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High protein mobility in skinned rabbit muscle fibres observed by ^{1}H NMR spectroscopy

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^{1}H NMR spectra of skinned rabbit muscle fibers show a group of relatively sharp resonance lines which presumably originate from highly mobile protein domains. Comparison with the spectrum of myosin subfragment 1 suggests that these signals may come at least partly from mobile regions of the myosin head. NMR could possibly be used to characterize the movements of crossbridges in force generation.

Muscle; Myosin; NMR; Skinned fibres; Mobility

1. INTRODUCTION

A real understanding of force production and shortening in muscle requires more detailed information on molecular structures and interactions. NMR methods can be used to study muscles at the macroscopic level (NMR-tomography and in vivo spectroscopy) and at the microscopic level (NMR-microscopy). At the molecular level, many NMR studies have been reported on isolated macromolecular components of the contractile machinery (for a recent review see Levine et al. [1]), but none on the proteins of intact skinned or living muscles. In the following, we will show the principal possibilities of high resolution ^{1}H NMR spectroscopic studies on proteins in intact skinned fibres.

2. MATERIALS AND METHODS

Bundles of fibres from psoas muscles of the rabbit were skinned by immersion in relaxing solution (70 mM K propionate, 8 mM Mg acetate, 5 mM EGTA, 7 mM ATP, 6 mM MOPS, pH 6.8) plus 0.5% Triton X-100 for 5 h at 4°C. They were then stored in relaxing solution/glycerol (50:50) at −20°C. For NMR measurements, smaller bundles were washed in a modified relaxing solution (30 mM KCl, 8 mM $MgCl_2$, 10 mM K phosphate, 2 mM EGTA, 7 mM ATP, pH 6.8 in D_2O), mounted on teflon rods, and closely packed into a sample tube fitted with a circulation system. Myosin subfragment 1 (S1) was prepared by chymotryptic digestion modified from Weeds and Taylor [2]. ^{1}H NMR spectra were recorded with a Bruker AM-500 spectrometer operating at 500 MHz. The water signal was suppressed by selective presaturation. The pH values were measured with a combination glass electrode, and the pH values given are not corrected for the isotope effect. The spectra were referenced either directly to the internal standard DSS or using the H-1′ resonance of free Mg·ATP in the solution as secondary standard (in our system 6.128 ppm relative to DSS).

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Abbreviations: DSS, 4,4-dimethyl-4-silapentane-sulphonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; MOPS, 3-(*N*-morpholino)propane sulphonic acid; NMR, nuclear magnetic resonance.

3. RESULTS AND DISCUSSION

Figure 1 shows the ^{1}H NMR spectrum of relaxed skinned fibres. In addition to the signals from small molecules present in the buffer solution (ATP and EGTA), a broad background from the skinned fibres themselves can be observed. This background is not completely unstructured as would be expected for a rigid macromolecular assembly, but shows relatively sharp lines with line widths of the order of 20–100 Hz. The high field part of the spectrum, which contains no signals from small molecules in the buffer solution, is shown in more detail in Fig. 2c. It is not clear which components of the molecular assembly of the skinned fibres are responsible for these signals. However, an obvious candidate is myosin, which is a major component of the fibres. It is known that myosin monomers in solution show a group of ^{1}H NMR resonance lines which are much sharper than expected for a molecule with such a high molecular weight [3–15]. These resonances originate either from the non-helical C-terminal region or from mobile domains in the head part of the myosin molecule. The C-terminus is immobilised after aggregation of myosin to form thick filaments [14,15], but the head part of the molecule is very likely to be mobile. Fig. 2a shows the NMR-spectrum of myosin subfragment 1 (S1) (corresponding to one of the two heads of myosin) in solution. It is to be expected that the effective dipolar correlation time of S1 will increase after formation of the thick filaments, and Fig.

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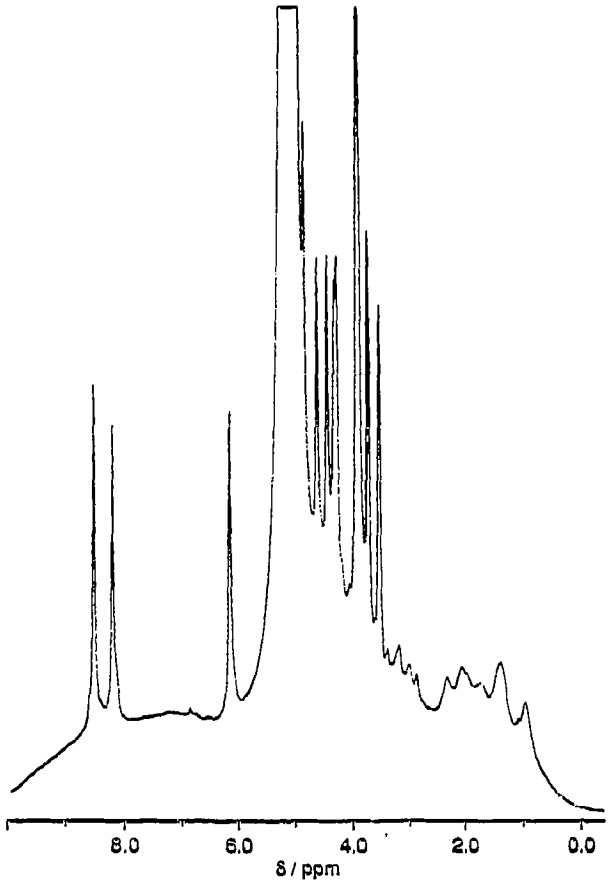

Fig. 1. ^{1}H NMR spectrum of skinned muscle fibres. 500 MHz spectrum from skinned rabbit muscle fibres in relaxing buffer. Temperature, 16°C. The sharp peaks downfield from 3.5 ppm arise from small molecules (ATP, EGTA, HDO); the remaining broader peaks are from proteins.

