Solution Structure of Cytochrome c6 from *Synechococcus elongatus*

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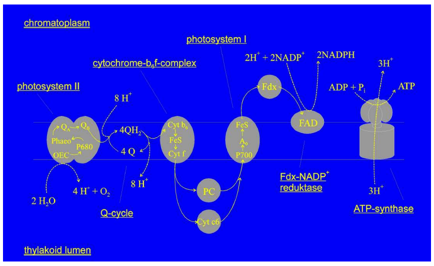
Abstract

Cytochrome c6 acts as a small, soluble electron carrier between the two membrane bound complexes cytochrome b_6f and photosystem I (PSI) in oxygenic photosynthesis. As a primordial thermophilic cyanobacterium that has no plastocyanin, *Synechococcus elongatus* is very interesting in terms of evolutionary aspects of electron transfer.

We determined the solution structure of cytochrome c6 from the thermophilic cyanobacterium *Synechococcus elongatus*. After resonance assignment based on homo- and heteronuclear NMR spectra, structure calculation was performed with restrained molecular dynamics using 1586 interresidual NOEs and 28 dihedral angle restraints as experimental restrictions. The overall fold exhibiting four α-helices is similar to other class I c-type cytochromes. In addition to the secondary structure in most homologues, a small antiparallel β-sheet is found in the vicinity of Met58, one of the axial heme ligands. This is the first solution structure of a cytochrome c6 from a prokaryotic organism.

A negatively charged surface region that was suggested to be one of two possible electron transfer sites in cytochromes is reduced in cytochrome c6 of *Synechococcus elongatus*. This confirms the hypothesis of only one electron transfer site around the heme being used in cytochrome c6 for electron transfer to PSI and cytochrome b_6f. As PSI is known in structural detail for this organism, the reported cytochrome c6 structure provides the basis for mutagenesis studies to delineate the mechanism of electron transfer between both.



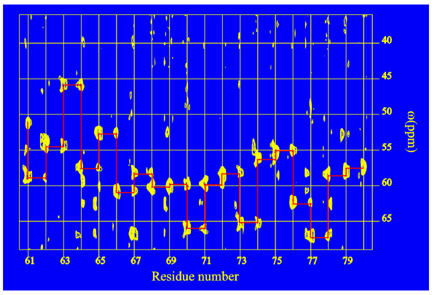
Location of cytochrome c6 on the lumenal side of the thylakoid membrane. It catalyzes the electron transport from the membrane bound cytochrome b_6f-complex to photosystem I. Cytochrome c6 was shown to be the only electron carrier in *Synechococcus elongatus*. In some blue or green alga both Plastocyanin (PC) and Cyt c6 are encoded and the alternative expression of the homologous proteins is regulated by the availability of copper. Both proteins are structurally very different, but they have the same function in those organisms. Whereas plastocyanin is a β-sheet protein with copper as the central ion, cytochromes c6 are highly α-helical heme containing proteins.

Structure determination

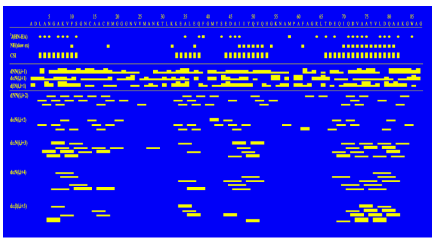
For the assignment of the resonances a combined assignment strategy was employed, relying upon 3D triple-resonance through-bond correlation experiments [Kay et al., 1990] and standard homonuclear techniques [Wüthrich, 1986]. Spin patterns were identified from the analysis of TOCSY and COSY spectra in H_2O and D_2O.

A total of 2300 NOE distance restraints was derived from the NMR spectra, which corresponds to an average of 26 NOEs per residue. For the structure calculations, the X-PLOR program package [Brünger, 1993] was used including a floating assignment of prochiral groups. The heme geometry was taken from the CHARMM 22 force field. A total of 60 structures was calculated by an *ab initio* simulated annealing strategy and the best 20 structures were selected for further analysis. None of the 20 structures showed single distance violations of more than 0.5 Å or systematic distance violations of more than 0.25 Å.

