THE IMPACT OF ELECTROLYTE COSOLUTES ON THE CONFORMATION OF A PEPTIDE FROM CYTOCHROME C HEME LYASE: A CIRCULAR DICHROISM STUDY

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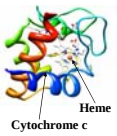
Introduction

• ***Mithocondrial cytochrome c heme lyase*** **(CCHL)** or **holocytochrome c synthetase** is an enzime which catalyzes the covalent attachment of the heme cofactor to the two cysteine residues in the well conserved CysXxxXxxCysHis heme binding motif of the cytochrome c. So, CCHL has a role in the translocation of the cytochrome c across the outer mitochondrial membrane.



• The mechanisms of this interaction are unknown.

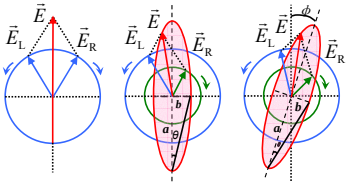
• The amino acids sequence of CCHL suggests that a region of about 25 amino acids close to the N terminus, which contains two heme-regulatory motifs CysProVal, is disordered.

• In this work, a peptide of 25 amino acids, including these two heme-regulatory motifs, has been synthesized and circular dichroism (CD) spectroscopy has been applied to study the conformational stability, as well as the effect of Hofmeister salts (such as NaF) on its conformation.

• In order to find the percentages of different secondary structure motifs, a computational study of the CD spectra has been done.

Circular dichroism

• Circular dichroism (CD) consists in the difference of the absorption of the left and right circularly polarized components of a light beam that propagates through an optically active substance.



$$\theta = \frac{b}{a} = \frac{E_L - E_R}{E_L + E_R} = 0.575\,\Delta A = 32.98^\circ\,\Delta A$$

θ - the ellipticity of the emergent electromagnetic wave;

$\Delta A = A_L - A_R$ - the circular dichroism of the sample;

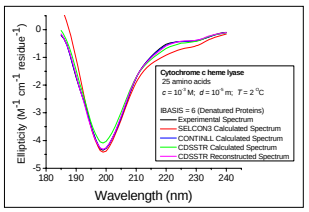
A_L and A_R - the absorbances of the sample for the left and right components, respectively.

• In order to investigate the structure of CCHL, we have made a computational analysis, using **CDPro** and **Dichro Web** softwares.

• Starting from a reference set of proteins with known structure and known CD spectra, and using the singular value deconvolution algorithm and variable selection procedures, the programs calculate the fraction of each secondary structure motif (α-helix, β-sheet, turn and unordered structure) that contributes to the protein's spectrum.

Results and discussion

Experimental and calculated CD spectra, using CDPro



The best fits obtained using CDPro software were for a reference set of proteins with desorder structure, like denatured proteins.

The secondary structure motifs calculated by CDPro and Dichro Web software packages and the influence of NaF cosolute

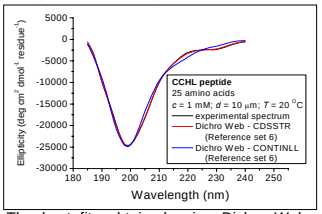
Package of programs	Sample	Ref. set	Program	Secondary structure motifs α_R (%)	α_D (%)	β_R (%)	β_D (%)	Turns (%)	Unordered (%)	Total (%)	Error NRMSD
CDPro	CCHL	6	CDSSTR	0.3	2.3	12.5	7.1	11.8	65.3	99.3	0.068
			CONTINLL	0.2	3	12.5	7.3	12.5	64.4	99.9	0.009
	CCHL + NaF	6	CDSSTR	1.9	2.7	4.4	2.8	5.3	82.5	99.6	0.071
			CONTINLL	2.2	3.7	2.7	3.1	8.9	79.4	100	0.012
DICHRO WEB	CCHL	6	CDSSTR	2	2	5	3	7	80	99	0.014
			CONTINLL	2.9	6.5	0	3.8	12.9	73.9	100	0.059
	CCHL + NaF	6	CDSSTR	4	3	19	9	22	41	98	0.004
			CONTINLL	5.4	6.9	0	2.7	0	85	100	0.082

Tertiary class of proteins	Score
All α	1
α+β	2
α / β	0
All β	3
"Denatured"	4

The CDPro and Dichro-Web results predict that the secondary structure of peptide mainly consists of unordered motifs. It can also contain turns, *β*-sheets and *α*-helices in smaller amounts.

