Properties and function of cytochrome c heme lyase (CCHL)

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Summary

Cytochrome c heme lyase (CCHL), also known as holocytochrome c synthetase is the maturation enzyme that attaches heme covalently to the mitochondrial c-type cytochromes. CCHL is a 269 amino acid long soluble protein of the mitochondrial intermembrane space and it is in strong interaction with the inner membrane. Only the primary structure of the enzyme is known, whence we know that close to its N-terminal end it contains two conserved CPV heme regulatory motifs that are important in its heme binding activity.

We have heterologously overexpressed and purified the mitochondrial maturation enzyme from yeast, that was proven to be catalytically active in the bacterial environment, for structural investigations and in vitro maturation studies. We have tested its purity by denaturing gel electrophoresis, and we have determined its yield (~0.25 mg/g wet cell mass). We have noticed that under denaturing conditions the protein band runs consistently at a higher molecular mass than expected from the sequence of CCHL.

Based on this (and other) facts we have predicted the structure of the protein by the program IUPred. This program can identify with high fidelity disordered regions in a protein. According to the prediction a contiguous segment on the N-terminal end, representing ~25% of the total sequence is disordered, and contains an excess of some of the amino acids which typically promote disorder. We have examined with the program the structure of its known homologous, and we found that the proportion of the disordered part in these proteins can be even higher, from 25 % to 61 %.

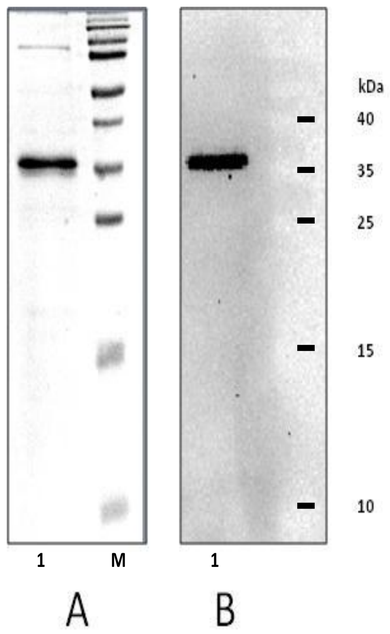
In order to further characterize the secondary structure we have measured the UV circular dichroism (CD) spectrum of CCHL and fitted it with the program CDTOOL. This analysis yielded a total of 35.7% contribution by segments not assuming ordered secondary structure. The results of the prediction and the CD experiment are in good agreements in terms of the existence of a sizeable disordered region in the protein.

We have carried out in vitro experiments with the purified CCHL and its heme substrate to study their interaction. Their incubation yielded biphasic spectral changes over time. We attribute the first phase to the disappearance of free heme, while the heme and the protein establish interaction. During the second phase, when an absorption spectrum typical of 6-coordinated low spin heme develops, the heme is likely to become coordinated by the cysteines of the heme regulating motifs of CCHL. Based on these results we can propose a model for the interaction of CCHL with heme. According to our model the disordered segment of CCHL, which includes also the heme regulating motifs, is able to wrap the heme due to its disorder-related flexibility.

We have also performed in vitro maturation experiments with the purified CCHL and its heme and apocytochrome substrates. We have demonstrated that under strongly reducing conditions our heterologously produced and purified heme lyase is able to mature cytochrome c in vitro. This was proven by the heme peroxidase activity of the holocytochrome band in denaturing gel as well as by the absorption spectrum of the reaction mixture.

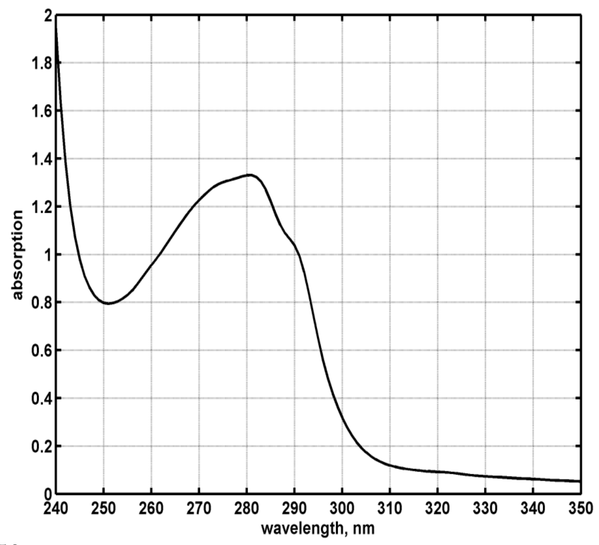
Our results open the way towards the better understanding of the structure and function of CCHL and its interaction with its two substrates, heme and apocytochrome c. We have reached the point where by producing site specific mutants of CCHL in the future we will be able to explore the mechanism of the maturation of the exceptionally important protein, mitochondrial cytochrome c.

