500 MHz ^{1}H-NMR study of the N-terminal *N*-trimethylalanine residue of LC-1 and LC-2 light chains in rabbit fast skeletal myosin solutions

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Received 2 April 1984; revised version received 8 May 1984

High-resolution proton NMR at 500 MHz has been used to study the *N*-trimethyl terminals of LC-1 and LC-2 light chains in rabbit fast skeletal myosin solutions. The observed resonance is a sum of two Lorentzians with $\Delta\nu = 5 \pm 1$ Hz, $\delta = 3.23$ ppm and $\Delta\nu = 12 \pm 1$ Hz, $\delta = 3.22$ ppm, respectively. By selective proteolytic modifications samples lacking either the N-terminal segment of LC-1 up to Lys-17 (papain modification) or the N-terminal segment of LC-2 up to Arg-7 (trypsin modification) were prepared. From the NMR spectra of the modified samples the narrower $-N^+(CH_3)_3$ methyl resonance is shown to originate from LC-1 and the broader from LC-2. Thus in solution LC-1 and LC-2 N-terminals do not behave identically and there exists between both terminals no interaction reflected by linewidth or chemical shift variation when either N-terminal is removed. In intact as well as in modified myosins the number of resonating protons corresponds within experimental error to the expected values. The comparison of the present NMR results with the rates of proteolytic cleavage suggests that in solution these light chains could be folded back along S1 but without impeding the motion of the N-terminal residues, while in filaments the LC-1 and LC-2 light chains would expose their median sensitive part to proteolytic enzymes.

^{1}H-NMR *Methyl relaxation* N-*Trimethylalanine* *Myosin* *Light chain modification*

1. INTRODUCTION

In most species (muscle cells as well as non-muscle cells) myosin is composed of two heavy chains and 4 light chains organized in a typical highly asymmetrical structure (review [1,2]). A long helically coiled coil (the tail or rod) includes about 3/5 of the two heavy chains intertwined on their C-terminal side. The rest of each heavy chain forms an oblong globular head carrying two light chains. Most myosins have two kinds of light chain (one of each kind on each head). One kind either mediates regulation (regulatory light chain) or simply bears regulatory attributes (pseudo-

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Abbreviations: DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; MLCK, myosin light chain kinase; Nbs2, 5,5-dithiobis(2-nitrobenzoic acid); AMP-PNP, adenylyl imidodiphosphate; NOE, nuclear Overhauser effect. PAGE, polyacrylamide gel electrophoresis; HMM, heavy meromyosin; LMM, light meromyosin; S-1, HMM subfragment-1; CT, chymotrypsin; P, papain; T, trypsin. Because of the influence of preparative methods on the structure of such typical subfragments or subunits of myosin as HMM, S-1, LC-1, LC-2, these subfragments or subunits are characterized in the following way: (a) information given in parentheses before the name of the fragment refers to the origin of the fragment, i.e., summarizes conditions under which it was obtained; (b) information given in parentheses after the name of the fragment refers to some essential characteristics of the species, e.g., myosin-[(Ca^{2+}, P)LC-1'] defines a myosin species in which the LC-1 light chain has been modified by papain in the presence of Ca^{2+}. Trypsin (bovine pancreas) (EC 3.4.21.4); papain (Papaya latex) (EC 3.4.22.2); chymotrypsin (EC 3.4.21.1)

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regulatory light chains) without exhibiting any clearly defined regulatory role (see [3]); the other kind has not been assigned any specific role and in one species its removal has been shown to leave the ATPase activity unimparied [4]. In vertebrate fast skeletal muscle the first kind belongs to the pseudo-regulatory type (Nbs2 or LC2 light chain) and the second can be divided into two sub-types according to their apparent M_r (A1 or LC1, 25 000; A2 or LC3, 17 000). In rabbits specifically these two subtypes have identical sequences over their 141 C-terminal residues, while A1 has a very characteristic additional sequence of 41 residues at its N-terminal end. Between this extra sequence and the sequence common to both light chains is an 8-residue homologous sequence with 5 amino acid substitutions [5]. Two interesting characteristics of the A1 extra sequence are a highly basic N-terminal segment followed by a quasi-regular 'Ala-Pro' repeat. Authors in [6] showed recently that the blocking N-terminal residue is *N*-trimethylalanine. This finding is interesting from both a technical and a speculative point of view. The *N*-methyl protons were shown to constitute a very sensitive NMR probe with which to observe the behaviour of the light chain extremity [6,7]. Moreover, the signal at 3.23 ppm typical of *N*-trimethyl protons was also observed in the pseudo-regulatory light chain (LC-2 or Nbs2-LC) as well as in LC-1 from bovine cardiac ventricular muscle, also an A1-type light chain [6].