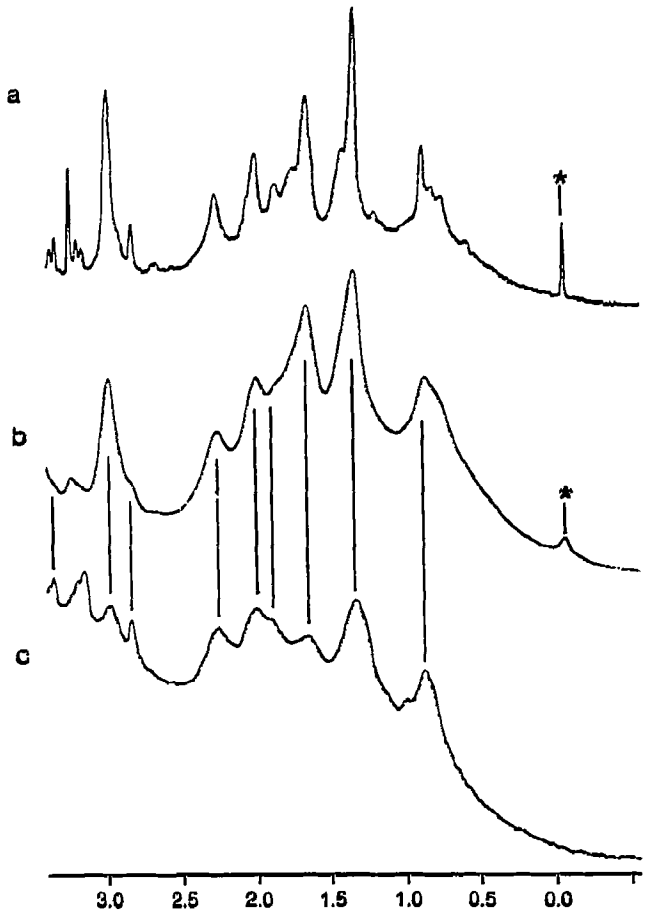

Fig. 2. Comparison of the ^{1}H NMR spectra from skinned muscle fibres and from myosin S1. (a) Part of a ^{1}H-NMR spectrum of myosin S1, with the signal-to-noise ratio improved by exponential multiplication leading to an additional line-broadening of 3 Hz. (b) Same data as shown in (a) but broadened by exponential multiplication with an additional line broadening of 40 Hz. (c) Part of the spectrum shown in Fig. 1, with a line broadening of 3 Hz. Temperature 16°C. Vertical lines show spectral features occurring at the same position in the two spectra. (*) internal standard DSS.

2b shows an artificially broadened spectrum of S1. There are striking similarities between this broadened spectrum of S1 and the spectrum of the complete skinned fibre (Fig. 2c): almost all peaks given by S1 are also observed in the fibre spectrum. However, the intensities and line widths differ substantially: this is not unexpected, since the line widths of the resonances from S1 in the filaments would probably not be broadened uniformly and since resonances from other proteins would probably also contribute to the spectrum. If the observed resonances are indeed resonances of mobile parts of S1 then three different effects could occur when S1 is bound to actin in the rigor state: (i) The signal is completely quenched by the interaction, (ii) only the effective relaxation times are influenced, all signals remaining visible, or (iii) as a combination of (i) and (ii) only some of the signals could be quenched with other signals still visible. Case (i) would be expected if the mobile parts of S1 became more rigid with the actin interaction, e.g. if they are in direct contact with actin. Case (ii) would result if the local mobility of the NMR-visible parts of S1 are not influenced significantly by the attachment of myosin to actin. Since it is known that most of the NMR-visible resonances of S1 arise from the N-terminal residues of the light chains, which probably do not interact directly with actin, case (ii) appears more likely. Indeed, preliminary experimental results are in line with interpretation (ii): a complex change in the dispersion of relaxation times appears to occur on going from the relaxed to the rigor state.

Observation of NMR features due mainly to myosin S1 could allow characterization of the ways of which myosin moves in muscles, both in itself and in relation to actin, and over a large range of time scales. These signals should also be observable by in vivo NMR methods, so that similar studies could be performed on living animals and even on human subjects. Even if the observed signals are shown not to arise from the especially important proteins actin and myosin, NMR

observations on proteins in intact muscle could be valuable in leading to the recognition of other protein motions or in permitting medical diagnosis of muscular disorders in vivo.

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