I. The purity of the His_6-tag fused CCHL :



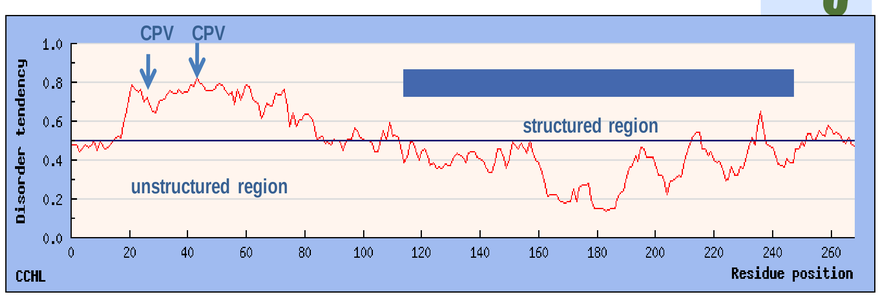
A: 15 % SDS - PAGE, stained with Coomassie BB G-250
B: Western blot assay of the His_6-tag fused CCHL (HRP conjugated His_6 -tag antibody).
1. His_6-tag fused CCHL (~8 µg).
M. Protein Ladder, Fermentas SM0671.

II. The UV absorption spectrum of the His_6-tag fused CCHL:

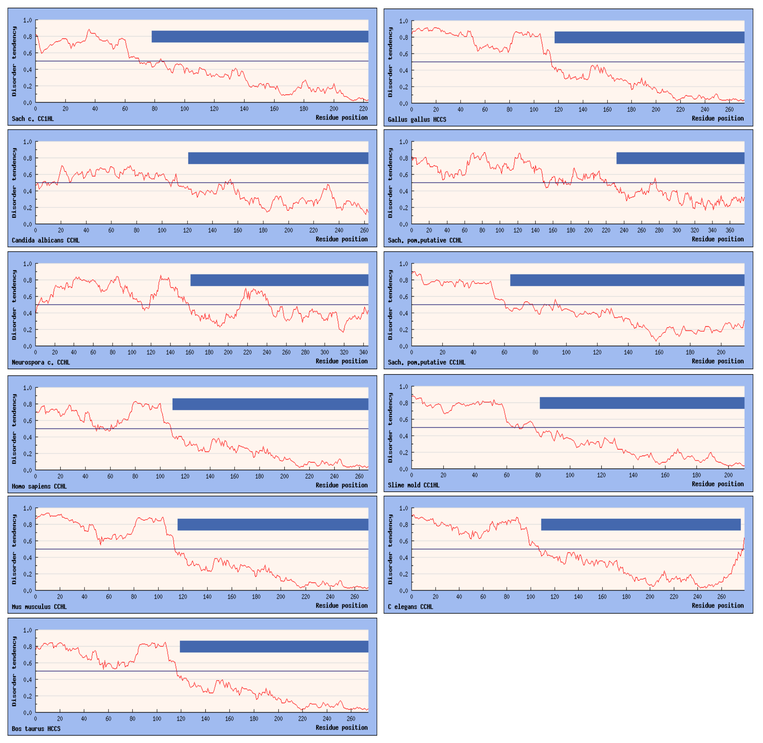


III. The structure of the CCHL enzyme as the IUPred program predicts it:

Dosztányi, Tompa, Simon, http://iupred.enzim.hu/



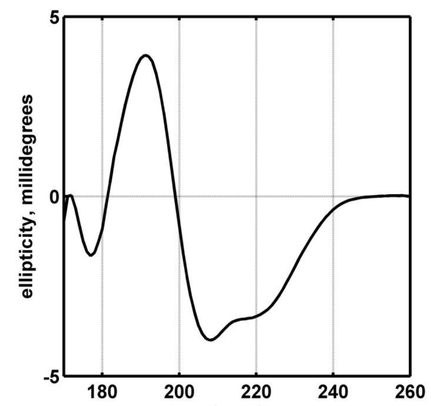
IV. The structure of CCHL homologs according to the IUPred disordered structure predictor program:



Saccharomyces cerevisiae CC_1HL
Candida albicans CCHL
Neurospora crassa CCHL
Homo sapiens HCCS
Mus musculus HCCS
Bos taurus HCCS

Gallus gallus HCCS
Schizosaccharomyces pombe putative CCHL
Schizosaccharomyces pombe putative CC_1HL
Slime mold CCHL
Caenorhabditis elegans CCHL

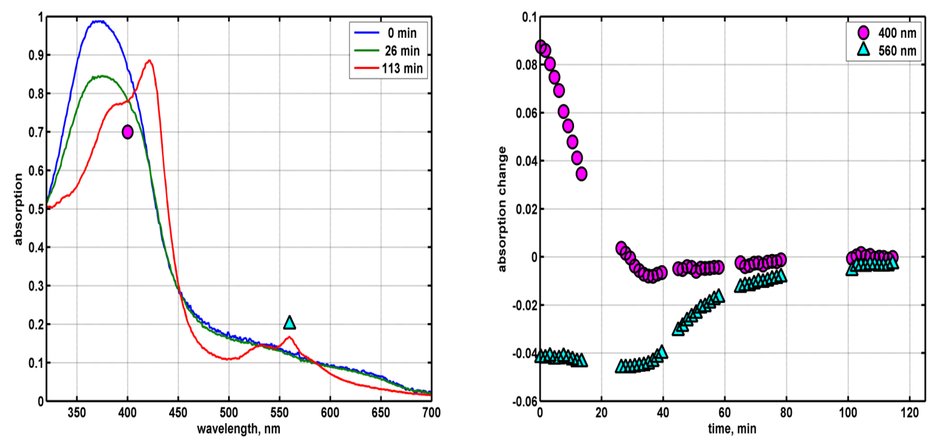
V. The UV Circular Dichroism (CD) spectrum of CCHL:



VI. The proportion of secondary structure elements of CCHL, calculated from fitting of the UV CD-spectrum:

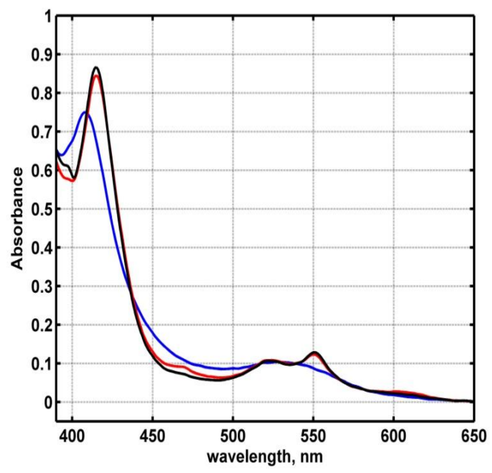
Alpha-Regular	Alpha-Distorted	Beta-Regular	Beta-Distorted	Turns	Unordered
13.7 %	12.2 %	16.5 %	8.6 %	13.3 %	35.7 %

VII. Interaction of CCHL with its heme substrate alone:

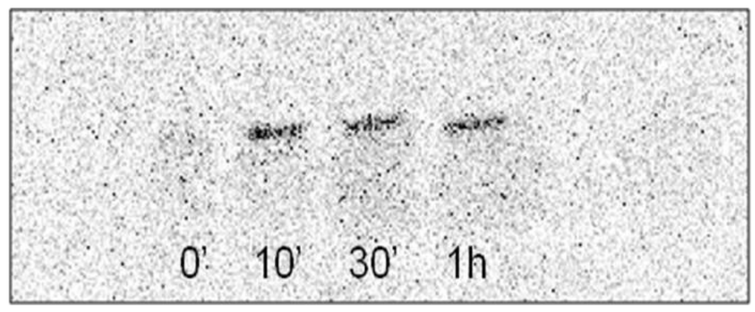


A: Time -dependent UV - Visible absorption spectra of heme and CCHL.
B: Time course of the spectral changes at two charactreistic wavelengths.

VIII. :In vitro maturation experiment. Interaction of CCHL with the heme and apocytochrome c substrates:



Absorption spectra befor reduction by Na-dithionite (blue), right after reduction (red), 40 minutes later (black).



„In-gel" ECL (enhanced chemiluminescence) signal detected as a consequence of heme peroxidase activity of the in vitro matured cytochrome c .