When isolated by suitable proteolytic digestion the myosin head is referred to as HMM-S1 (or S-1 or SF-1) [8]. This part, containing both the ATPase active site and a region interacting with actin, is often isolated and studied as a model for whole myosin in the molecular contractile system. However, it is important to point out that in this state the myosin head has been inevitably submitted to at least one of the following structural alterations: nicking of the heavy chain [9–11], excision of an N-terminal portion of both LC-1 and LC-2 light chains [12,13] or complete loss of one light chain [14,15]. Moreover, this fragment is no longer able to assemble into the filament structure functionally essential in muscular contraction. Parallel investigations should therefore be carried out whenever possible with whole myosin.

This report describes the effect on the *N*-trimethyl proton resonance at 3.23 ppm of selective light chain modifications carried out on inact solubilized myosin. In fact this 3.23 ppm line is shown here to be the sum of two Lorentzians. By selective modification of either LC-1 or LC-2 light chain each Lorentzian was assigned unambiguously to the N-terminal blocking methyls of the remaining LC-2 or LC-1 light chain. The lines have slightly different chemical shifts (δ) and the linewidth ($\Delta\nu$) is larger for LC-2 than for LC-1 *N*-methyls, an indication that in solution the LC-1 and LC-2 than for N-termini do not behave identically.

2. MATERIALS AND METHODS

2.1. Reagents

Reagent solutions and buffers were prepared with deionized glass-distilled water with the highest grade commercial chemicals. Trypsin from bovine pancreas, lyophilized and salt-free and papain as a crystalline suspension in acetate buffer were obtained from Boehringer-Mannheim (France). A preliminary activity check was carried out under the conditions of use with subsequent analysis of the products by SDS–PAGE. Type 1S lyophilized trypsin inhibitor (soybean) was supplied by Sigma. D_2O (99.8% isotropic enrichment) was provided by ORIS, CEA, France.

2.2. Protein preparations

All operations were carried out at 2–4°C unless otherwise specified. Myosin was extracted from fast-twitch rabbit skeletal muscle and purified as in [16].

Myosin-[$(Ca^{2+}$, P)LC-1'] was prepared from purified myosin by brief digestion with papain, shown earlier to cleave LC1 specifically at Lys-17 [17]. Myosin (10 mg/ml) suspended in 0.06 M NaCl, 0.02 M ammonium acetate (pH 7.0), 0.2 mM $CaCl_2$ was digested with papain (0.018 mg/ml, 24°C, 25 s); the digestion was stopped with iodoacetic acid (1 mM). An equal volume of cold water was added to the reaction mixture which was then centrifuged at 70 000 × *g* for 30 min.

Myosin-[$(Mg^{2+}$, T)LC-2'] was prepared as in [17]. Myosin suspended in 0.06 M NaCl, 0.02 M PO_4 (pH 7.0), 5 mM $MgCl_2$ was digested with trypsin (24°C, 40 s); the digestion was stopped with trypsin inhibitor (0.06 mg/ml).

Protein concentrations were measured by spectrophotometry taking $E_{280}^{1\%} = 5.3$ for myosin as

well as for the two modified species. Gel electrophoreses and densitometric analysis were performed as in [13].

