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The structure of γ-*N*-methylasparagine in C-phycocyanin from *Mastigocladus laminosus* and *Agmenellum quadruplicatum*

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The crystal structures of C-phycocyanin from *Mastigocladus laminosus* and *Agmenellum quadruplicatum* have been reinvestigated to accommodate the recently found γ-*N*-methylasparagine residue at β-72. In both structures, position β-72 could be modelled as γ-*N*-methylasparagine. The molecular phycocyanin structures were corrected accordingly. Possible roles of γ-*N*-methylasparagine are discussed on the basis of the three-dimensional structure.

γ-*N*-Methylasparagine; Posttranslational modification; Phycocyanin; Crystal structure; (*Mastigocladus laminosus, Agmenellum quadruplicatum*)

1. INTRODUCTION

Phycobilisome organelles are light-harvesting complexes found in cyanobacteria and red algae. Normally they are built up of three components, namely APC which is close to the surface of the photosynthetic lamellae and is a component of the central core of the phycobilisome. C-PC lies in the middle and PE or PEC in the outermost position of the antenna substructure of the organelles. Phycobiliproteins of known three-dimensional structure are composed of (αβ) monomers associated as disc-shaped trimers and hexamers. The hexamers are joined by linker proteins which are believed to be located in the central cavity of the hexamer. Each α- or β-chain carries one or two covalently bound open-chain tetrapyrrole (bilin) prosthetic groups (for reviews see [1–3]).

Recently, Minami et al. [4] reported a post-translationally modified aspartyl residue at position β-71 in allophycocyanin from *Anabena cylindrica*. Klotz et al. [5] characterized an NMA β-71 in *Anabena variabilis* allophycocyanin and reported the presence of this modified residue at position β-71 in allophycocyanin from *Synechococcus* PCC 6301, *Porphyridium cruentum* and in the homologous position β-72 in R-phycoerythrin from *Gastroclonium coulteri*. Furthermore, NMA has been found in a variety of other phycobiliproteins [6–8] at homologous positions.

The X-ray crystal structures of two phycocyanins, PCML and PCAQ, have been determined and refined so far [9–11]. According to protein (PCML, [12]) and DNA (PCAQ, [13]) sequences, β-72 was reported as serine in PCML and as asparagine in PCAQ. The recent reinvestigation and finding of Ruembeli et al. [7,8] of an *N*-methylated asparagine in the β-chains of APC and C-PC from *Mastigocladus laminosus* led us to reinvestigate the crystal structures of C-PC from *Mastigocladus laminosus* and *Agmenellum quadruplicatum*.

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Abbreviations: APC, allophycocyanin; C-PC, C-phycocyanin; PE, phycoerythrin; PEC, phycoerythrocyanin; NMA, γ-*N*-methylasparagine; PCML, C-phycocyanin from *Mastigocladus laminosus*; PCAQ, C-phycocyanin from *Agmenellum quadruplicatum*; F_o, F_c, observed and calculated structure factor amplitude

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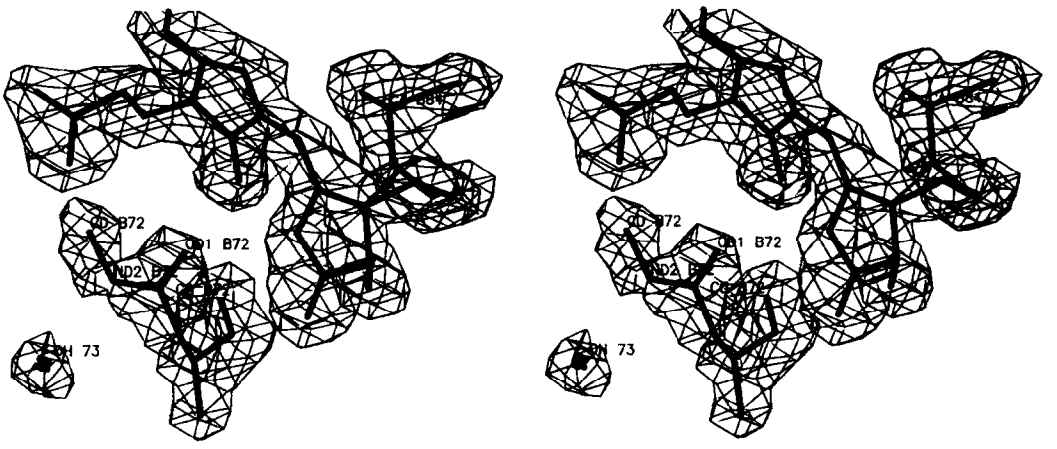

Fig.1. Stereoscopic plot of the electron density of NMA β-72 and pyrrole rings A and B of chromophore β-84 in PCML. The water molecule OH 73 and the side chain atoms of NMA β-72 did not contribute to the phase calculation for the Fourier synthesis.

