Effects of pH and molecular charge on dipolar coupling interactions of solutes in skeletal muscle observed by DQF, ^{1}H NMR spectroscopy

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Abstract

In this study we tested the effect of molecular charge and chirality as well as tissue pH on dipolar coupling interaction in skeletal muscle. These results were demonstrated by double quantum filtered, DQF, ^{1}H NMR spectra acquired on permeable skeletal muscle samples dialyzed against buffered solutions containing three classes of solutes—electrolytes (lactate and Tris), zwitterions (alanine and glycine), and non-electrolytes (dioxane and ethanol)—as a function of pH ranging from 5.0 to 8.5. The results show that charge density on the protein filaments strongly influences dipolar coupling of solutes in muscle whereas charge on the solutes themselves has only a small effect. The frequency splitting of the dipolar coupled peaks for all the molecules tested was strongly affected by muscle pH. Higher pH increased negative charge density on the filaments and resulted in weaker dipolar coupling for anions and zwitterions but stronger coupling for the cation TRIS. Molecular charge per se or chirality did not affect the frequency splitting of the dipolar coupled peaks. The molecules, lactate, ethanol, and alanine, have scalar coupled spins and consequently a double quantum signal in solution. However, spectra acquired from these molecules in muscle showed an additional frequency splitting due to additional dipolar coupling interactions. Due to lack of scalar coupling, spectra from Tris, glycine, and dioxane showed no double quantum signal in solution but did when in muscle. All these observations can be explained by the fact that the net charge on protein filaments dominates the mechanism of dipolar coupling interactions in the highly anisotropic structures in muscle.
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1. Introduction

An increasing number of studies have shown that ^{1}H NMR signal from biological tissues is influenced by the cellular and tissue structure leading to several kinds of ordering effects. Such effects include compartmentation, transport phenomena, susceptibility effects, diffusion anisotropy, temporal binding to macromolecules, and motional restriction [1]. The latter has been shown through observation of dipolar coupled peaks in ^{1}H NMR spectra of several metabolites in skeletal muscle [2,3]. In solution, dipolar coupling is the main mechanism through which relaxation occurs, but the isotropic motion of molecules causes these interactions to average to zero. Consequently, solution NMR spectra lack any splitting attributable to dipolar coupling. However, in ordered systems where molecular motion is restricted, dipolar interactions are not averaged to zero giving rise to additional frequency splittings. The larger the motional restriction, the stronger the dipolar coupling constant. The observed frequency splitting is effected by the static coupling between the spins, the order parameter, and the sample orientation with respect to B_O. The intensity of the dipolar coupled peaks is dependent on the concentration of the ordered solute, T_2 effects, and how well the DQ selection is tuned [4].

Several studies [2–6] have made use of this phenomenon to detect anisotropic motion of water molecules, lactate, creatine, and sodium ions in a variety of tissues, such as ligaments, tendons, muscle, cartilage, nerves, and blood cells. In a former study, we showed how dipolar coupling affects quantification of metabolites using ^{1}H NMR [7]. However, there is virtually no information on the nature of the interactions underlying this anisotropic motion of molecules in the tissue.

The goal of this study was to test whether dipolar coupling results directly from charge interactions. Muscle tissue is a good test bed for these experiments because the nanostructure of its filaments is well known, as is the dependence of the lattice spacing of these filaments on pH, osmotic pressure and ionic strength [8]. It is thus possible to vary charge and charge density on the muscle filaments by varying muscle pH to study ordering effects of various solutes in muscle. This ordering is detected by the presence of dipolar coupled peaks in the ^{1}H NMR spectra. The tissue is highly organized from the macroscopic to the nano-structural level such that its orientation with respect to B_O is easy to verify. We varied the charge on the solutes by selecting molecules with appropriate pK_a values for the pH range of 5–8.5 to provide: an anion (lactate), a cation (Tris), zwitterions (alanine and glycine), and non-electrolytes (dioxane and ethanol). We also varied the charge density of the protein filaments by increasing the pH above its isoelectric point, pH ~5 [8]. The underlying hypothesis was that charge distribution along the main structural proteins in muscle is responsible for the net dipolar coupling observed.

The study also addresses the question of generality of dipolar coupling effects in muscle. Specifically, are these anisotropic interactions specific to certain molecules, or are they a generic feature of skeletal muscle, and by extrapolation a general property of anisotropic cells? An understanding of the nature of these interactions will not only broaden their use in practical applications, such as spectroscopic imaging, but also provide insight into phenomenological processes at the molecular and cellular level.

2. Methods

2.1. Experimental design

DQF, *J*-resolved, ^{1}H NMR spectra [3] were acquired from six different molecules in muscle with buffered pH values ranging from 5.0 to 8.5. The choice of molecules was based on their structure and pK_a values, so that, in the given pH range, they would be either predominantly charged (lactate and Tris), or zwitterionic (alanine and glycine), or neutral non-electrolytes (dioxane and ethanol).