2.3. Sample preparations

At the concentrations used for measurements (20–30 mg/ml) the solutions were highly viscous, and care was needed to obtain homogeneous samples. From the centrifugation pellet stage onwards, all buffer solutions were carefully degassed. For each separate species the pellet was dissolved in 0.177 (v/w) 3 M NaCl in D_2O then diluted with 12 vols cold D_2O and left standing in a closed vessel in a dry box for 4–5 h at 4°C to promote H/D exchange. The precipitate was centrifuged (30 min at 70000 × *g*) and redissolved in 0.177 (v/w) 3 M NaCl, 0.06 M PO_4 (pH 6.7) in D_2O. The samples were then extensively dialyzed for 18 h in a closed vessel against the appropriate buffer solution.

2.4. NMR spectroscopy

NMR spectra were obtained by using a 500 MHz Bruker spectrometer operating in the Fourier mode. The solvent (HDO) line was eliminated by selective decoupling with NOE suppression. Chemical shifts are quoted in ppm relative to the internal standard, DSS. The temperature was regulated at 283 ± 1 K. For samples containing about 30 mg/ml of intact or modified myosin good spectra were obtained with 256 scans.

3. RESULTS

The selective light chain modifications described offer a potential source of information on the whole myosin molecule and possible responses to limited well-defined defects. In this general approach the advantage of NMR lies in the fact that both LC-1 and LC-2 contain a convenient sensor which, by the selective modification method, can be removed alternately from one light chain or the other.

3.1. Sample characteristics

Each sample of 'integral' or 'intact' myosin (see section 2) was checked for light chain characteristics (fig.1) and stoichiometry by a densitometric analysis of SDS–PAGE (table 1). These samples

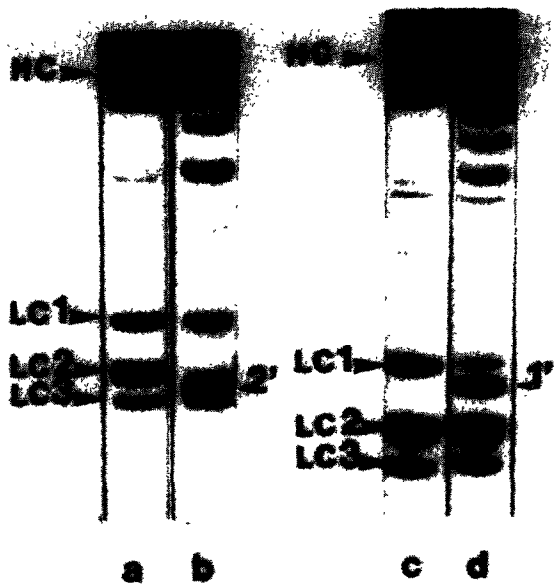

Fig.1. SDS–PAGE of various natural and modified myosin species. (a) Natural (unmodified) myosin; (b) myosin-[(Mg^{2+}, T)LC-2'], myosin modified specifically on the LC-2 light chain by brief digestion with trypsin; (c) natural (unmodified) myosin; (d) myosin-[(Ca^{2+}, P)LC-1'], myosin modified specifically on the LC-1 light chain by brief digestion with papain.

have characteristics in accordance with the type of preparation used [17]. A trace impurity was present in the actin region but it is suspected to be a protein other than actin. Potein F was eliminated whereas proteins B, C and the H,I doublet were still present [18]. For purposes of comparison two specifically modified myosins were prepared as described in section 2. Myosin-[(Ca^{2+}, P)LC-1'] was shown to contain an LC-1 light chain cleaved on its N-terminus Lys-17 [17]. As checked previously, the corresponding N-terminal segment is no longer present in purified preparations. Myosin-[(Mg^{2+}, T)LC-2'] was shown to have lost the LC-2 N-terminal segment up to Arg-7 [17]. The characteristics of these species actually used here are given in table 1. It should be noted that a limited cleavage occurs in one of the heavy chains for about 10–15% of the myosin population. However, the preparation procedure guarantees that the myosin species used here retain their ability to form filaments under the usual pH and ionic strength conditions.

Table 1

Characteristics of Ala-$N^+(CH_3)_3$ proton resonances for unmodified and modified myosins in various media.

