn and α^{AP} from M.l. are indicated by asterisks. (–) Deletion. Cysteinyl residues 84 (C) involved in bilin attachment. Amino acids in brackets are placed by homology. The amino acids are numbered according to [13]. Underlined sequences indicate stretches identified by automated Edman degradation of the complete α^{APB} subunit (a), the peptides obtained after cleavage with BNPS-skatole (b), and the peptides generated by digestion of α^{APB} with endoproteinase Lys-C (c).

phycobiliproteins this residue can be placed as the C-terminal one of the complete α^{APB} subunit. The peptide starting with Leu^{59} was sequenced up to Gln^{81}.

In fig.1 the sequence established for α^{APB} is compared to that of *M. laminosus* α^{AP}, arranged as in [13]. The homologies between α^{APB} and *M. laminosus* phycobiliproteins are shown in table 1. The closest relationship was found between α^{AP} and α^{APB}. Probably these two subunits share a direct common ancestor, as do β^{AP} and $\beta^{16.2}$ [16].

Table 1

Homologies (%) between α^{APB} from *Synechococcus* 6301 and (a) the phycobiliprotein subunits from *Mastigocladus laminosus* and (b) the AP subunits from *Synechococcus* 6301

Phycobiliprotein subunit	Compared residues	% homology
a		
α^{APB}	40 N-terminal	83
$\beta^{16.2}$	all	29
α^{AP}	all	51
β^{AP}	all	33
α^{PC}	all	25
β^{PC}	all	28
α^{PEC}	all	22
β^{PEC}	all	29
b		
α^{AP}	all	53
β^{AP}	all	33

Since all phycobiliproteins are homologous to each other, they probably have similar tertiary structures. The three-dimensional structure of *M. laminosus* C-phycocyanin trimer, $(\alpha^{PC}\beta^{PC})_3$, has been established recently [17]. To find candidate amino acid residues responsible for the red-shifted absorption and fluorescence spectra of α^{APB}, we compared the primary structure of α^{APB} with the structures of all phycobiliproteins from *M. laminosus* in their possible three-dimensional arrangements analogous to phycocyanin. These comparisons indicated that residues Trp^{60} and Trp^{90}, which occur only in α^{APB}, could possibly interact with the chromophore with a resultant production of the red shift.

M. laminosus α^{APB} was isolated as a 1:2 mixture, α^{APB}:α^{AP}. The N-terminal 40 residues of α^{APB} could be determined by degradation of this mixture. They exhibit an 83% homology to the 40 N-terminal residues of *Synechococcus* 6301 α^{APB}.

The N-terminal sequences of α^{AP} and β^{AP} from *Synechococcus* 6301 were also established (fig.1). They agree with the complete amino acid sequence deduced from the respective genes [18]. The homologies of these subunits to α^{APB} are 53 and

33%, respectively, nearly the same as the homologies of the AP subunits of *M. laminosus*. It was seen that the difference in amino acid sequence between α subunits of different species was smaller than that between the sequence of the α and β subunits of the same organism.

The data obtained in this study also show that the 56 N-terminal residues of β^{AP} isolated from $(\alpha^{AP}\beta^{AP})_3$ and $(\alpha^{APB}\beta^{AP})_3$ are identical. This is a strong indication that the β^{AP} subunits from the two complexes are identical.

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