Charge Influences Dipolar Coupling Interactions of Lactate and Alanine in Skeletal Muscle

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INTRODUCTION

The orientation dependence of ^{1}H NMR spectra of several metabolites from skeletal muscle, such as lactate and Cr/PCr, has been shown to be due to residual dipolar coupling interactions [1,2]. The purpose of this study was to: 1) investigate how charge and chirality affect the dipolar coupling of metabolites in muscle, 2) to test whether dipolar coupling interactions can be detected in ^{1}H NMR spectra of alanine in muscle.

METHODS

NMR Spectroscopy: Double quantum (DQ), J-resolved spectra (64 t_1 values) were acquired on a Bruker (GE Omega) 4.7 T (200.1 MHz) CSI spectrometer, with 10 G/cm gradients using the single-turn solenoid as described in [1]. *Sample preparation*: L-lactate, D-lactate and L-alanine were acquired from SIGMA. L-lactate and D-lactate (3 M) and L-alanine (0.7 M) solutions were prepared at pH 7.1. Muscle samples were obtained from bovine abdominal muscle [1]. The fibers from this muscle are uniformly oriented and free of visible fat. Cylindrical samples cut from this muscle were leached free of small molecules by immersion in distilled water for 3 hours. DQ, J-resolved spectra acquired on leached samples prior to reconstitution with exogenous solutions yielded no signal in the region of interest (0-5ppm). Each leached sample was then injected with aliquots of one of L-lactate, L-alanine or D-lactate solution. For each injected sample, DQ, J-resolved spectra were acquired for two orientations (parallel and perpendicular) of the fibers relative to Bo. Sufficient time (2 hours) was allowed for diffusion into the sample [1]. *Data processing*: Free induction decays of the two-dimensional data were apodized with a 5 Hz Gaussian in the t_2 dimension and with a $\sin^2$ function (10° phase shift spanning 80% of the data set) in the t_1 dimension. Baseline correction was used prior to Fourier transformation in both t_1 and t_2 dimensions.

RESULTS

Observation of dipolar coupling interactions for both alanine and lactate in muscle appears to be dependent upon the charge of the molecule as shown in figure 1. For L-lactate (figure 1a), there is a 24 Hz splitting due to dipolar coupling in addition to the 7 Hz J-coupled peaks [1]. However, for L-alanine (figure 1b) no dipolar coupled peaks are observed. In both figure 1a and 1b, muscle fibers were parallel with Bo. Upon rotation of the fibers perpendicular to Bo, the 24 Hz splitting of lactate collapsed to an unresolved peak as expected [1]. In contrast, spectra acquired from samples spiked with the L-alanine solution showed no orientation dependence.

Preliminary results show that spectra acquired on samples injected with acidic lactate solution do not contain the dipolar coupled pool. However, spectra from samples injected with basic alanine solution do show a 17 Hz splitting in addition to J-coupling when fibers are parallel to Bo. This 17 Hz splitting gets reduced to 2 Hz when fibers are perpendicular to Bo. A quantitative experiment to measure the transition in pH values between dipolar coupling and its absence has not yet been done.

Spectra acquired on a sample injected with D-lactate solution contained both the dipolar and the J-coupled pools of lactate as did spectra from samples injected with L-lactate under identical conditions.

DISCUSSION

The main conclusion of this study is that charge plays a key role in the dipolar coupling of small metabolites in muscle. At pH 7.1 where lactate exists as an anion, spectra show a significant dipolar coupled pool. However, alanine, a zwitterion at pH 7.1, shows no dipolar coupling.

Since both lactate stereoisomers show dipolar coupled pools, chirality, can be eliminated as the cause of such interactions. Also, the fact that dipolar coupling interactions are observed in leached samples rules out interactions between small metabolites as an explanation for such interactions.

One hypothesis to explain these results arises from the fact that muscle has a highly regular protein structure that would order the surface charges to set up an electrical field [3].Thus, an anion, such as lactate at normal physiological pH's (~7), would be subject to an orientational electrostatic force. However, this interaction would be too weak for alanine at pH ~7 where it exists as a zwitterion, a dipole with no net charge. We are currently investigating this hypothesis with molecules of varying charge and lipophilicity within a range of physiologically relevant pH's.

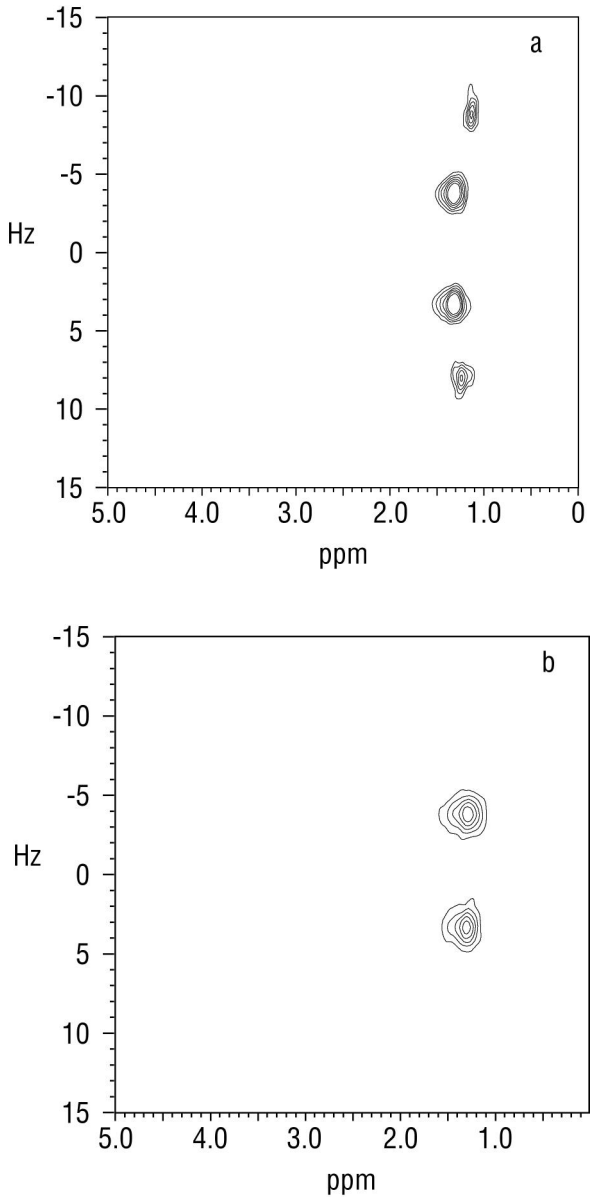

Figure 1:DQ, J-resolved proton NMR spectra from muscle samples injected with a) L-lactate solution and b) L-alanine solution.

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