Sample designation	Medium including					Molar concentration (μM)	$-N(CH_3)_3$ of LC-1			$-N(CH_3)_3$ of LC-2			No. of protons observed[a]
	Mg^{2+} (mM)	Ca^{2+} (mM)	EGTA (mM)	EDTA (mM)	AMP-PNP (mM)		$\Delta\nu$ (Hz)	No. of protons		$\Delta\nu$ (Hz)	No. of protons		
								Expected	Observed		Expected	Observed	
Unmodified myosin	1	–	1	–	0.68	71.4	4	12 (100)	12 ± 1	12.5	18 (100)	18 ± 2	30 ± 3
Unmodified myosin	10	–	1	–	0.68	71.2	4.5	12 (100)	12 ± 1	11.0	18 (100)	18 ± 2	30 ± 3
Unmodified myosin	–	0.1	–	–	–	74.4	5	12 (100)	11 ± 1	11.0	18 (100)	17 ± 2	29 ± 3
Unmodified myosin	10	0.1	–	–	–	83.5	4.5	12 (100)	12 ± 1	13.0	18 (100)	19 ± 2	32 ± 3
Unmodified myosin	–	–	–	0.5	–	78.2	5	12 (100)	13 ± 1	11.5	18 (100)	20 ± 2	33 ± 3
Unmodified myosin	5	–	–	–	–	74.0	6.5	12 (100)	13 ± 1	13.0	18 (100)	21 ± 2	33 ± 3
Myosin-[(Mg^{2+}, T)LC-2']	1	–	1	–	0.68	59.7	5.0	12 (>95)	12 ± 1	–	0 (<3)	–	15 ± 1
Myosin-[(Mg^{2+}, T)LC-2']	1	0.1	–	–	–	60.1	4.5	12 (>95)	11 ± 1	–	0 (<5)	–	16 ± 2
Myosin-[(Ca^{2+}, P)LC-1']	–	–	–	0.5	–	69.3	6.0	3 (25)	3 ± 1	13.0	18 (>95)	21 ± 2	22 ± 2
Myosin-[(Ca^{2+}, P)LC-1']	5	–	–	–	–	69.9	7.0	3 (25)	3 ± 1	13.5	18 (>95)	21 ± 2	24 ± 2

The standard medium was: 0.6 M NaCl, 0.02 M PO_4; pH values as read on the pH-meter were between 6.4 and 6.7. The linewidths are obtained from decompositions of resonances into a sum of two pure Lorentzians. The number of protons is obtained from these decompositions or from integration by cutting and weighing traces[a] using for calibration the area corresponding to the resonance of the $>N-(CH_2)_2-N<$ protons of EDTA when present. $\Delta\nu$ is the linewidth at half-maximum peak height, the accuracy is ±0.5 Hz. Percentage of intact chains is given within parentheses

3.2. *NMR spectroscopy*

The ^{1}H-NMR spectrum of intact myosin resembles those in [7,19], however with more detailed features. Superimposed on a broad envelope typical of such a large molecule are sharper resonances indicative of domains with greater degrees of mobility (fig.2). The sharp resonance at 3.23 ppm [6] originates from the methyl protons of Ala-$N^+(CH_3)_3$, an N-terminus blocking residue of LC-1. This residue is also present in LC-2 but in [6] this light chain was removed in the preparation process. The present experimental conditions are such that in intact myosin both LC-1 and LC-2 termini are present. The 3.2 ppm resonance could be resolved into two Lorentzian components in several spectra obtained with independent preparations of intact myosin (fig.3). Typical results are given in table 1. For the two components the chemical shifts are 3.23 ppm for the narrower and 3.22 ppm for the broader.

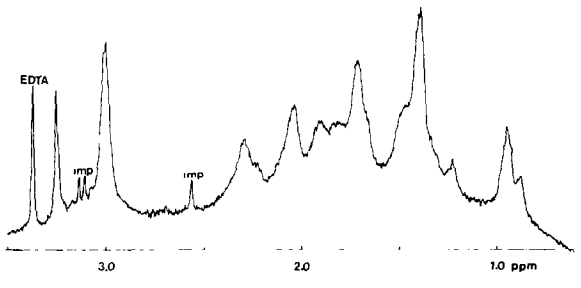

Fig.2. ^{1}H-NMR spectrum at 500 MHz (aliphatic region) of unmodified rabbit skeletal myosin (0.078 mM) in 20 mM phosphate buffer (pH 6.4) containing 0.6 M NaCl and 0.5 mM EDTA in ^2H_2O at 10°C. Chemical shifts are relative to internal DSS.