2.2. Sample preparation

Cylindrical samples (14 mm diameter, 19 mm average height) from bovine abdominal muscle were dialyzed free of small molecules by immersion in buffered solution at pH = 7. The solution was kept at 3 °C and was changed every 2 h for a total of 6 h. Prior to spectra acquisition, the dialyzed sample was put in a 200 mM solution of a particular molecule buffered at the pH of choice for an additional 6 h at 3 °C. The pH of the solutions was measured before and after the dialyzing process and it was found not to vary more than ±0.06. Analysis of the diffusivity of solutes with diffusion coefficients on the order of $5 \times 10^{-6} \, cm^2 \, s^{-1}$ shows that the concentration along the axis of the muscle samples (radius 0.7 cm) reaches <10% of the external concentration of solute in ~2 h for dialyzing solutes out of the muscle [9]; similarly concentrations along the axis reaches >90% for dialyzing solutes of interest into the tissue.

Electrophoretic gels: Proteins from three different muscle samples at pH = 5, 7, and 8.5, respectively, were electrophoretically separated following the procedure described in [3]. This analysis tested for stability of the muscle structure to proteolysis and dissolution under our experimental conditions.

Solutions: Buffered solutions of 200 mM L(+)-lactate, D(−)-lactate, Tris (Tris[hydroxymethyl]amino-methane), L-alanine, L-glycine, dioxane, and ethanol were prepared and stored at 3 °C. In addition to the molecule under investigation, each solution contained 0.002 M $MgCl_2$ and 0.225 M KCl to bring the overall ionic strength to 0.25. Biological buffers, (20 mM) MES (pH = 5.0 and 6.0), ACES (pH = 7.0), and Bicine (pH = 8.0 and 8.5), (SIGMA Catalog Nos.: M5287, A9758, and B8660) were used to buffer each solution at a given pH value.

2.3. NMR spectroscopy

Spectra were acquired on a Bruker (GE Omega) 4.7 T CSI spectrometer, with 10 G/cm maximum gradient strength. The same coil as described in our previous work [3,7] was used. This is a single-turn solenoid coil with 25-mm diameter and 12-mm height.

After shimming, DQF, *J*-resolved, ^{1}H NMR spectra were acquired using the same pulse sequence as in [7]. To increase the signal to noise, the pulse sequence was used to selectively excite the methyl protons of alanine and lactate (AX_3 spin systems) and of ethanol (A_2X_3 spin system). The echo times, τ_1 and τ_2, were set either to $1/2\Delta$ to refocus the sum, Δ, of both dipolar and scalar coupling or to $1/2J$ thus refocusing only the scalar coupling [3,7]. For the rest of the molecules, Tris, glycine, and dioxane, which in solution have no observable DQ signal, the pulse sequence was used non-selectively to excite any coupling that might be occurring. In these cases the optimum values for τ_1 and τ_2 were found by

trial and error and then set to the appropriate, $1/2\Delta_{dc}$ value, with Δ_{dc} denoting the dipolar coupled splitting (Hz) observed for each case.

Samples were studied with the muscle fibers oriented parallel or perpendicular to B_O [3]. Only the results from the parallel orientation are discussed because the dipolar effects to be described collapsed to an unresolvable peak in the perpendicular orientation.

To check the validity of the dialyzing process, five control muscle samples were dialyzed in buffered solution at pH = 7. Spectra were acquired from these samples prior to reconstitution with the molecule of interest. These spectra showed no resonance peaks in the region of interest (0–4 ppm) except for some breakthrough of the water resonance.

2.4. Data processing

Prior to Fourier transformation, free induction decays were baseline corrected in both t_1 and t_2 dimensions followed by an apodization with $\sin^2$ function (10° phase shift of 90% of the data set) and 8 Hz Gaussian function in each dimension, respectively, as used previously [7].

3. Results

Validity of the method. Spectra acquired to validate the dialyzing process are shown in Fig. 1. Fig. 1a shows DQF, *J*-resolved ^{1}H NMR spectra acquired from an endogenous muscle sample with fibers oriented parallel to B_O showing both the dipolar and scalar coupled peaks of the lactate methyl protons ($\sim$1.2 ppm). The spectra also show some leak from the water protons ($\sim$4.7 ppm), as well as contributions from the methyl protons of trimethylamines and possibly creatine ($\sim$3.0 ppm) as previously reported [3]. However, spectra
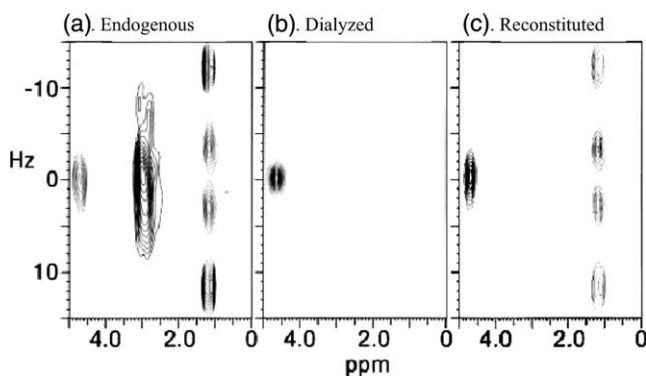
acquired from the same sample after the dialysis (Fig. 1b) lack any signal coming from these metabolites. Fig. 1c shows spectra acquired after the muscle sample was reconstituted with 50 mM L(+)-lactate solution. Note the reappearance of both dipolar ($\sim$24 Hz) and scalar coupled ($\sim$7 Hz) peaks of lactate in these spectra.