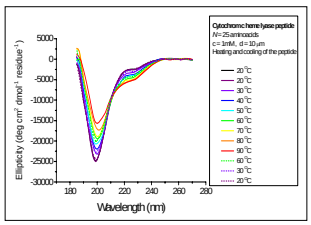
The CLUSTER program of CDPro package predicts that the peptide tertiary structure belongs to a class of proteins with irregularly tertiary structure (like denatured proteins).

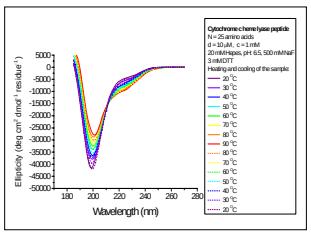
Experimental and calculated CD spectra, using Dichro Web



The best fits obtained using Dichro Web software were for a reference set of proteins with disordered structure, like denatured proteins.

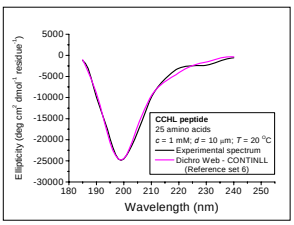
Experimental CD spectra at different temperatures and the NaF cosolute influence

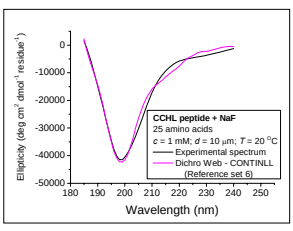




The peptide has high thermal stability. The NaF presence doesn't change the positions of the CD bands, but increases the CD, making the peptide more asymmetrical.

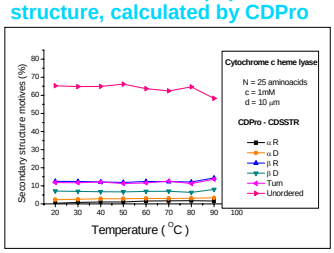
Computed CD spectra of the native structure (T = 20 °C) and the NaF cosolute influence

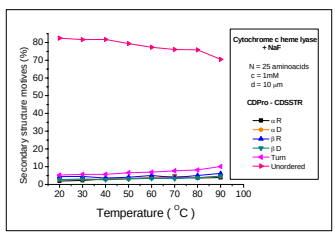




The NaF presence modifies the peptide structure and the fits obtained using CONTINLL program tend to not be so good.

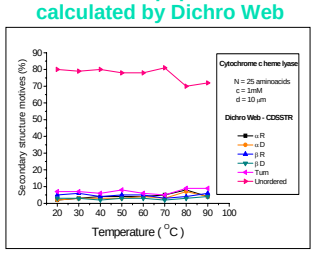
Thermal denaturation and NaF influence on the peptide structure, calculated by CDPro

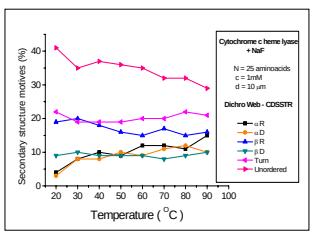




The CDPro results predict that the NaF presence seems to increase the amount of the unordered structures.

Thermal denaturation and NaF influence on the peptide structure, calculated by Dichro Web





The Dichro Web results predict that the NaF presence seems to decrease the amount of the unordered structures.

Conclusions

• For the native structure of CCHL peptide (T = 20 °C) and for the heated peptide (T = 30 °C – 90 °C), the CDSSTR and CONTINLL programs of CDPro and DICHRO WEB software packages give very good fits, using reference sets 6, which contain proteins with disordered structure, like denatured proteins.

• The computational analysis indicate that peptide contains mainly unordered structures, in good agreement with the structural prediction based on the amino acid sequence of CCHL.

• The thermal stability of CCHL is very high and the NaF cosolute doesn't change the thermal stability of the peptide.

• Heating the peptide (T = 30 °C – 90 °C), the amount of the unordered structures decreases, the peptide becoming more ordered. This effect can be due to the hydrophobic interactions between the peptide and water.

• In the presence of NaF, the fits obtained with CONTINLL program tend to not be so good.

• The CDPro results predict that the NaF presence seems to increase the amount of the unordered structures, during the Dichro Web results predict a strong decreasing of the unordered structures content in the presence of NaF cosolute.

• We need further studies to get inside the NaF influence on the amounts of the secondary structure motives during the thermal denaturation of CCHL peptide.

Acknowledgements

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