MS82.O02

UHR PX and NPC studies of H-FABP water network with tiny perdeuterated crystals

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We have obtained very detailed information about the internal water molecules in the large internal cavity inside fatty acid binding (FABP) proteins , in the presence of bound fatty acids (FA), by Ultra High Resolution X-Ray Crystallography (UHR) to 0.7 Å and Neutron Protein Crystallography (NPC) to 1.9 Å using a "radically small" (V=0.05 mm3) crystal. These waters form a very well ordered dense cluster of 12 molecules, positioned between the hydrophilic internal wall of the cavity and the fatty acid molecule. This information has been used for a detailed electrostatic analysis based on the charge distribution description modeled in the multipole formalism and on the Atoms in Molecules theory. This information is also being used in molecular dynamics simulations of H-FABP and its complex with FA in order to quantify the energetic contribution of these internal waters to the binding energy. The experiment has been done with oleic acid, coming with the protein expressed in E. Coli. The results have been analyzed in order to understand the interactions between the FA, the internal water and the protein, and in particular the role played by the water molecules in determining the potency and specificity of FA binding to FABPs. The major tool for visualizing the water molecules inside the H-FABP cavity is UHR X-Ray Crystallography combined with NPC. UHR crystallographic structures give the positions of hydrogen and oxygen atoms for well-ordered water molecules. NPC determines hydrogen atom positions, particularly of water molecules which have multiple conformations, leading to the best possible crystallographic model. This model was then complemented by a transferred charge distribution to accurately determine the electrostatic and topological properties in the binding pocket, providing a description of the way water molecules in hydration layer contribute to the binding of ligand, which is essential to understand and model ligand binding.

Keywords: Neutron Protein crystallography, High resolution X-Ray crystallography, H-FABP