Electrophoretic gels (data not shown) indicated no differences in the weight distribution and separation pattern of proteins in all samples tested (pH = 5.0, 7.0, and 8.5) compared to control tissue. Gel patterns showed no evidence of proteolysis or protein solubilization; the present results were not distinguishable from those shown in our previous work [3]. Thus, the experimental manipulations did not disrupt the major muscle proteins even after a prolonged spectroscopic experiments.

3.1. DQF ^{1}H NMR spectroscopy

3.1.1. Solutions

As expected, DQF, *J*-resolved, ^{1}H NMR spectra from solutions of L(+)-lactate (Fig. 2a) and L-alanine (Fig. 4a) showed only the 7 Hz scalar coupled methyl peaks at $\sim$1.2 and 1.3 ppm, respectively. Single quantum spectra from methyl protons of ethanol give rise to a triplet at 1.0 ppm (J_{HH} = 7 Hz) through scalar coupling to the methylene protons. However, as shown in Fig. 7a, the DQF, *J*-resolved spectra from ethanol show only two peaks due to antiphase cancellation of the central peak [10]. Tris, glycine, and dioxane in solution have only equivalent protons precluding *J*-coupling and any observable DQ transitions; the solution spectra of these molecules showed no peaks in the 0–5 ppm region.

3.1.2. Dipolar coupling of charged molecules

Lactate and Tris were chosen because, in the given pH range, they exist predominantly in their ionic forms. The pK_a values for lactate (carboxyl) and Tris (amino) are $\sim$3.8 and 8.1, respectively [11]. Consequently, throughout the experimental pH range (5.0–8.5), around 99% of lactate molecules are in their anionic form. At pH = 5.5, $\sim$99% of Tris is in its cationic form, a value which decreases to $\sim$50% at pH = 8.0.

Lactate anion: The dipolar coupling constant of lactate, and hence the strength of the preferred orientation, decreases as pH is increased. Fig. 2b–d, show DQF, *J*-resolved, ^{1}H NMR spectra from muscle samples reconstituted with 200 mM L(+)-lactate solution buffered at pH = 5.0, 7.0, and 8.5, respectively. In all cases, muscle fibers were oriented parallel to B_O and τ_1 and τ_2 were set to refocus both couplings. Note that while the dipolar coupled peaks are barely detected for pH = 5.0 (Fig. 2b), these peaks become predominant for pH = 7 (see Fig. 2c–d). The frequency splitting of the dipolar coupled peaks decreased along the pH series: 26 Hz (pH = 5.0), 24 Hz (pH = 6.0), 21 Hz (pH = 7.0), and
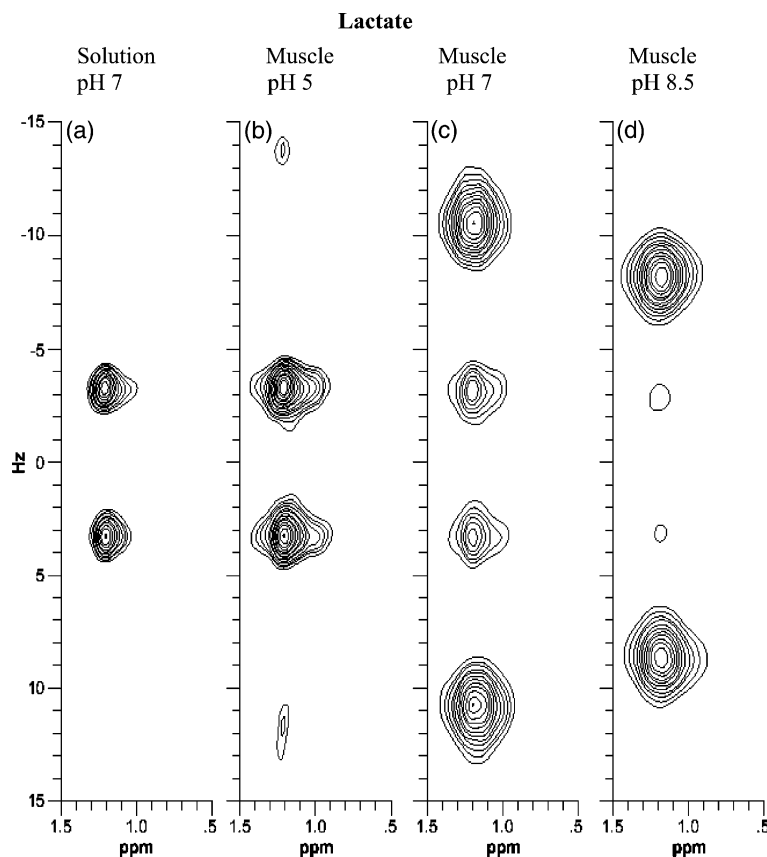

Fig. 1. DQF, *J*-resolved ^{1}H NMR spectra from a muscle sample with fibers oriented parallel to B_O in its (a) endogenous state, (b) after dialysis, and (c) after reconstitution with 50 mM L(+)–lactate solution buffered at pH = 7.0. Note the reappearance of both dipolar (24 Hz) and scalar coupled (7 Hz) peaks of lactate in (c).

Fig. 2. DQF, *J*-resolved, ^{1}H NMR spectra from (a) lactate solution, and (b)–(d) from muscle samples reconstituted with 200 mM L(+)-lactate solution buffered at pH = 5.0, 7.0, and 8.5, respectively. Note the decrease in the amount of splitting for the dipolar coupled peaks with increasing pH. The splittings were 26 Hz (pH 5), 21 Hz (pH 7), and 17 Hz (pH 8.5).

