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Characterization of water of hydration fractions in rabbit skeletal muscle with age and time of post-mortem by centrifugal dehydration force and rehydration methods

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Abstract

Centrifugal dehydration force (CDF) and rehydration isotherm (RHI) methods were used to measure and characterize hydration fractions in rabbit psoas skeletal muscle. The CDF method assessed fluid flow rate from rabbit muscle and hydration capacity of the fractions. Bulk and multiple non-bulk water fractions were identified. The non-bulk water was divisible into the following fractions: two outer non-bulk fractions, a main chain proteins backbone or double water bridge fraction, and a single water bridge fraction. The total non-bulk water amounts to about 85% of the total water in the muscle. The sizes of the water fractions (in g water/g dry mass) agree with a recently proposed molecular stoichiometric hydration model (SHM) applicable to all proteins in and out of cells (Fullerton GD, Cameron IL. Water compartments in cells. Methods Enzymol, 2007; Cameron IL, Fullerton GD. Interfacial water compartments on tendon/collagen and in cells. In: Pollack GH, Chin WC, editors. Phase transitions in cells. Dordrecht, The Netherlands: Springer, 2008). Age of the rabbit significantly slowed the flow rate of the outer non-bulk water fraction by about 50%. Also, muscle of the older rabbit (26 weeks vs. 12 weeks old) had less bulk water and less outer non-bulk water but the same amount of main chain backbone water compared to muscle of the younger rabbit. Increase in time post-mortem from 30 min to 4 h resulted in rigor mortis and a significantly slower flow rate of water from the outer non-bulk water fraction, which is attributed to muscle contraction, increased packing of contractile elements and increased obstructions to flow of fluid from the muscle fibers.

Keywords: Hydration; Myocytes; Muscle; Flow rate; Dehydration; Aging; Post-mortem

1. Introduction

The extent of water of hydration vs. the extent of bulk-like water in cells has been a subject of much debate. Cell physiologists and protein chemists have commonly accepted a water of hydration, or "bound water," value in the range of 0.2–0.4 g water/g dry mass (Ling, 1972, 2006; Cameron and Fullerton, 2008). This common assumption, either expressed or just tacitly assumed, is that all water above that of this "bound water" value has the physical properties of liquid water in bulk. Evidence is mounting to refute this common assumption about the extent of water of hydration on proteins and in cells (Ling, 2001, 2004; Pollack, 2003; Cameron et al., 1997, 2007; Fullerton and Cameron, 2007).

Recently published reports provide physical evidence for the formation of 4 different non-bulk water fractions/compartments on proteins and in cells (Fullerton et al., 2006a,b; Fullerton and Amurao, 2006; Fullerton and Rahal, 2007; Cameron et al., 1988a,b, 1997, 2007a,b; Cameron and Fullerton, 2008; Fullerton and Cameron, 2007). Data in these reports on collagen, globular protein and cells lead to the hypothesis of a molecular stoichiometric hydration model (SHM) applicable to all proteins in and out of cells (Fullerton and Cameron, 2007). This molecular model of protein

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hydration fractions or compartments matches estimates made by multiple methods, all yielding water of hydration values up to and much beyond 0.2–0.4 g water/g dry mass value.

This report deals with determination of the sizes of multiple water of hydration fractions and flow rates using CDF and RHI methods (see Cameron et al., 2007a,b) on psoas skeletal muscle of rabbits of different ages and of time post-mortem. Discussion focuses on the fit with the SHM method, and how age and post-mortem changes affect the hydration fractions of muscle water.

