Effect of different Hofmeister-salts on the unfolding of Trp-Cage miniprotein followed by Circular Dichroism Spectroscopy

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Introduction

From the pioneering work of *Franz Hofmeister* [1], it is well-known that neutral salts have remarkable effect on the solubility of proteins. The effects are dominated by anions. Based on his observations, Hofmeister set up a series of salts that significantly alter the structural stability and consequently, the solubility of proteins. The anions are ordered in a series ("Hofmeister series"), as follows:

$$F^- \approx SO_4^{2-} > HPO_4^{2-} > acetate > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$

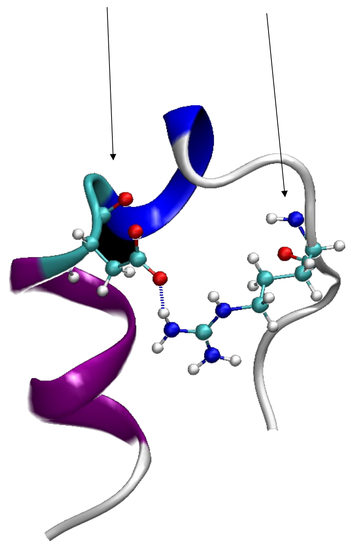
Early members (kosmotrope salts) of the series increase the protein-water interfacial tension, in effect, they strengthen the hydrophobic effect, decrease solubility ("salting out" effect), and normally decrease conformational fluctuations. By contrast, later (chaotrope) salts decrease the interfacial tension, weaken hydrophobic interactions, therefore they destabilize the protein structure and normally increase fluctuations ("salting in" effect). Cl^- is considered to be Hofmeister-neutral [2,3].

Temperature induced unfolding of the Trp-cage in different Hofmeister-salts ($NaClO_4$, NaCl, NaF) was investigated by means of Circular Dichroism Spectroscopy. Transition temperature between the folded and unfolded states, as well as the helicity of the protein were calculated from experimental data and by analyzing the trajectories of molecular dynamics simulations using the built-in DSSP routine of Gromacs [4].

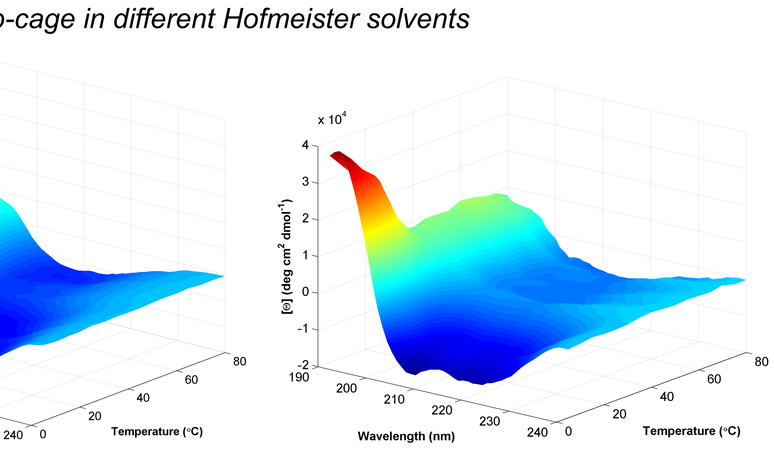
Our model system, the Trp-cage peptide

A 20-residue polypeptide with well-defined secondary structure.

Salt-bridge between **Asp 9** and **Arg 16**



Trp-cage in water (dots) surrounded by **Na^+** and **Cl^-** ions (colored spheres)



Experiments

Protein concentration	1 mg/ml (≈ 461 μM)
Salt concentration	500 mM
Wavelength range	190-240 nm
Temperature range	5-80°C (incr. 5°C)

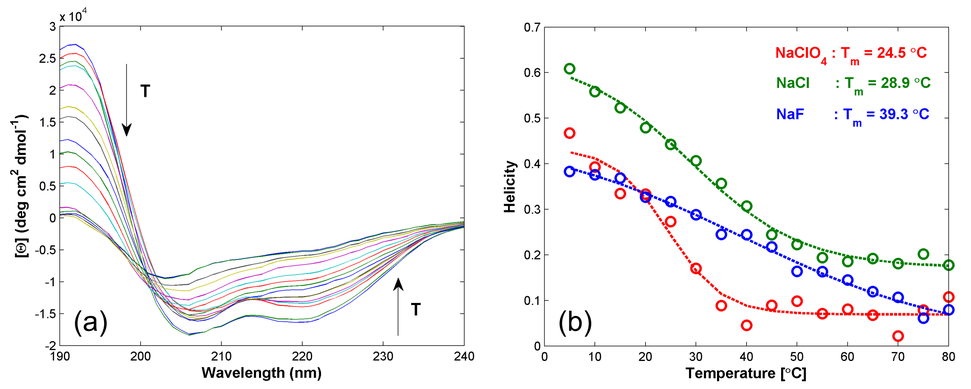
3D Circular Dichroism spectra of Trp-cage in different Hofmeister solvents

Amongst other parameters, **helicity** is a good measure of thermal stability of helix-containing structures. With increasing temperature, **thermal fluctuations can destabilize the H-bonds** of an α-helix, causing the denaturation of the protein.