In samples modified by trypsin, i.e., lacking the first 7 residues of LC-2, the spectra reveal a single Lorentzian line with $\Delta\nu = 5 \pm 0.5$ Hz in the various media used for this study (table 1), thus having the same linewidth and chemical shift as the narrower component of intact myosin. Samples modified with papain, i.e., lacking the N-terminal segment up to and including Lys-17 in 75% of the LC-1 population, display only one broad line which can be resolved into two components (table 1). The broader has the same $\Delta\nu$ and δ as the broad component in intact myosin while the narrower has the characteristics ($\Delta\nu$, δ, intensity) of the remaining 25% of the LC-1 population.

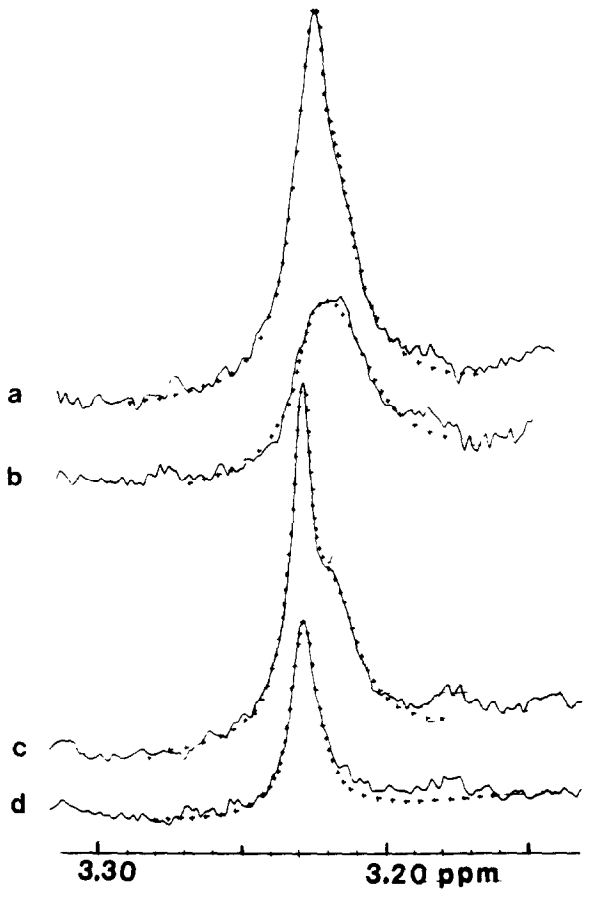

Fig.3. ^{1}H-NMR *N*-trimethyl resonances of unmodified and modified rabbit skeletal myosins, the characteristics of samples are given in table 1. (a) Unmodified myosin (0.078 mM) and (b) myosin-[(Ca^{2+}, P)LC-1'] (a myosin species selectively modified in LC-1 by papain) (0.069 mM) in medium including EDTA (0.5 mM). (c) Unmodified myosin (0.071 mM) and (d) myosin-[(Mg^{2+}, T)LC-2'] (a myosin species selectively modified in LC-2 by trypsin) (0.060 mM) in medium including EGTA (1 mM), Mg^{2+} (1 mM), AMP-PNP (0.68 mM). The superimposed + correspond to calculated values for: (a) two Lorentzians with $\Delta\nu = 5$ Hz, $\delta = 3.23$ ppm, 13 ± 1 protons and $\Delta\nu = 11.5$ Hz, $\delta = 3.22$ ppm, 18 ± 1 protons, respectively; (b) two Lorentzians with $\Delta\nu = 6.5$ Hz, $\delta = 3.23$ ppm, 3 ± 1 protons and $\Delta\nu = 13.0$ Hz, $\delta = 3.22$ ppm, 21 ± 2 protons, respectively; (c) two Lorentzians with $\Delta\nu = 4.0$ Hz, $\delta = 3.23$ ppm, 12 ± 1 protons and $\Delta\nu = 12.5$ Hz, $\delta = 3.22$ ppm, 18 ± 2 protons, respectively; (d) one Lorentzian with $\Delta\nu = 5.0$ Hz, $\delta = 3.23$ ppm, 12 ± 1 protons.