17 Hz (pH = 8.5). Thus, two properties of lactate in muscle are uncovered by the experiments displayed in Fig. 2. First, the frequency (and thus the strength) of the dipolar coupling decreases as pH is increased. Second, from these spectra, it seems that the relative intensities of the dipolar coupled and uncoupled fractions of lactate in muscle are a function of pH. However, it should be noted that the relative intensity of the peaks is also a function of T_2 for any given experimental condition [7]. Changes in T_2 were not studied as a function of pH for any solute in this work and we make no interpretation of changed intensity.

In addition, DQF, *J*-resolved spectra acquired from both L(+)- and D(−)-lactate stereoisomers in reconstituted muscle at pH = 7.0 showed the dipolar coupled peaks ($\Delta = 21$ Hz). This experiment provides evidence that the observed anisotropy is independent of molecular stereospecificity. Under physiological conditions, however, only the L(+) stereoisomer is present and recognized by the natural enzyme in muscle.

Tris cation: While solution spectra of Tris showed no DQ signal in the (0–5) ppm region, dipolar coupled peaks were observed in Tris spectra acquired from muscle samples oriented parallel to B_O. The appearance of a DQ signal in the spectra from Tris in muscle can

only originate from dipolar coupling interactions that are not averaged to zero in the presence of an ordering effect of tissue. Typical DQF, *J*-resolved, ^{1}H NMR spectra from muscle samples reconstituted with 200 mM Tris solution at pH = 6.0, 7.0, 8.0, and 8.5 are shown in Fig. 3a, b, c, and d, respectively. The dipolar coupling constant of Tris, and hence the strength of the preferred orientation, increased as pH increased. The frequency splitting for Tris increased from ∼5.0 Hz at pH = 5.0 to a maximum value of 16 Hz for pH = 8.5.

3.1.3. Zwitterions

L-Alanine and L-glycine were chosen because in the pH range of these experiments they exist as polar molecules with a net electrical dipole moment but no net charge. With pK_a values ∼2.4 (carboxyl) and 9.8 (amino), 95% of both alanine and glycine are zwitterionic over the chosen pH range [8].

Alanine: Spectra of alanine in muscle showed dipolar coupled peaks which were strongly dependent on the muscle pH. Comparison of DQF, *J*-resolved, ^{1}H NMR spectra acquired from L-alanine in solution (Fig. 4a) versus muscle (Fig. 4b and c) revealed the additional frequency splitting due to dipolar coupling. For the parallel orientation of the muscle fibers with B_O, this
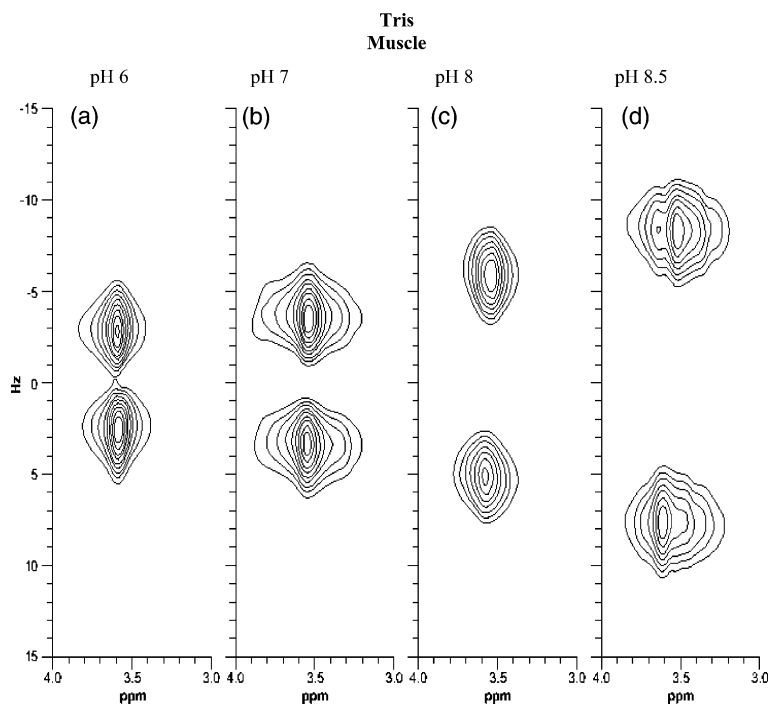
Fig. 3. DQF, *J*-resolved, ¹H NMR spectra from muscle samples reconstituted with 200 mM Tris solution buffered at (a) pH = 6.0, (b) pH = 7.0, (c) pH = 8.0, and (d) pH = 8.5. Note that the frequency splitting for Tris increases with muscle pH. The splittings were 5 Hz (pH 6), 7 Hz (pH 7), 11 Hz (pH 8), and 16 Hz (pH 8.5).
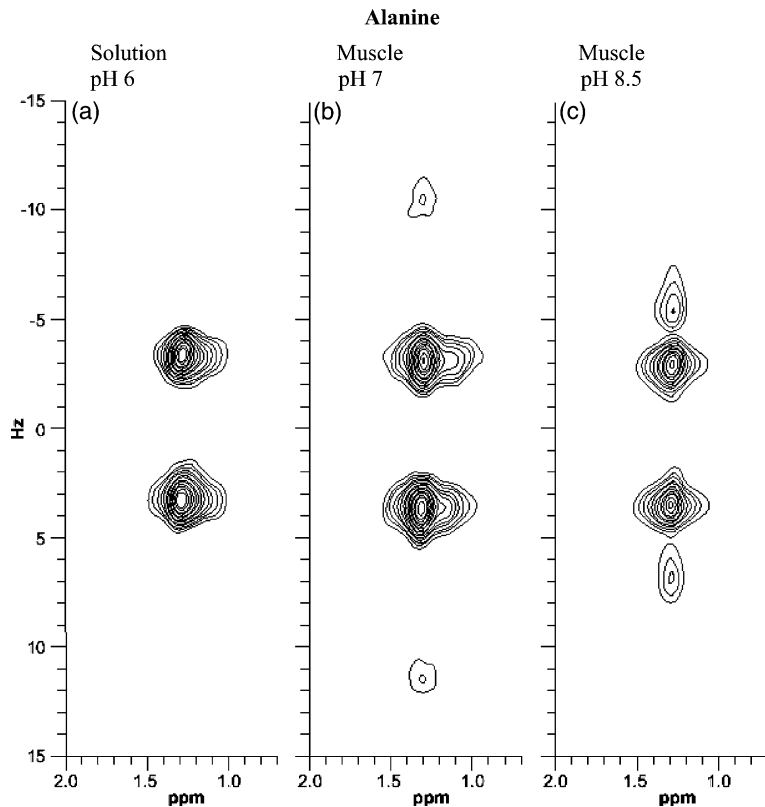

