Water-related phenomena in spectroscopic studies of biological macromolecules

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Here we would like to report our (sometimes unintended) observations related to interaction of water and biological macromolecules (proteins and polysaccharides). Our studies concerning small biologically active solutes (like ATP, NO, metal cations) have revealed that many of their effects on protein behavior are related to changes in protein's hydration shell.

In particular, over a temperature range from 25°C to 80°C and at constant pH (7.4), we measured diffusion coefficients of different proteins using quasi-elastic light scattering (QELS) in the presence of different solutes and low molecular weight compounds. The main discoveries were that 1) spermine NONOate (known as a potent nitric oxide (II) donor) persistently decreased the hemoglobin aggregation temperature independently on the Na+/K+ environment, 2) ATP alone had rather mild stabilizing effect on the studied protein's but it facilitated protein's hydration water destabilization in the presence of spermine NONOate and 3) mutual effects of ATP and NO were strongly influenced by buffer ionic compositions.

Furthermore, circular dichroism spectrometry (CD) measurements have brought the following major results: 1) spermine NONOate persistently decreased the hemoglobin unfolding temperature, irrespectively of the Na+/K+ environment, 2) ATP being combined with Na+ (but not K+) showed unfolding of Hb at room temperature and increased the unfolding temperature by 3°C in both sodium-based and potassium-based buffers and 3) mutual effects of ATP and NO were (again) strongly influenced by particular buffer ionic compositions.

Yet another promising tool for investigation of the self-structuring and structuring effects of water on biological macromolecules is spectrofluorimetry. 3D fluorescence spectra of different water samples show different diagonal features resulting from Raman scattering of 1st and 2nd order as well as Rayleigh scattering of 1st and 2nd order. These and other spectral signatures of water, usually considered as annoying artifacts, may carry useful and interesting information about the hydrogen bonding and the degrees of freedom of water molecules. Our studies with highly diluted agarose samples have demonstrated very distinct changes in the spectra features of water related to melting and solidifying of agarose gels.

Also macroscopically, we were able to produce and observe the water exclusion zone (EZ) phenomenon repeatedly by adding of 5µm microparticles to gels of different chemical nature submerged in aqueous solutions. The properties of the formed EZ's have been investigated in respect of pH-, ATP and K+/Na+ sensitivity and it has been shown that ATP as well as ions show significant effects on EZ formation.