

Title: Ion Specific Hofmeister Effects on Peptides and Proteins

Author: Ing. Jana Hladílková

Department: Physical and Macromolecular Chemistry

Advisor: Prof. Pavel Jungwirth, DSc., IOCB AS CR

Advisor's email address: pavel.jungwirth@uochb.cas.cz

Abstract: Classical molecular dynamics simulations in combination with advanced methods of analysis were used to shed light on missing parts of our molecular understanding of the Hofmeister series. In tandem with various experimental techniques, real proteins as well as model systems were investigated in aqueous salt solutions in order to identify and quantify ion-protein interactions either leading or not leading to the canonical cationic and anionic Hofmeister ordering.

The potassium cation was found to significantly enhance the BHMT enzymatic activity in contrast to the rest of the common monovalent cations. In the quest to rationalize this behavior, a key potassium binding site in the vicinity of the active site was discovered and described. Moreover, the exceptionally strong effect of K^+ on the enzymatic activity was explained by hydration properties of the cations within the limited space of the active site in interplay with their attraction to the nearby negatively charged residues. By contrast, only a small and indirect influence, which follows the cationic Hofmeister series, was established for the LinB dehalogenase. The binding hot spot for all the cations was assigned at the mouth of the tunnel leading to the active site. This assumption was further supported by single point mutations at the tunnel mouth of this enzyme.

A systematic study of anion-peptide interactions was realized for a variety of model systems with aid of NMR experiments. Examination of the (VPGVG)₁₂₀ polypeptide revealed a dominant role of anion-backbone interactions in neutral biological systems. On top of that, it was shown that anions are not attracted to the nonpolar side chains of residues like valine. In order to quantify our previous findings, capped triglycine was investigated as a model system for the peptide bond in aqueous solutions of five sodium salts. It was confirmed that the more weakly hydrated the anion is, the more it interacts with the peptide backbone ($SCN^- > I^- > Br^- > Cl^- > SO_4^{2-}$). Consequently, thiocyanate and iodide act like salting-in agents, bromide and chloride are neutral in contrast with sulfate which is repelled from the backbone surface and shows a salting-out behavior.

To capture the effect of charged residues, anion-peptide binding sites for uncapped aqueous triglycine were explored. In this case, charge-charge interactions dominate, resulting in a reversed Hofmeister series ($SO_4^{2-} > Cl^- > Br^- > I^-$), i.e., the more strongly hydrated the anion is, the larger its affinity to the positively charged N-terminus. Interestingly, SCN^- does not fully follow this rule as a consequence of a synergy between charge-charge and anion-backbone interactions of this non-spherical ion. A direct comparison of our results with experimental data published in the 1970s led to a discovery of an error in the original publication. It was proven that an inefficient synthetic procedure caused assignment of the measured salting-out constants to a fully capped triglycine molecule instead of a half-capped version, which matched with our computational results.

Finally, an innovative way of studying ion-protein interactions was demonstrated on the example of electrophoretic measurements and calculations of neutral model systems and electroosmotic flow markers.

Keywords: molecular dynamics, proteins, peptides, ions, Hofmeister series.