In order to find the percentages of different secondary structure motifs on its conformation. 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.

In this work, a peptide of 25 amino acids, including these two heme-regulatory motifs Cys-Pro-Val, is disordered. 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 Cys-Pro-Val, 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 the conformational stability, as well as the effect of Hofmeister salts (such as NaF) on its conformation. In order to investigate the structure of CCHL, we have made a computational analysis, using CDPro and Dichro Web software packages, and the influence of NaF cosolute on the structure of CCHL was calculated by Dichro Web application. Starting from a reference set of proteins with known structure and known CD spectra, and using the singular value decomposition algorithm and variable selection procedures, the programs calculate the fraction of each secondary structure motif (%-helix, %-sheet, turn and unordered structure) that contributes to the protein's spectrum. For the native structure of CCHL peptide (T = 30 OC – 90 OC), the amount of the unordered structures decreases, the peptide becoming more ordered. The CDPo and Dichro-Web results predict that the secondary structure of peptide mainly consists of unordered motifs. 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). The best fits obtained using CDPro software were for a reference set of proteins with disordered structure, like denatured proteins. The Dichro Web results predict that the NaF presence seems to increase the amount of the unordered structures.

Conclusions

- For the native structure of CCHL peptide (T = 20 OC) and for the heated peptide (T = 30 OC – 90 OC), 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 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 OC – 90 OC), 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 not to be so good. The CDPro results show 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.

References


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