It is thus clear that of the two lines observed for intact myosins the narrower, also found in myosins altogether lacking LC-2 N-termini, must originate from LC-1 *N*-trimethyls. The broader component at 3.22 ppm is then to be attributed to LC-2 *N*-trimethyls.

Moreover within the limits of experimental error the linewidths are identical in intact myosin and in the corresponding modified species. It can then be concluded that: (i) the linewidths and chemical shifts observed are intrinsic properties of LC-1 and LC-2 *N*-trimethyl termini; (ii) since these linewidths and chemical shifts are the same in modified samples and intact myosin no significant interaction between LC-1 and LC-2 N-termini which could give rise to changes in their linewidths and/or chemical shifts occurs in soluble myosin. Furthermore, it is shown that divalent metals (Ca^{2+} or Mg^{2+}) or nucleotides (AMP-PNP) have little or no effect on the linewidths (table 1).

The number of protons resonating at 3.22 and 3.23 ppm was estimated by reference to a single line at 3.44 ppm corresponding to $>N-(CH_2)_2-N<$ protons of EDTA present in some of our samples at a concentration of 0.5 mM. Two different approaches (simulation of spectra or integration by cutting and weighing traces) led to the results in table 1. In all intact myosin samples the number of protons so obtained was 33 ± 3 per myosin molecule. The expected value is 30, taking 1.35 LC-1 and 2 LC-2 per myosin molecule. In papain-modified myosin the agreement between experimental and calculated values is excellent. For the trypsin modification the values obtained by spectrum simulation agree well with the expected values if only one Lorentzian is assumed. In fact, the best fit is obtained if another Lorentzian with $\Delta\nu \cong 5$ Hz, $\delta = 3.21$ ppm and corresponding to about 3 protons is added. The number of protons derived from integrations exceeds the expected value by 2 or 3 protons, if only *N*-methyl groups are taken into account.

For the 3.00 ppm resonance, containing the ϵ-CH_2 resonances of Lys residues, the number of protons estimated as before is 94 ± 5 in unmodified myosin and only 88 ± 4 in papain-modified myosin, with or without magnesium added. The difference is consistent with the removal of 5 Lys residues, indicating that the 4 N-terminal Lys residues at least are mobile.

4. DISCUSSION

4.1. Interrelationship between light chain N-termini

Under our conditions the N-terminal blocking residues -Ala-$N^+(CH_3)_3$ of LC-1 and LC-2 are present in intact myosin and were actually observed. After selective removal of one or other of these residues it was possible to characterize each separately in situ in solubilized whole myosin. The quantitative agreement between expected and observed numbers of protons shows without ambiguity that all the *N*-methyl protons of N-terminal residues for both LC-1 and LC-2 light chains give rise to observable resonances in intact solubilized myosin. Because no alteration of the linewidths and chemical shifts could be observed after each type of modification it may be concluded that no significant interaction takes place between these light chain extremities, either directly or indirectly through conformational changes in the supporting structure (S-1 heavy chain). It is also shown that an occasional break in one of the heavy chains (e.g., at the HMM-LMM junction in myosin-[(Mg^{2+}, T)LC-2']) hardly alters the behaviour of the intact LC-1. The same conclusions apply to the intact LC-2 in the papain modification. In this sense it is confirmed that the two 'artificial' myosin isozymes [17] are reliable species for structure-function studies.

