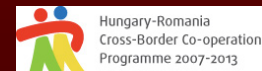


Influence of NaF, NaCl and NaClO₄ salts on the stability of Trp-cage miniprotein: a computational study

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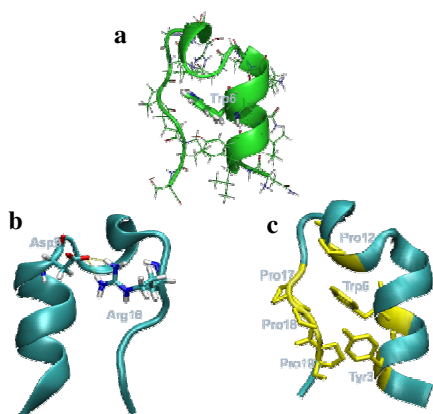
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Introduction

Since the famous observation of Hofmeister, it is well-known that solubility of proteins can be influenced by adding salts to the solution. Ions can be ranked according to extent of this influence (*i.e.* Hofmeister series). These series start with the kosmotropic, structure-stabilizing and end with the chaotropic, structure-destabilizing ions. Despite the wide-spread use of Hofmeister effect, its interpretation has remained a matter of debate. Together with experimental investigations molecular modeling can help to understand the atomic level processes behind this effect.

In a recent study, Dzubiella [1] pointed out that the effect of Hofmeister active salts on the stability of simple model peptides (like charged Ala-based helices) can be simulated using non-polarizable force fields. In this study, we investigated the effect of kosmotropic (F⁻) and chaotropic (ClO₄⁻) ions on the stability of a more protein-like model peptide, the Trp-cage miniprotein (Tc5b, a). It is a 20-residue-long polypeptide and shows several important characteristic features of proteins. It has a stabilizing salt bridge (b), a hydrophobic core (b) and a well-defined, stable secondary and tertiary structures.



Results and Discussion

The influence of investigated salts on the stability of the structure of Trp-cage miniprotein is characterized by the fractional helicity, the RMSD value (from the experimental structure) of heavy atoms in the backbone, and the solvent accessible surface area (SAS). The last 30 ns of trajectory was used for the calculation of average values of these quantities. The fractional helicity of miniprotein has decreased from 55 % to approximately 10 %, while the temperature was risen from 300 K to 450 K (Fig. 1). This phase transition can be characterized by the melting temperature values obtained from sigmoidal curve fitting. The melting temperature obtained for the kosmotropic F⁻ ion is the highest (383.4 K) and for the chaotropic ClO₄⁻ ion is the lowest (343.4 K). The Hofmeister inactive Cl⁻ gave the closest value (365.6 K) to the case of pure water (357.5 K). The RMSD of the heavy atoms of main chain and solvent accessible surface area (SAS) curves (Figs. 2 and 3) have similar characteristic features as the fractional helicity curve.

Although the order of the calculated values of melting temperatures is in accordance with the position of applied anions in the Hofmeister series, the absolute values are shifted upwards. The sigmoidal fit is also possible in the case of other state-describing features, such as the RMSD of the heavy atoms of main chain, the SAS and the minimum distance between the heavy atoms of two salt-bridge-forming residues. Comparing the fitted curves, which scaled to interval (0,1), and the transition temperatures with the results of fractional helicity fit (Fig. 4), significant differences can be found between the three considered salts, indicating different pathways of structural destabilization. The system containing F⁻ ion shows the lowest transition temperature for the minimum distance of salt-bridge-forming residues, while in the case of ClO₄⁻ ion, the corresponding feature is the fractional helicity. The highest transition temperatures belong to the SAS for each salt, which quantity describes compactness of the hydrophobic core.

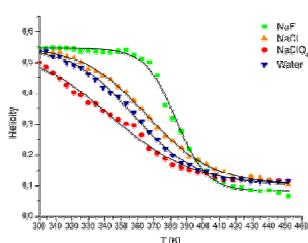


Fig. 1 The fractional helicity as a function of temperature.

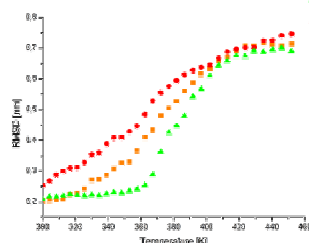


Fig. 2 The RMSD of the heavy atoms of main chain as a function of temperature.

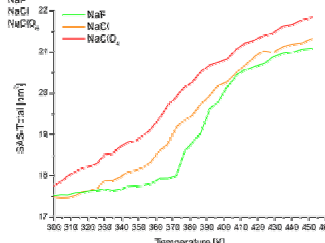


Fig. 3 The solvent accessible surface area (SAS) as a function of temperature.

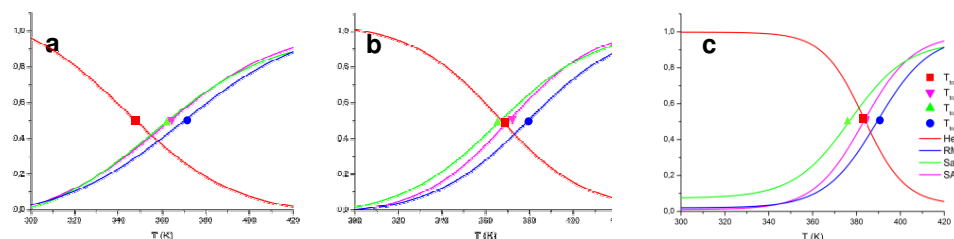


Fig. 4 The fitted curves of fractional helicity, RMSD, SAS and minimum distance between the heavy atoms of the side chains of Asp⁹ and Arg¹⁶, as well as their transition temperatures in the presence of three different salts: (a) NaClO₄, (b) NaCl and (c) NaF.

Methods

In our investigations, the Amber ff99SB-ILDN force field and the TIP3P water model together with the ion parametrization of Joung *et al.* [2] and the perchlorate parametrization of Baaden *et al.* [3] were used in four 90 ns long REMD simulations with Gromacs simulation package. There were 32 different temperatures, ranged between 300 K and 450 K. The considered systems contained ≈2200 water molecules in a cubic simulation box.

Electrostatic interactions had a 10 Å cutoff and the temperature regulation was controlled by the *v*-rescale method. Periodic boundary conditions and the LINCS algorithm (bonds involving hydrogen are constrained) were also used in these calculations.

In the first simulation, the model peptide was dissolved in water, while in the other cases, NaF, NaCl and NaClO₄ salts were added to the solution in 1 M final concentration.

The systems were prepared for the REMD simulation by an energy minimization followed by a 10 ns long fixed solute geometry NPT simulation and finally an also 10 ns long ensemble dynamics at the 32 temperatures.

References

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Conclusions

The influence of Hofmeister active ions on the structural stability of Trp-cage miniprotein can be studied computationally using replica exchange molecular dynamics simulations with non-polarizable ff99SB-ILDN force field and the TIP3P water model together with a proper ion parametrization.

The temperature dependence of the fractional helicity, the RMSD from the experimental structure, as well as the solvent accessible surface area show the expected shifts if we add anions with different Hofmeister activity to the solution.

The order of melting temperatures calculated from the fractional helicity curve is in accordance with the position of applied anions in the Hofmeister series.

In the presence of various salts, the destabilization can be characterized by different initiation steps, as follows. For the F⁻ ions, the transition temperature of the minimum distance of salt-bridge-forming residues is the lowest, whereas in the case of ClO₄⁻ ions, the transition temperature of the helix content is the corresponding feature. For the investigated salts, the highest transition temperature was observed with regard to the SAS.