Temperature induced unfolding of the protein was monitored by measuring the **mean residue ellipticity ([Θ], [deg cm² dmol⁻¹]** at **222 nm**, one of the characteristic (negative) CD-peak corresponding to an **α-helix**.

The helical content ($f_{H,T}$) of the protein was calculated using the following empirical formula with appropriate parameters [5]*:*

$$[\Theta]_{222} = f_{H,T}\left(1 - \frac{n_\alpha}{n}\right)\left[[\Theta]_\alpha + [\Theta]_{\alpha T}(T - T_0) + \frac{[\Theta]_{\alpha W}}{n}\right] + (1 - f_{H,T})\left[[\Theta]_r + [\Theta]_{rT}(T - T_0) + \frac{[\Theta]_{rW}}{n}\right]$$

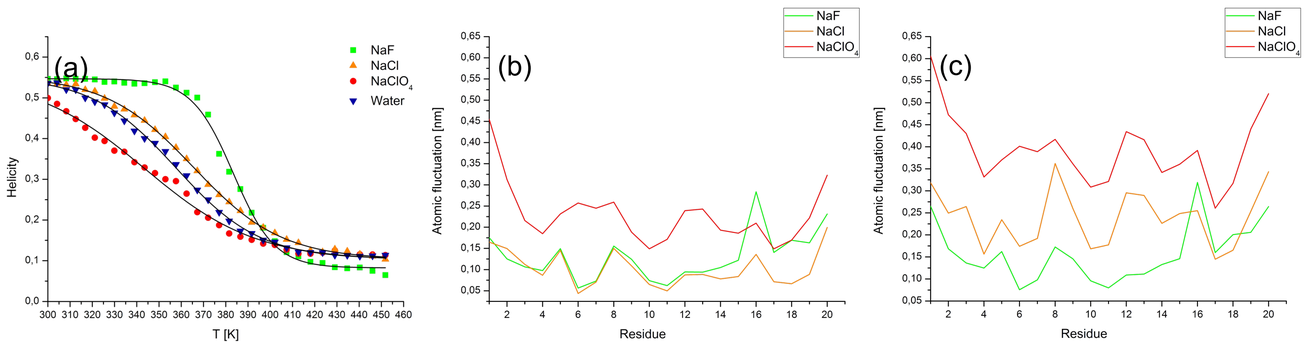


Typical CD spectra of Trp-cage in NaF with increasing temperature (a) and the calculated helical contents (b) in different solutions

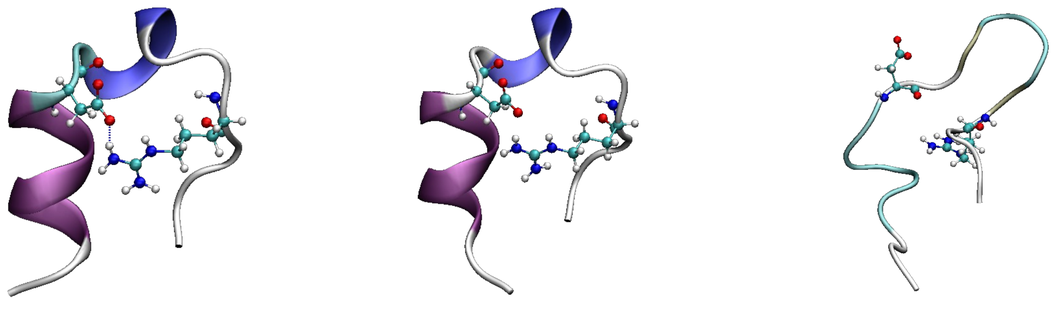
Theoretical investigation of temperature induced unfolding

Replica Exchange Molecular Dynamics (REMD) simulations were also carried out to model the effect of Hofmeister salts on the stability of Trp-cage. The calculated helical contents found to be comparable with the measured ones.

Plots of the average atomic fluctuations at different temperatures clearly show the structure-stabilizing property of kosmotrope (NaF) salts.



Helix-content (a) together with average atomic fluctuations at T=300K (b) and T=340K (c) from REMD simulations



Structure of the peptide at different temperatures. At high T, the salt-bridge is broken and the secondary structure is completely lost

Conclusions

CD spectroscopy was used to find experimental proof of the effect of Hofmeister anions on proteins. Differences in the calculated „melting" temperatures and helicities indicate that in solutions containing kosmotrope salts (e.g. NaF), due to the enhanced hydrophobic interaction and interfacial tension, the well-defined secondary structure of Trp-cage is conserved at a higher degree than that in chaotrope (e.g. $NaClO_4$) salts.

Molecular dynamics simulations have also confirmed our experiments. The calculated helical content and its temperature-dependence is similar to the experimental findings. Increase of the melting temperature in case of kosmotrope salts indicate higher structural stability against thermal denaturation.

Analysis of calculated atomic fluctuations are in accordance with the experimental results. At high simulation temperatures the RMSD values of Trp-cage in $NaClO_4$ solution exceed the fluctuations typical for a structure that can be called stable. The same values remain almost unaltered in case of NaF solution.

Further investigations are in progress to give a thermodynamical description of temperature-induced unfolding of Trp-cage.

References

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