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γ-*N*-Methylasparagine in phycobiliproteins from the cyanobacteria *Mastigocladus laminosus* and *Calothrix*

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Reinvestigation of the amino acid sequences of all phycobiliproteins from *Mastigocladus laminosus* showed that there is a post-translationally modified asparagine residue at position 72 of the phycobiliprotein subunits β^{PC}, β^{AP} and $\beta^{16\,2}$. This residue was identified as γ-*N*-methylasparagine and it was also found in β^{PE} of *Calothrix*. This study also revealed some differences in the amino acid sequences of β^{AP} and β^{PC} compared to the published data.

γ-*N*-Methylasparagine; Posttranslational modification; Phycobiliprotein; Amino acid sequence; (*Mastigocladus laminosus, Calothrix*)

Phycobiliproteins are pigmented proteins which are organized into light-harvesting complexes called phycobilisomes (review [1]). Minami et al. [2] found a modified aspartic acid residue in the amino acid sequence of the phycobiliprotein subunit β^{AP} from the cyanobacterium *Anabaena cylindrica* and Klotz et al. [3] identified γ-*N*-methylasparagine at position 71 in the amino acid sequence of β^{AP} from *A. variabilis*. The occurrence of this post-translationally modified asparagine was also reported in β^{AP} from *Synechococcus* PCC 6301, *Porphyridium cruentum* and in R-phycoerythrin from *Gastroclonium coulteri* [3].

We therefore reinvestigated the amino acid sequences of all phycobiliproteins from *Mastigocladus laminosus* including the core-associated linker polypeptide $L_C^{8,9}$. Total hydrolysis of β^{PC}, β^{AP} and $\beta^{16.2}$ yielded 1 mol methylamine/mol protein which could be quantified by amino acid analysis. No methylamine was detectable in hydrolysates of β^{PEC}, the α-subunits of AP, PC, PEC and the linker polypeptide $L_C^{8,9}$. Fragmentation of β^{PC}, β^{AP} and $\beta^{16.2}$ with endoproteinase Lys-C produced methylamine-positive peptides which were isolated by gel filtration and sequenced by automated Edman degradation [experimental details will be presented elsewhere (in preparation)]. The phenylthiohydantoin derivative of the amino acid residue at position 72 of β^{PC}, β^{AP} and $\beta^{16.2}$ eluted

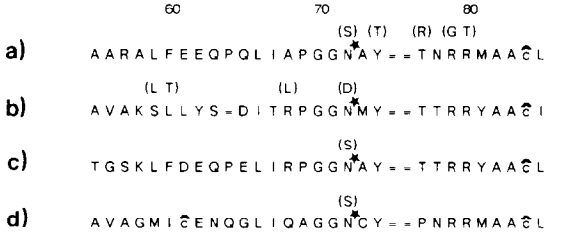

Fig.1. Amino acid sequences of phycobiliprotein fragments containing γ-*N*-methylasparagine (N*) from (a-c) *Mastigocladus laminosus* and (d) *Calothrix* as determined by automated Edman degradation. (a) β^{PC}, (b) β^{AP}, (c) $\beta^{16\,2}$, (d) β^{PE}. The residues in parentheses are those in [11], [12], [13] and [4], respectively. ĉ, Cys-chromophore; =, deletion.

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Abbreviations: AP, allophycocyanin; PC, C-phycocyanin; PEC, phycoerythrocyanin; PE, C-phycoerythrin; α and β, subunits of phycobiliproteins; $L_C^{8,9}$, linker polypeptide of the AP core

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with the same retention time as that of serine from the HPLC column but amino acid analysis of this hydrolyzed PTH derivative and of the sequenced fragments showed that this residue has to be interpreted as γ-*N*-methylasparagine (fig.1). This post-translational modification is not restricted to β^{AP} but is also found in the highly homologous core component $\beta^{16.2}$ and in β^{PC} in the phycobilisome rods.

The amino acid sequences of the subunits of C-phycoerythrin from *Calothrix* were both established by sequencing the protein [4] and the DNA [5]. These two sequences differed only in residue 72 of β-PE; asparagine was determined from the DNA sequence and serine from Edman degradation of the protein. Our reinvestigation of this part of the sequence showed that residue 72, encoded as asparagine, is modified to γ-*N*-methylasparagine in the protein (fig.1).

During sequence analysis of the peptides of β^{AP} and β^{PC} some sequence errors could be eliminated and those found in β^{PC} by Schirmer et al. [6] were confirmed (fig.1). Comparing the corrected sequence of β^{PC} from *M. laminosus* with that from *Anabaena* 7120 [7]. *Synechococcus* 6301 [8] and *Agmenellum quadruplicatum* [9,10] now reveals a segment of 27 amino acid residues (from Leu 66 to Tyr 94) with complete homology throughout these four organisms. No other so extended highly conserved region can be found within C-phycocyanin sequences and therefore this part of the molecule may be essential for the function of this light-harvesting protein. The amino acid sequences of β^{PC} from *A. quadruplicatum* [9,10] and *Anabaena* 7120 [7] which were deduced from the DNA sequences both revealed as asparagine residue at position 72. Further investigations on these proteins and on β^{PC} of other organisms will show if this Asn 72 generally is post-translationally methylated. The position of γ-*N*-methylasparagine in the three-dimensional structure of C-phycocyanin and a possible role of this modified residue will be discussed later (in preparation).

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