2. RESULTS

The starting model coordinates of the structure of PCML were taken from Schirmer et al. [11]. The $2F_o - F_c$ map calculated at 2.1 Å with phases from a model where the β-72 side chain had been omitted showed a weak, but clearly defined extension which can accommodate an NMA side chain (fig.1). The $F_o - F_c$ difference-Fourier map displayed an elongated peak of positive density (0.69 e/Å^3, σ is 0.07 e/Å^3) in this region, which is the highest peak in the map and confirms this interpretation. Ser β-72 was replaced by an NMA and the resulting structure was subjected to one macro-cycle of constrained energy refinement (EREF [14]) with a strong constraint on the starting model, so that the model was not allowed to undergo significant changes. This is reflected in the R value (21.7%) of the final model, which remained unchanged. A subsequent least squares refinement of the thermal parameters showed high temperature factors of B = 38 (Å^2) for the side chain of NMA β-72 in accordance with the weak electron density at this position.

The side chain of residue β-72 is located in the loop between helices B and E. Atom OD1 of NMA β-72 is located near the nitrogen N21 of pyrrole ring B of chromophore CYC β-84 (distance 2.9 Å) and near atom OG1 of Thr β-124 (distance 2.8 Å). The plane of the N-methyl carboxamide group at NMA β-72 is almost parallel to the main plane of chromophore β-84 and shields ring B of the chromophore against solvent. An additional water molecule (OH 73) was observed in the electron density and added into the new model. It lies within hydrogen bond radius of atom ND2 β-72 (fig.2).

The situation at β-72 in PCAQ is less clear, due to the lower resolution (2.5 Å) of this crystal structure. Because of the three-fold redundancy in this crystal structure, the electron density map was averaged, using the procedures described by Schirmer et al. [10]. Fourier- and difference Fourier maps, calculated with phases from a model where the side chain β-72 had been omitted indicated a slightly larger side chain than asparagine at β-72. An NMA residue was built into the PCAQ model with a similar conformation as in the PCML model. The water molecule found near ND2 of NMA in PCML is lacking in PCAQ. Instead another water molecule, which interacts with the main chain atom N β-72 is very well defined in the electron density of PCAQ but is absent in PCML.

3. DISCUSSION

The biosynthesis and function of NMA in phycobiliproteins remain to be investigated. It is generally accepted, that asparagine β-72 is methylated via S-adenosylmethionine under consumption of ATP [6,15]. Surface accessibility calculations (see [16] for a review of this topic),