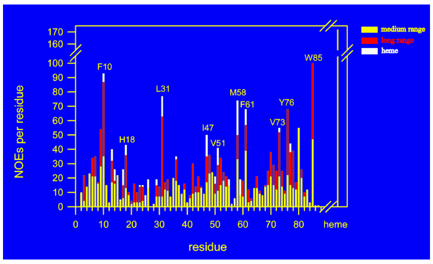
The overall fold of *S. elongatus* cytochrome c_6 resembles the topology of class I cytochromes c. Four a-helices are observed as elements of regular secondary structure with the typical arrangement of the a-helices at the N- and C-termini enclosing an angle of approximately 90°. The second and third helix, which are not observed in all members of the family are located on different sides of the heme. Whereas the two C-terminal and the N-terminal helices are well defined, the second helix is not so well-defined as already expected from the experimental data. In addition to a-helices, two 3_{10}-helical turns (15-17, 81-83) are observed. Another element of regular secondary structure observed is a short antiparallel b-sheet in the vicinity of the axial heme ligand Met58.



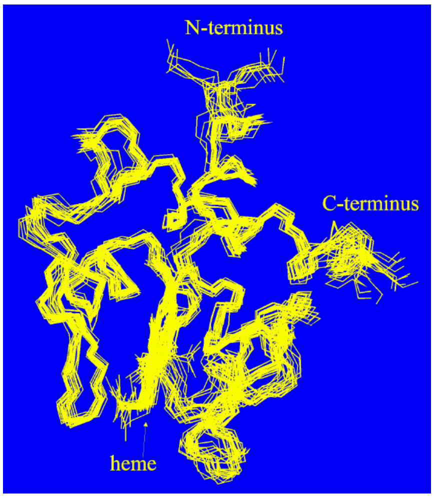
Strip plot of a HNCA-spectrum. The chain tracing from residue 61-80 is indicated by red lines.



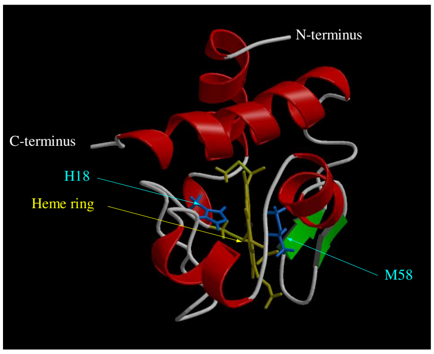
Schematic representation of the experimental data indicative of secondary structural elements. $^3J_{HN-HA}$ coupling constants < 6.5 Hz or > 9 Hz are marked by (●) or (■), respectively. In D_2O slowly exchanging amide protons (□) and proton chemical shift indices (CSI) for HA together with ^{13}C chemical shift indices for CA and C', characteristic for α-helical regions are represented by (⇧). Sequential and selected medium range NOEs are represented by bars between the coupled residues. The thickness of the bars is a measure for the NOE intensity.



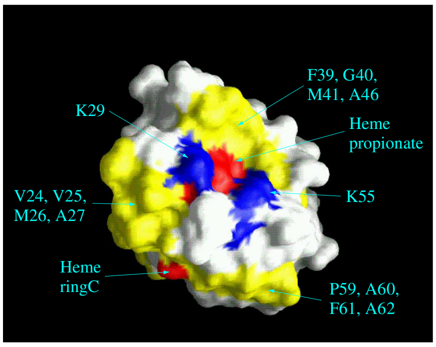
Distribution of medium (i-j ≤5) and long (i-j >5) range NOEs over the sequence. The height of the bars indicates the number of medium range (yellow) or long range (red) NOEs per residue. White bars represent NOEs between amino acids and the heme group.



