Direct MicroCT Measures of Collagen Interfacial Water Density and Protein Conformational Response to Hydrating Solvent

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Purpose: Improved understanding of the role of water in protein conformational response to adjacent solvent environments has far reaching importance in medicine, biology and biotechnology. This presentation introduces a new molecular measurement method capable of directly measuring hydration properties of proteins using 10^6 to 10^8 amplification factor of semi-crystalline tendon to evaluate shape response of the collagen molecule. **Methods:** Bovine extensor tendon was first dialyzed against neat water to remove mobile ions and metabolites and then either evaluated directly or following molecular infusion by a test solution such as 0.2 M glucose as reported here. MicroCT measures of relative diameter d/d_0, relative length l/l_0, and relative volume $V//V_0$ as a function of hydration *h(g water/g dry solid)*were combined with accurate mass measurements to calculate tendon density ρ and differential (removed water) density ρ_Δ both with and without glucose infusion. All relative values are referenced to measurements on completely dry tendon. These tendon quantities were validated as equivalent to relative measures of molecular collagen response by comparison to X-ray crystallography. **Results:** The density of first layer water ranged from 1.08 ± 0.03 SE (native) g/ml on hydrophobic surfaces to 1.53 ± 0.14 SE (native) g/ml on hydrophilic surfaces. These values agree well with the range from recent MDS (molecular dynamic simulation) calculations and neutron diffraction studies showing 10 to 50% density elevation. Elevated density from MDS results are attributed primarily to the interaction of water with large (immobile) macromolecular surfaces (75% of change) and electrostriction (25% of change). Asymmetric shape changes of tendon and collagen agree with predictions of the Stoichiometric Hydration Model SHM. **Discussion:** To our knowledge this is the first report of direct measures of first monolayer water density using the ~10^8 amplification factor of the semi-crystalline tendon structure. The method also provides direct evidence of stabilized acyclical and excluded cyclical glucose in water filled cavities between collagen surfaces. Acyclical glucose is compressed during dehydration from a diameter of ~7Å to 3.9 Å by electrostatic constriction.