Fig. 4. DQF, *J*-resolved, ¹H NMR spectra acquired from (a) ʟ-alanine in solution versus muscle at (b) pH = 7, and (c) pH = 8.5. Note that the frequency splitting due to dipolar coupling, absent in solution, is present in muscle and decreases as muscle pH is increased. The splittings were 22 Hz (pH 7) and 13 Hz (pH 8.5).
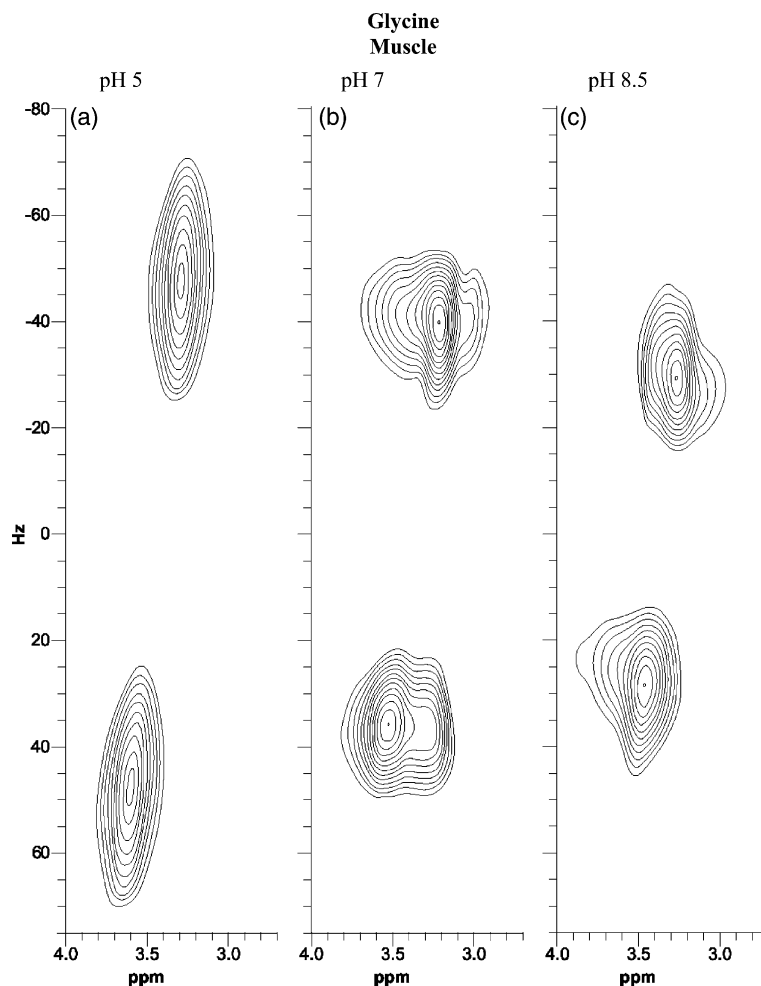
Fig. 5. Typical DQF, *J*-resolved, ¹H NMR spectra acquired from muscle samples reconstituted with 200 mM glycine solution buffered at (a) pH = 5.0, (b) pH = 7.0, and (c) pH = 8.5. Note that the dipolar coupling frequency splitting for glycine is much higher than for the other molecules. Also, this splitting decreased as muscle pH was increased. The splittings were 95 Hz (pH 5), 75 Hz (pH 7), and 55 Hz (pH 8.5).

frequency splitting of 22 Hz at pH = 7 decreased to 13 Hz at pH = 8.5. However, unlike lactate, for alanine, spectra were dominated by the *J*-coupled peaks for the entire experimental pH range with the dipolar coupled peaks undetectable for pH < 6.5.

