

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

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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.

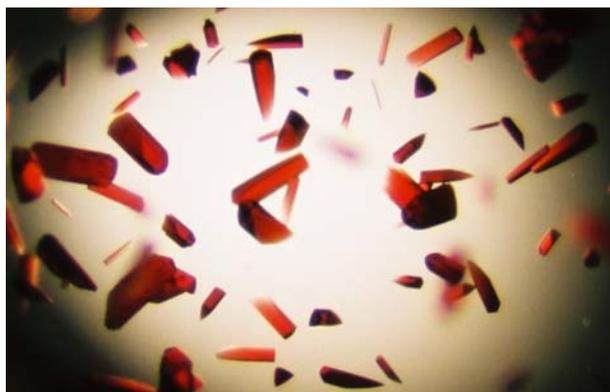


Figure 1. Crystals of reduced cytochrome *c₆* from *Synechococcus* sp. PCC 7002.

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 cation... π interactions have been detected between Tyr₄₃, Tyr₅₆, Phe₆₈ and, respectively, Lys₃₇, Lys₄₈ and Arg₇₁ (Fig. 2).

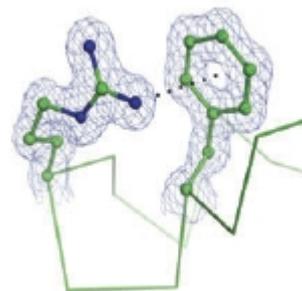


Figure 2. N-H... π interaction between the only Arg and Phe68 ($2F_o - F_c$ electron density map contoured at 1.2σ).

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.

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