CENTRIFUGATION DEHYDRATION AND REHYDRATION METHODS TO DETERMINE WATER COMPARTMENTS

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To validate any new method it is important to use a material that has been well studied and characterized using established and accepted methods. Thus tendon/collagen was chosen for this verification study. This report begins with an experimentally tested molecular model of collagen hydration (Fullerton and Rahal 2006).

This model reveals four hydrated compartments on native tendon/collagen: The Ramachandran single water bridge = 0.065g H_20/gDM, the double water bridge = 0.197 g/g which equals a total bridge water of 0.26g/g as Berendsen's predicted as a single chain that perfectly matches the spacing of hydrogen bonding sites on the collagen main chain with a spacing of 0.47mm between bonds or d_c = 0.235 mm per water molecule chain link. This equals 4 water molecules (18 Daltons each) per every 3 protein residues (mean 91.2 Daltons each) or 0.262g water/g collagen for a single chain of water in each groove or cleft of collagen triple helix, primary hydration over hydrophilic surfaces = 0.8 g/g, and secondary hydration over hydrophobic surfaces = 0.8g/g, and secondary hydration over hydrophobic surfaces = 0.8g/g. Total hydration of native tendon/collagen = 1.6g/g. Weight loss change of the tendon, resting on a filter at times during centrifugation was used to assess fluid flow rates. Break points in flow rate and final dry mass of the sample allowed determination of water compartment sizes. The rehydration rate of a carefully dried tendon revealed the size of inner water of hydration compartments. The sizes of the four measured water compartments, was in excellent agreement with the molecular model. This newly validated centrification method has now been applied to skeletal muscle tissues. The sizes of three outer water compartments were determined and showed no changes for four hours post-mortem. Later times post-mortem showed increase in flow rate of water from the tissue that was correlated to decrease in ultrastructural obstructions. This centrifugation method is now being applied to globular proteins and to cell suspensions using smaller pore size filters.

An aim of the above described methods is to understand the molecular basis of water solute interactions in systems that are not as well characterized as tendon/collagen.

Fullerton, GD, Rahal, A. Collagen structure the molecular source of tendon magic angle effect. J. Mag Reson Imag. (2006) In press.