Glycine: In contrast with solution spectra, devoid of any coupling, spectra acquired from glycine in muscle show an orientation dependent dipolar coupling which is pH dependent. Glycine spectra showed coupling ranging from 95 Hz (pH = 5) to 55 Hz (pH = 8.5). Typical spectra from a pH series (Fig. 5a–c) reveal this variation wherein the dipolar coupling constant was much higher than the values obtained for the other molecules.

3.1.4. Neutral molecules

1,4-Dioxane and ethanol are molecules normally not present in muscle under physiological conditions, but were chosen due to their lack of charge within the experimental pH range. In addition, dioxane has zero dipole moment and ethanol has a small dipole moment.

Dioxane: While there are no DQ transitions for dioxane in solution, DQF, *J*-resolved ¹H spectra acquired from dioxane in muscle showed an orientation dependent, dipolar coupled peak at ∼3.5 ppm. The frequency splitting of the dipolar coupled peaks decreased from ∼6 Hz (pH = 7, Fig. 6a) to ∼4 Hz (pH = 8.5, Fig. 6b). Spectra acquired with pH < 7.0 did not show any detectable DQ signal.

Ethanol: Comparison of spectra from ethanol in solution (Fig. 7a) with ethanol in muscle at pH = 8 (Fig. 7b) show dipolar coupling interactions of ethanol in muscle. However, no dipolar coupled peaks could be detected for ethanol in muscle at pH < 8.0. Spectra acquired at pH = 8.5 were, however, not significantly different from those shown in Fig. 7b acquired at pH = 8.0.

3.1.5. Summary of results

Spectra acquired from all the molecules tested, independent of their charge, polarity, and chirality, showed anisotropic effects observed by dipolar coupling
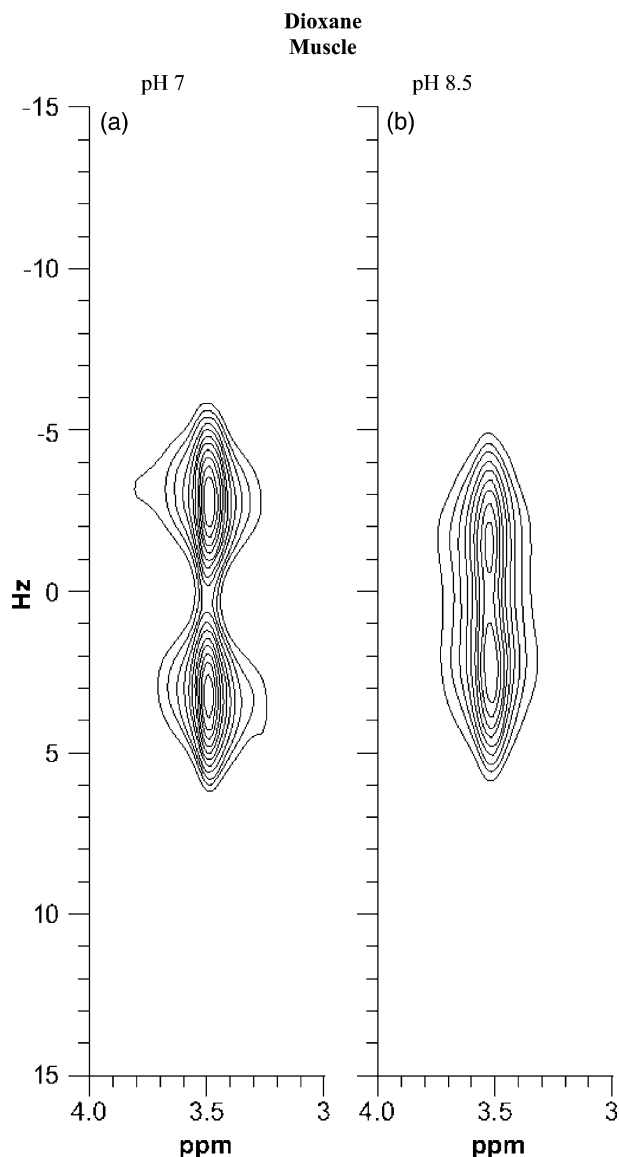
Fig. 6. DQF, *J*-resolved ¹H spectra acquired from dioxane in muscle reconstituted with dioxane solution buffered at (a) pH = 7.0 and (b) pH = 8.5. Spectra acquired with pH < 7.0 were similar to those acquired from dioxane in solution in that they did not show any detectable DQ signal. Note that the frequency splitting due to dipolar coupling decreases as muscle pH is increased. The splittings were 6 Hz (pH 7) and 4 Hz (pH 8.5).

interactions in muscle. Most importantly, muscle pH strongly influenced the strength of dipolar coupling constant as observed by strong variations in the frequency splitting of the dipolar coupled peaks with changes in muscle pH.

