ATOMIC-RESOLUTION STRUCTURE OF CYANOBACTRIAL CYTOCHROME C6 WITH AN UNUSUAL SEQUENCE INSERTION

S. Krzywda 1*, W. Białek 2, A. Szczepaniak 2 and M. Jaskólski 1

1 Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland
2 Faculty of Biotechnology, University of Wrocław, Poland

Keywords: high-resolution structure, cytochrome, photosynthesis

During photosynthesis, electron transfer between two membrane-bound complexes, cytochrome b6f and photosystem I can be accomplished by the copper-containing protein, plastocyanin, or the heme protein, cytochrome c6 [1]. Cytochromes c6 are water-soluble, low-spin heme-containing proteins involved in the high-potential (340-390 mV) electron transport chain. They are characterized by low molecular mass, 80-90 amino acid residues in the mature protein, and have a covalently bound heme group.

Cytochrome c6 from the mesophilic cyanobacterium Synechococcus sp. PCC 7002 is unique among all known cytochromes c6 due to the presence of an unusual seven-residue insertion, K44DGSKSL50. Furthermore, the present protein is unusual because of its very high content (36%) of the smallest residues (glycine and alanine) [2].

The crystallization experiments used the hanging-drop vapor-diffusion method. Red crystals (Fig. 1) suitable for X-ray analysis were obtained from 10 mM sodium Hepes, pH 6.2, and 2.2 M ammonium sulfate over a period of one week.

Figure 1. Crystals of reduced cytochrome c6 from Synechococcus sp. PCC 7002.

The structure of the reduced form of cytochrome c6 has been determined at 1.2 Å and refined to an R factor of 0.107. It reveals that the overall fold of the protein is similar to that of other class I c-type cytochromes despite the presence of the specific insertion. The insertion is located within the most variable region of cytochrome c6 sequence, i.e. between helices II and III. The first six residues (K44DGSKS49) form a loop, whereas the last residue, Leu50, extends the N-terminal beginning of helix III. Several specific non-covalent interactions are found inside the insertion as well as between the insertion and the rest of the protein. Energetically significant cationπ interactions have been detected between Tyr43, Tyr56, Phe68 and, respectively, Lys37, Lys48 and Arg71 (Fig. 2).

Figure 2. N-Hπ interaction between the only Arg and Phe68 (2Fo – Fc electron density map contoured at 1.2σ).

The crystal structure contains three copies of the c6 molecule per asymmetric unit and is characterized by unusually high packing density, with solvent occupying barely 17.58% of the crystal volume.

Acknowledgements: Some of the calculations were carried out in the Poznan Metropolitan Supercomputing and Networking Center. This work was funded in part by grants number N N204 245635 and N N303 3856 33 from the Ministry of Science and Higher Education.

*) e-mail:szymon@amu.edu.pl

References