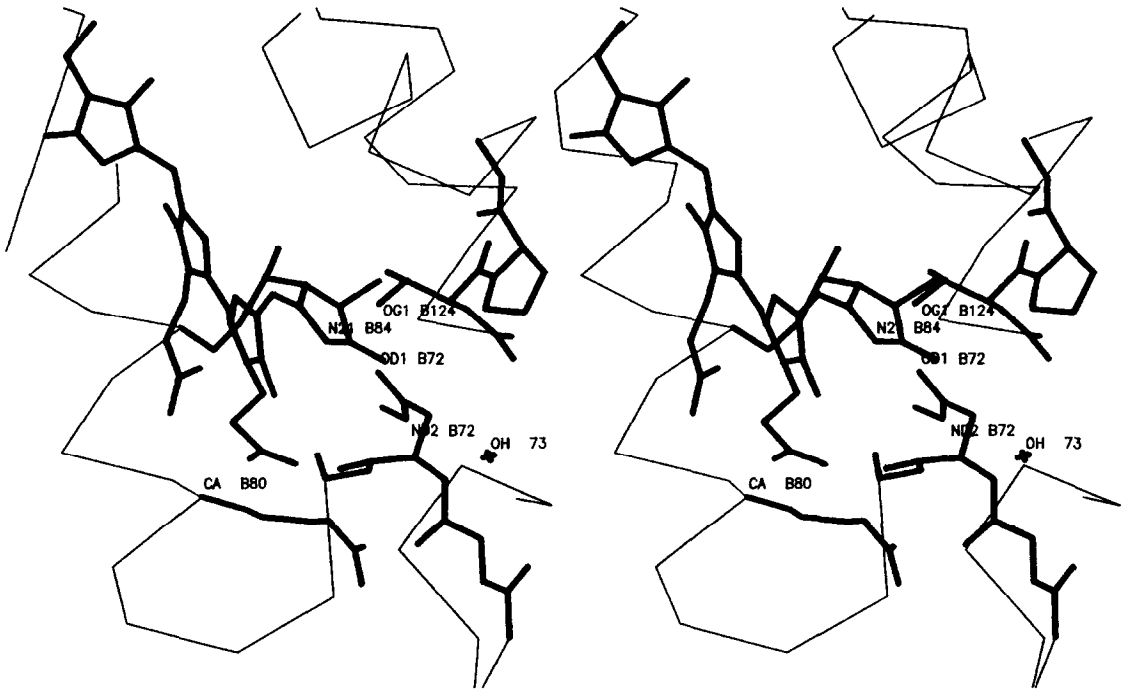

Fig.2. Structure of NMA β-72 with surrounding backbone (thin line) and interacting side chains (thick lines). The end of helix B is in the lower right corner and helix E lies on the left side of the plot.

carried out with the program HYBAC showed a relatively high accessibility of the methyl group in *N*-methylasparagine β-72 in PCML. The trimeric structure may therefore be methylated after its formation. Even in the hexameric aggregation state in PCAQ the access of residue β-72 to solvent molecules is not decreased. It should be noted however that both phycocyanins were crystallized without linker, which is known to interact with chromophore β-84 in the native state and is therefore close to β-72. Further structural studies have to show a possible interaction between the side chain of β-72 and linker polypeptides.

The absence of NMA in the α-subunits suggests that the methylated residue may also play a role in modifying the absorption characteristics of the chromophore β-84. The introduction of a methyl group may prevent water molecules from interacting with atom ND2 β-72 and influence the microscopic dielectric constant in this region. It is known that β-84 is the chromophore absorbing the longest wavelengths, the *f*-chromophore, responsible for inter-hexamer energy transfer [11,17].

Other possible roles of NMA β-72 are in proper protein folding of the β-subunit. NMA β-72 is located in a complex turn between two α-helices. It may also be involved in directing the proper biosynthetic attachment of the β-84 chromophore which is spatially adjacent.

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REFERENCES

[1] Glazer, A.N. (1984) Biochim. Biophys. Acta 768, 29–51.
[2] Zuber, H. (1985) Photochem. Photobiol. 42, 821–844.
[3] Zuber, H. (1986) Trends Biochem. Sci. 11, 414–419.
[4] Minami, Y., Yamada, F., Hase, T., Matsubara, H., Murakami, A., Fujita, Y., Takao, T. and Shimonishi, Y. (1985) FEBS Lett. 191, 216–220.
[5] Klotz, A.V., Leary, J.A. and Glazer, A.N. (1986) J. Biol. Chem. 261, 15891–15894.
[6] Klotz, A.V. and Glazer, A.N. (1987) J. Biol. Chem. 262, 17350–17355.
[7] Ruembeli, R., Suter, F., Wirth, M., Sidler, W. and Zuber, H. (1987) FEBS Lett. 221, 1–2.

[8] Ruembeli, R., Suter, F., Wirth, M., Sidler, W. and Zuber, H. (1987) Biol. Chem. Hoppe-Seyler 368, 1401–1406.
[9] Schirmer, T., Bode, W., Huber, R., Sidler, W. and Huber, H. (1984) J. Mol. Biol. 184, 257–277.
[10] Schirmer, T., Huber, R., Schneider, M., Bode, W., Miller, M. and Hackert, M.L. (1986) J. Mol. Biol. 188, 651–676.
[11] Schirmer, T., Bode, W. and Huber, R. (1987) J. Mol. Biol. 196, 677–695.
[12] Frank, G., Sidler, W., Widmer, H. and Zuber, H. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1085–1096.
[13] Pilot, T.J. and Fox, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 6983–6987.
[14] Jack, A. and Levitt, M. (1978) Acta Crystallogr. sect. A 34, 931–935.
[15] Paik, W.K. and Kim, S. (1980) Protein Methylation, John Wiley & Sons, New York.
[16] Richards, F.M. (1977) Annu. Rev. Biophys. Bioeng. 6, 151–176.
[17] Schirmer, T. and Vincent, M.G. (1987) Biochim. Biophys. Acta 893, 379–385.