4. Discussion and conclusions

Six small molecules (46–121 Da) with either positive, negative or zero ionic charge show dipolar coupling interaction in muscle pointing to a generality of dipolar
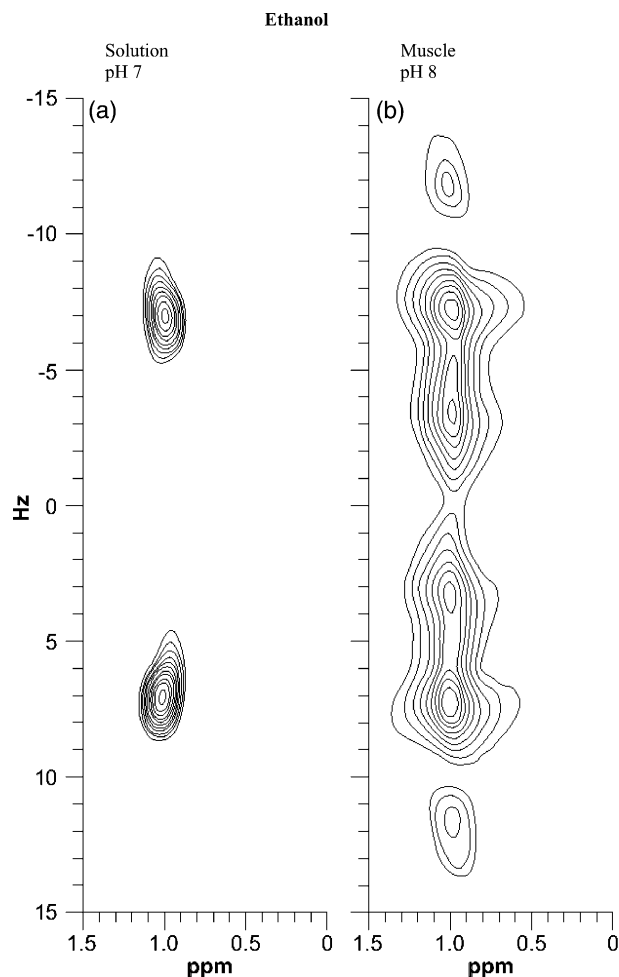

Fig. 7. DQF, *J*-resolved ¹H spectra from ethanol in solution (a), are compared with those acquired from a dialyzed muscle sample reconstituted with 200 mM ethanol buffered solution at pH = 8.0 (b). In the spectra acquired from muscle note the presence of additional, dipolar coupled peaks, with splittings of 7 and 24 Hz at pH 8. In solution the splitting due to scalar coupling was 14 Hz.

coupling in solutes in muscle and, hence, anisotropic motion. Further, the net charge density of the highly ordered filaments provides a lattice for this restriction; the charge on the solutes themselves has smaller effects. The experiments also revealed solute-specific effects, such as increased dipolar coupling in Tris versus decreased dipolar coupling in lactate with increased pH and the much larger coupling constant for glycine versus alanine. While dipolar coupling in an anisotropic tissue like muscle is a common feature of proton spectroscopy, our new results, taken together, show that the properties and structural interactions must be investigated specifically for each molecule. These results raise two further questions. What is the nature of the interaction causing the solutes not to tumble freely and isotropically but, on a time average, have a preferred orientation which, in turn, causes dipolar coupling interaction not to average to zero? What is the identity of the coupled spin-systems which give rise to dipolar coupled peaks? However, our

experiments were not designed to establish the coupling partners nor the variation of the order parameter with pH. The following section discusses several mechanisms that could account for our results, but we cannot establish a detailed physical chemical picture of the orientating forces involved. We can only conclude it is an electrostatic interaction. Our results show these electrostatic forces within the muscle filaments are modified as a function of pH and suggest that electrostatic modulation of the strength of orienting force effects intra-molecular dipolar coupling of solutes as it does with lactate.

Restriction of isotropic motion: One explanation for the observed motional restriction relies on simple, ionic interaction: Anions would be repelled from the negative charges on the filaments, and cations would be attracted; polar zwitterions may also be oriented with respect to the filament charges. This mechanism should depend on the magnitude of the charge density of the muscle filaments, which increases as pH increases. For example, Millman [8] calculated the charge density increasing from one electron per nanometer of thick (myosin-containing) filament at pH 5.5 to 100 electrons per nm at pH 8.5.

The results support the hypothesis that the orientating force causing dipolar coupling effects in proton NMR spectra originates from the charge distribution along the major structural proteins of muscle. Skeletal muscle is a highly structured tissue, from the nanostructure of the intracellular myofilaments to the macromolecular fiber proteins. Because the filament proteins are in register, an ordered distribution of surface protein-charge results. Under physiological pH ($\sim$7.0) and ionic strength ($\sim$150 mM) both thick and thin filaments are negatively charged [8]. The net negative charge on the filaments decreases with decreasing pH to the isoelectric point (pH $\sim$5.0) to become net positive as the pH decreases further [8]. Therefore, decreasing the pH of the filament lattice reduces the electrostatic repulsion between the filaments to cause both the lattice and the fibers to shrink. Increasing pH will increase the negative charge density, increase the electrostatic repulsion and cause the lattice and fibers to swell. The increased charge density would explain the weaker ordering effects (lower dipolar coupling constant) of the anion, lactate, and zwitterions, glycine, and alanine, at higher pH. However, for Tris, which is the only positively charged molecule tested, the direct electrostatic force between the positive molecular charge and the negative charge on filaments would increase with increasing pH, which would explain why for Tris, unlike the result for the other molecules, the dipolar coupling strength increased as pH increased.