Besides these recently discovered *N*-trimethylated blocking residues myosin contains other *N*-methylated residues: Lys-34, 60% consisting of ϵ-monomethyllysine and two ϵ-methyllysines of which one is thought to be buried in the hydrophobic pocket of the active site (residue 129) [20]. A methylhistidine [21] is also found at position 766. All these *N*-methyl protons might be expected to resonate in the 3.2 ppm region, but as estimated below they would have to possess a considerable degree of freedom to be detectable and indeed are not observed among narrow N-CH_3 resonances. However, a small fraction, endowed with extra mobility as a result of trypsin modification, could possibly account for the slight excess of protons present after this operation.

4.2. Linewidth characteristics

The *N*-trimethyl protons give extremely narrow resonance lines (5–12 Hz) for a molecule of this size which means that these protons have long transverse relaxation times T_2, since $1/T_2 = \pi\Delta\nu$ (neglecting field inhomogeneities (~0.1 Hz, i.e., well below the observed line width) and two-bond coupling with ^{14}N (~0.6 Hz). For protons, and in the absence of paramagnetic species, as is the case here, relaxation occurs only through inter- and intramolecular dipolar interactions with other protons. Since experiments were performed in D_2O the relaxation is assumed to be due to rotational diffusion. In the absence of any segmental motion in the molecule the transverse relaxation time T_2 of a proton i relaxed by j protons is [22,23]:

$$\frac{1}{T_2} = \frac{3}{20}\gamma^4\hbar^2 \sum_j r_{ij}^{-6}\left[3\tau_c + \frac{5\tau_c}{1+\omega_0^2\tau_c^2} + \frac{2\tau_c}{1+4\omega_0^2\tau_c^2}\right]$$

where γ and $\hbar$ have their usual meanings, r_{ij} is the interproton distance, $\omega_0 \cong 3 \times 10^9$ Hz, and τ_c is the rotational correlation time for the Brownian motion of the molecule. Very short-range interactions only are effective; moreover, as τ_c is shorter so T_2 is longer. For a spherical globular molecule with the same M_r as monomeric myosin and tumbling as a whole entity τ_c is about 10^{-6} s, depending upon the temperature and viscosity through the well-known Stokes-Einstein equation. The corresponding linewidths for protons are increased further by the anisotropy effect of the molecule whose shape departs very largely from a sphere [24]. The free rotation of each N-terminal methyl about its symmetry axis can result at most in a 4-fold reduction of the linewidth imposed by the isotropic tumbling of this symmetry axis [25]. Assuming that each methyl proton is relaxed only by its two neighbours on the same carbon, an estimation may be made for the 'effective τ_c' [26] corresponding to the reorientation of each methyl axis; for LC-1 terminus, $\tau_{c,\mathrm{eff}} \sim 10^{-9}$ s [27], assuming free rotation about this axis. The motion is not restricted by the viscosity of the myosin solution since the microscopic viscosity reflected by the EDTA linewidth (6.5 Hz) is the same as in the corresponding dialysis buffer. But from inspection of CPK models for LC-1 and LC-2 N-termini it may be seen that, due to the presence of the adjacent residue Pro-2 together with that of the Ala-1 methyl group, the rotation of the -$N(CH_3)_3$ group about the C_α(Ala)–N bond is highly restricted. To account for the narrowness of the trimethyl resonances extensive segmental motions of the chains are required. This implies also that the N-termini be surrounded by solvent molecules to move freely.

4.3. Light chain conformation and proteolytic susceptibility

A comparison of proteolytic cleavages of both LC-1 and LC-2 has shown [28] that the rates are significantly different for myosin filaments (in a low ionic strength medium) and for monomeric myosin (high ionic strength). In the N-terminal region up to about 20 residues the observed rate of cleavage is greater in filaments as compared to monomeric myosin. Thus for both LC-1 and LC-2 light chains in the filament where the polymeric structure would be expected to hinder accessibility to light chains for proteolytic enzymes the reverse is in fact observed, suggesting that the extremities can act as 'antennae' engaged in some interaction with an as yet undefined part of the structure, perhaps the shaft, and therefore extend and expose their median sensitive part to proteolytic enzymes.