2. Methods and materials

2.1. Muscle samples

Rabbits 12 weeks and 26 weeks of age, weighing 3 and 4.75 kg, respectively, were used. The rabbits were killed by a blow to the head, decapitated, evisceration, and the wall of the abdomen cut open to expose the psoas muscle. The psoas was removed and transected halfway between the origin and insertion. Two-millimeter sections across the muscle fibers were blotted with Whatman number one filter paper. The initial blotted weight of specimens ranged from 0.19 to 0.21 g. The remainder of the psoas was wrapped in clear polyethylene at room temperature of 21 °C for 4 h, unwrapped and a fresh 2 mm transection through the body of the muscle was taken 0.5 cm from the first transection, blotted with filter paper and weighed prior to centrifugation. In one experiment, fresh 2 mm transections of muscle were placed in deionized water at 21 °C and stirred for 2.5 h, blotted, weighed and subjected to the centrifugation procedure as described below.

2.2. Centrifugal dehydration force method

The method used for centrifugation dehydration was described previously (Cameron et al., 2007a,b). Weighed pieces of muscle from 0.19 to 0.21 g were placed in a micro-filterfuge tube (Rainin Instrument Co. Oakland, CA, Cat. No: 7016-022) containing a filter membrane of 0.45 μm pore size. The specimens did not cover the entire surface of the filter. Samples were centrifuged for intervals up to 150–210 min with the specimens are 5.7 cm from the rotor center, giving a total force of 14,000 × *g* and a stress of about 4.0 MPa. (MC 1400 Microcentrifuge, Hoefer Scientific Instruments, San Francisco). Intermediate weights of the tissues were recorded at each centrifugation interval. Following centrifugation, all samples were dried to weight equilibrium at 80–90 °C in a vacuum oven and the final dry weight was measured to allow the water content of each sample to be expressed as grams of water per gram dry weight. After the centrifuge run, the bottom section of the centrifuge tubes were dried in the vacuum oven, weighed, washed free of any solutes, dried as before and reweighed. This procedure allows determination of loss of solutes from the tissue during centrifugation.

The water content of the tissue was calculated by subtracting the final dry weight from the weight of the sample at each interval of time during centrifugation and then by expressing the data in g water/g dry mass. Plotting the data against time under centrifugal load reveals the shape of the curves defining the resistance to fluid flow or water flow rate through and out of the tissue. Thus the slope of the curve provides a numerical measure of the resistance to fluid flow.

In one experiment, fresh psoas muscle from the 26-week-old rabbit washed in deionized and stirred water for 2.5 h resulted in muscle swelling. Application of the CDF method to the water washed and filter paper blotted muscle revealed a larger fast-flowing water fraction during the first minute than it did from 4 min of centrifugation onward. Swelling caused a 2.3-fold increase in the size of this faster flowing water fraction, leading to the conclusion that this initial faster flowing water compartment has the characteristics expected of bulk water.

2.3. Rehydration isotherm method

A water sorption isotherm as a function of time was measured for dried muscle. Proteins are very heat-labile when wet due to the small free energy difference between the native conformation of the protein and many possible random orientations of the protein chain when fully hydrated in a dielectric fluid. Thus, the protein must be first dried at room temperature in a vacuum chamber to hydration less than 0.26 g water/g dry mass before raising the temperature slowly to 90 °C. The sample was dried at 90 °C for ∼7 days until no further decrease in mass was observed. The specimen was exposed to room temperature of 21 °C in an atmosphere with relative humidity 45%. The specimen was periodically weighed on an analytical balance. Data at each time of weighing were expressed in g water/g dry mass and all data were used to obtain the curve fit of the data. The difference between hydrated mass and dry mass divided by the dry mass was recorded.

2.4. Statistical analysis

The Graph Pad Prism statistical program was used for curve fits, ANOVA and multiple range tests. Lack of overlap of the 95% confidence interval values between means indicated significant differences.

3. Results

3.1. Centrifugation dehydration force results

The muscle water content from 8 min onward during the centrifugation procedure was used for exponential curve fit analysis. The 8–120 min data from each muscle gave excellent fits to exponential decay (i.e. r^2 values for individual specimens ranged from 0.9967 to 0.9995). Extrapolation of these best fit curves to the zero time water content values was determined.

All data points from 0 to 4 min fell above the exponential fit from 8 min of centrifugation onward. This fact indicates the presence of a faster flowing water compartment, the size of