A second interaction which could yield restriction in motion stems from hydration of the actin/myosin filament lattice. Increased hydration with increasing pH

could form steric restrictions on the solutes in response to increased charge density. However, the experiments described here showed that dipolar coupling varied with muscle pH even when the molecular charge, hence the amount of molecular solvation, did not change. For example, lactate was $\sim$99% charged throughout the pH range and yet the observed dipolar coupled peaks varied in both amount of frequency splitting and relative intensity with the muscle pH. Furthermore, a simple calculation shows the hydrated solutes are much smaller than the spacing between filaments. Bond lengths and bond angles of the solutes studied gave a maximum radius of $\sim$3 nm for the molecules which, compared to the spacing between actin/myosin filaments, around 20 nm, suggests that steric restriction is unlikely. The calculation was done accounting for the given bond lengths and bond angles of the molecules with a hydration number of 5 and a radius for H_3O^+ of 0.28 nm [9,11].

A third type of intermolecular interaction, the specific binding of the solutes with proteins present in muscle, and/or a specific metabolic interaction in muscle, is also refuted by this study. For example, specific binding of lactate to lactate dehydrogenase (LDH) is excluded because of the absence of an effect of chirality of lactate on the results. Binding to LDH is also excluded by a similar increase in both the dipolar coupled pool (24 Hz) and scalar coupled pools (7 Hz) when exogenous lactate is added several-fold in excess of the physiological range; at this high concentration specific binding sites would be saturated [3]. Further, dipolar coupling is seen in achiral molecules, such as dioxane and ethanol, for which the likelihood for stereospecific interactions is small. In addition, the experiments described here were conducted on muscle freed of small molecular weight metabolites by dialysis, thus excluding interaction with unknown metabolites as a plausible explanation for dipolar coupling.

Nature of coupling partners: In a prior study [3] we showed that for lactate, the dipolar coupling originates mainly from a CH_3–CH coupling with some contribution from the coupling of the intramethyl protons. The similarity of the alanine and lactate spin systems suggests that the same holds true for alanine as well. Ethanol has a more complex spin system compared to the rest of the molecules tested. In solution, the triplet at the methyl resonance results from coupling of the methyl protons to the methylene ones. Spectra acquired from ethanol in muscle suggest the presence of a doublet of doublets (Fig. 7b). One explanation would be the orientation of the methylene protons which makes them non-equivalent spins; i.e., the methyl protons see each of the methylene ones separately and hence a doublet of doublets. One explanation for the order of magnitude greater coupling seen in glycine is geminal coupling of the two protons on the alpha carbon, which are closer than the coupling partners in the other solutes studied.

These details must remain speculative because our experiments were not designed to study these interactions. Peak intensities of the dipolar coupled peaks was also changed with pH but these results could be simply due to T_2 effects and/or the distribution of the total amount of solute between the "isotropic" and "anisotropic" pools in muscle [3,7]. Study of these effects was also not part of the experiments described here.

Conclusion: This study showed that charge distribution along the major structural proteins of skeletal muscle plays a key role in the observed anisotropy of dipolar coupling interactions of solutes in this tissue. The frequency splitting of the dipolar coupled peaks was strongly dependent on muscle pH while independent of molecular charge and chirality of the solutes. Thus, the mechanism behind this anisotropic ordering of molecules in muscle appears to affect all types of solutes, independent of charge or polarity of the solutes; therefore the same mechanism should affect at least some of the solvent molecules, water.

Acknowledgments

References

[1] Ch. Boesch, How MRI and MRS can probe tissue microstructures far below the spatial dimensions of a voxel, NMR Biomed. 14 (2) (2001) 112–132.

[2] C. Boesch, R. Kreis, Dipolar coupling and ordering effects observed in magnetic resonance spectra of skeletal muscle, NMR Biomed. 14 (2) (2001) 140–149.

[3] I. Asllani, E. Shankland, T. Pratum, M. Kushmerick, Anisotropic orientation of lactate in skeletal muscle observed by dipolar coupling in ^{1}H NMR spectroscopy, J. Magn. Res. 139 (1999) 213–224.

[4] G. Navon, H. Shinar, U. Eliav, Y. Seo, Multiquantum filters and order in tissues, NMR Biomed. 14 (2) (2001) 112–132.

[5] Y. Assaf, G. Navon, Y. Cohen, In vivo observation of anisotropic motion of brain water using 2H double quantum filtered NMR spectroscopy, Magn. Reson. Med. 37 (1997) 197–2003.

[6] A.H. Aletras, L. Tsoref, G. Navon, In vivo ^{1}H double quantum filtered MRI of human wrist and ankle, Magn. Reson. Med. 43 (2000) 640–644.

[7] I. Asllani, E. Shankland, T. Pratum, M. Kushmerick, Double quantum filtered ^{1}H NMR spectroscopy enables quantification of lactate in muscle, J. Magn. Res. 152 (2001) 195–202.

[8] B.M. Millman, The filament lattice of striated muscle, Phys. Rev. 78 (2) (1998) 359–391.

[9] J. Crank, The Mathematics of Diffusion, Oxford University Press, Oxford, 1975.

[10] L.A. Trimble, J.F. Shen, A.H. Wilman, P.S. Allen, Lactate editing by means of selective-pulse filtering of both zero- and double-quantum coherence signals, J. Magn. Res. 86 (1990) 191–198.

[11] L. Stryer, Biochemistry, fourth ed., W.H. Freeman, San Francisco, 1995.