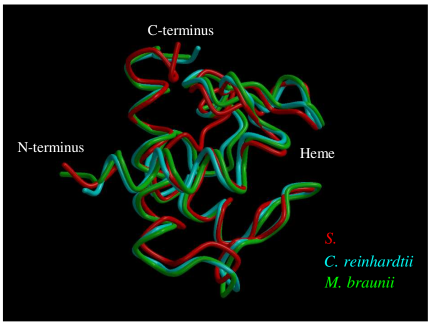
Backbone overlay of a family of 20 cytochrome c6 structures including the heme group. The structures were accepted by the criterion of the best energy function after the restrained molecular dynamics calculation. The RMSD values for the backbone atoms and all heavy atoms are 0.77 Å and 1.06 Å, respectively.



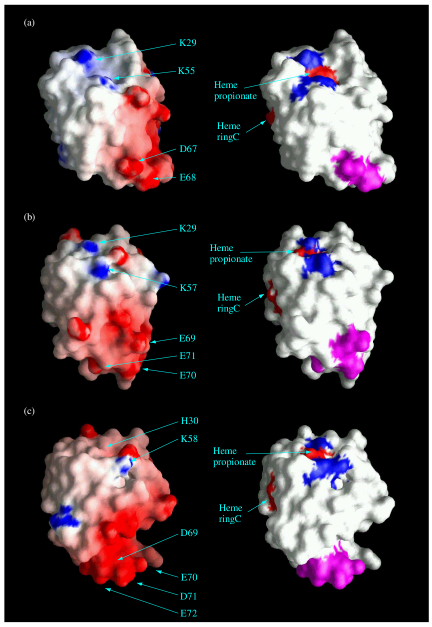
MOLSCRIPT/Raster3D ribbon drawing indicating the elements of regular secondary structure. The heme and its axial ligands Met58 and His18 are shown in stick representation.



Detailed view of the hydrophobic patch (yellow) close to the heme site that forms the docking site for photosystem I. The solvent accessible surface area of the heme is on average 6.3 %. The exposed parts of the heme (red) are the methyl groups at the edge of pyrrole ring C (CBC, CMC) and the propionate at ring D (O1D, O2D, CGD). These groups are candidates for a direct electron transfer from the heme to photosystem I. The negative charge of the propionate is counterbalanced by two semiconserved basic residues flanking the heme site (K29, K55).



MOLSCRIPT/Raster3D backbone overlay of *Synechococcus elongatus* (red), *Chlamydomonas reinhardtii* (blue), and *Monoraphidium braunii* (green) cytochrome c6 in tube representation.



Electrostatic properties and regions supposed to be involved in electron transfer. Electrostatic surface potential (left) of the cytochromes c6 from *Synechococcus elongatus* (a), *Chlamydomonas reinhardtii* (b) and *Monoraphidium braunii* (c). Red zones correspond to negative potentials and blue zones to positive. Residues discussed for importance in electron transfer pathways (right). Surface exposed regions of the heme (red), conserved positively charged residues near the heme propionates (blue), and »acidic patch« around amino acids 70 (pink).

Conclusions

As a primordial thermophilic cyanobacterium that has no plastocyanin, *Synechococcus elongatus* is very interesting in terms of evolutionary aspects of electron transfer. As PSI is known in structural detail for this organism, the reported cytochrome c_6 structure provides the basis for mutagenesis studies to delineate the mechanism of electron transfer between both.

The solvent accessible surface area of the heme is on average 6.3 %. The exposed parts are the methyl groups at the edge of pyrrole ring C (CBC, CMC) and the propionate at ring D (O1D, O2D, CGD). These groups are candidates for a direct electron transfer from the heme to potential electron transfer partners. The negative charge of the propionate is counterbalanced by two semiconserved basic residues flanking the heme site. A metal site surrounded by a hydrophobic surface patch including positive charges was reported previously to be a preferred docking site to redox partners. Interestingly, the heme crevice is flanked by the two not well conserved methionines at positions 26 and 41, that might play a role in protecting the cytochrome from oxidation.

There is a concentration of acidic amino acids around residue 70 which involves residues 67 and 68 in *S. elongatus* cytochrome c_6. In comparison to other cytochromes c6 this acidic patch is reduced, confirming the assumption that only one electron transfer pathway is used in cyanobacteria. This was already suggested previously from the absence of a positive patch in cyanobacterial photosystems.

References

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