As regards the extremity of LC-1 another candidate for the interaction is actin, since the LC-1-Ala-$N(CH_3)_3$ is immobilized when the acto-S-1 complex is formed [7,29]. Further studies will be needed to show whether light chain extremities constitute in whole or in part the actual active interacting structure in the filaments; alternatively they might only be squeezed and their motion hindered by the proximity of the effectively interacting structural elements, although the proteolytic susceptibility study in [28] does not favour the latter hypothesis.

ACKNOWLEDGEMENTS

Many thanks are due to Miss Béatrice Gilbert for technical assistance and Dr Fazakerley for a critical reading of the manuscript.

REFERENCES

[1] Harrington, W.F. (1979) in: The Proteins, vol. 4 (Neurath, H. and Hill, R.L. eds) pp. 246–411, Academic Press, New York.
[2] Bagshaw, C.R. (1982) Muscle Contraction, Chapman and Hall, London.
[3] Scholey, J.M., Taylor, K.A. and Kendrick-Jones, J. (1981) Biochimie 63, 255–271.
[4] Wagner, P.D. and Giniger, E. (1981) Nature 292, 560–562.
[5] Frank, G. and Weeds, A.G. (1974) Eur. J. Biochem. 44, 317–334.
[6] Henry, G.D., Dalgarno, D.C., Marcus, G., Scott, M., Levine, B.A. and Trayer, I.P. (1982) FEBS Lett. 144, 11–15.
[7] Prince, H.P., Trayer, H.R., Henry, G.D., Trayer, I.P., Dalgarno, D.C., Levine, B.A., Cary, P.D. and Turner, C. (1981) Eur. J. Biochem. 121, 213–219.
[8] Lowey, S., Slayter, H.S., Weeds, A.G. and Baker, H. (1969) J. Mol. Biol. 42, 1–29.
[9] Hayashi, Y. (1972) J. Biochem. 72, 83–100.
[10] Cardinaud, R., Dassin, E. and Pelletier, F. (1973) Biochem. Biophys. Res. Commun. 52, 1057–1063.
[11] Stone, D. and Perry, S.V. (1973) Biochem. J. 131, 127–137.
[12] Cardinaud, R. (1979) CR Acad. Sci. 288, ser. D, 1481–1484.
[13] Cardinaud, R. (1980) Biochimie 62, 135–145.
[14] Yagi, K. and Otani, F. (1974) J. Biochem. 76, 365–373.
[15] Weeds, A.G. and Taylor, R.S. (1975) Nature 257, 54–56.
[16] Cardinaud, R. and Drifford, M. (1982) J. Muscle Res. Cell Motil. 3, 313–332.
[17] Cardinaud, R. (1982) Eur. J. Biochem. 122, 527–533.
[18] Starr, R. and Offer, G. (1971) FEBS Lett. 15, 40–44.
[19] Highsmith, S., Akasaka, K., Konrad, M., Goody, R., Holmes, K., Wade-Jardetzky, N. and Jardetzky, O. (1979) Biochemistry 18, 4238–4244.
[20] Tong, S.V. and Elzinga, M. (1983) J. Biol. Chem. 258, 13100–13104.
[21] Lu, R., Sosinski, J., Balint, M. and Sreter, F. (1978) Fed. Proc. 37, 1695.
[22] Kubo, R. and Tomita, K. (1954) J. Phys. Soc. Jap. 9, 888–919.
[23] Solomon, I. (1955) Phys. Rev. 559–565.
[24] Woessner, D.E. (1962) J. Chem. Phys. 37, 647–654.
[25] Woessner, D.E. (1962) J. Chem. Phys. 36, 1–4.
[26] Lanir, A. and Nayon, G. (1971) Biochemistry 10, 1024–1032.
[27] Woessner, D.E., Snowden, B.S. Jr and Meyer, G.H. (1969) J. Chem. Phys. 50, 719–721.
[28] Cardinaud, R. (1984) in: Recent Advances on Contractile Proteins (Arena, A.E.E. and Russo, M.A. eds) Praeger Scientific, New York, in press.
[29] Dalgarno, D.C., Prince, H.P., Levine, B.A. and Trayer, I.P. (1982) Biochim. Biophys. Acta 707, 81–88.