EFFECT OF MUSCLE pH AND MOLECULAR CHARGE ON DIPOLAR COUPLING INTERACTIONS IN MUSCLE OBSERVED BY DOUBLE QUANTUM 1H MRS

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ABSTRACT
Double quantum (DQ) 1H NMR spectra were acquired on permeable muscle samples dialyzed against solutions containing three classes of solutes: cations or anions, zwitterions and non-electrolytes as a function of pH ranging from 5 to 8. All classes of compounds showed frequency splittings due to dipolar coupling. Frequency splitting and the intensity of these peaks increased as pH increased. As expected, Tris, glycine and dioxane show no DQ signal in solution. Therefore, the observed anisotropy could only arise from ordering effects in the filament lattice of the muscle. Thus, the net charge on protein filaments dominates the mechanism of dipolar interactions in muscle.

INTRODUCTION
1H NMR spectra of several metabolites in skeletal muscle show orientation-dependent frequency splittings due to dipolar coupling interactions [1]. These splittings are absent in solution where these molecules tumble freely with no preferred orientation. However, skeletal muscle is a well ordered biological structure. The ordered protein filaments impose a structured charge distribution. Studies in our lab are focusing on elucidating the mechanism(s) of muscle lattice orientation of solutes and metabolites. In particular, the experiments described here are investigating the role of muscle pH and molecular charge on dipolar coupling interactions of various probe molecules in muscle.

METHODS
NMR Spectroscopy: Selectively excited, DQ filtered, J-resolved spectra were acquired using the pulse sequence as described in [1] on a Bruker (GE Omega) 4.7 T (200.1 MHz) CSI spectrometer, with 10 G/cm gradients using a single-turn solenoid.
Sample preparation: To vary the effect of pH and molecular charge on dipolar coupling interactions, 200 mM buffered solutions of probe molecules of L-lactate, Tris, L-alanine, glycine, dioxane and ethanol were prepared at pH values of 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Cylindrical samples from bovine abdominal muscle (commonly referred to as flank steak) were dialysed free of small molecules by immersion in buffered solution at pH=7 to discriminate the interaction with filament structure from any other interactions. Each sample was then reconstituted with a probe molecule at a given pH value. DQF, J-resolved spectra were acquired for two different orientations (parallel and perpendicular) of the muscle fibers with Bo.
Data processing: Base line corrected, free induction decays of the two-dimensional data were apodized with a 5 Hz Gaussian in the t2 dimension and with a sin2 function (10° phase shift spanning 80% of the data set) in the t1 dimension.

RESULTS
DQF, J-resolved spectra were acquired on three classes of compounds. In the pH range of these experiments, lactate and Tris were negatively and positively charged respectively; alanine and glycine did not have a net charge but possessed a net electrical dipole moment; ethanol and dioxane were chosen as molecules with no net charge or electrical dipole moment. At pH=7, 7.5 and 8, all the above molecules showed dipolar coupled peaks in the DQF, J-resolved 1H NMR spectra. The intensity of the peaks, as well as the frequency splitting, increased as the pH increased. However, no dipolar coupled peaks could be detected for pH=5, 5.5 and 6.0 for any of these molecules.

DISCUSSION
Dipolar coupling, unlike scalar coupling which is transmitted through covalent bonds, is transmitted through space. Isotropic molecular motion averages dipolar coupling to zero. Therefore, the dipolar coupled peaks showed in these spectra reveal a general restriction to motion within the fiber lattice. This result may have profound effects in the way MRS is applied in ordered tissues.

REFERENCES