# Bending of smooth muscle myosin rod

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Electron microscopy of mammalian smooth muscle myosin rods showed them to be 153 ± 7 nm (SD) long, and to bend sharply (> 90°) but infrequently, and pH independently (range 6.5–9.5), at a single site 45 ± 4 nm from one end of the molecule. Light meromyosin (LMM) preparations were 99 ± 10 nm long, and showed no bends. Intrinsic viscosity *vs* temperature plots for rods and LMM indicated that neither fragment changed in flexibility in the range 4–40° C. Peptide mapping in the presence and absence of SDS established that the proteolytic susceptibility of the hinge at the N terminus of LMM reflects the presence of locally different structure, and not simply a clustering of susceptible residues. The isolated smooth muscle myosin rod thus contains only a single hinge, having significant stiffness, and lacks the second bend seen under certain conditions in the intact molecule.

*Smooth muscle myosin* *Myosin rod* *Myosin hinge*

## 1. INTRODUCTION

The twin heavy chains of the myosin molecule wrap together over much of their length to form a rod-like $\alpha$-helical coiled coil, parting near their N-termini to fold into twin prolate heads. In muscle the tails of the molecules pack into a linear polymer, the thick filament, from which the heads project in a helical array [1]. In the model for muscle contraction in [2], a flexible hinge is postulated to exist at a trypsin-sensitive site in the myosin rod [3]. This allows the heads, together with the adjacent SF 2 section of the rod, to swing clear of the thick filament surface and bind to actin over a range of separation distances between the actin and myosin filaments. Authors in [4] obtained evidence for such a hinge ~45 nm from the head–tail junction of skeletal myosin. Bends in other positions have been seen in this [5] and other [6] myosins. The flexibility of the myosin tail represents a major constraint upon possible crossbridge mechanisms, and it is therefore likely that if a flexible hinge is required in the contraction mechanism, then it will represent a conserved structural feature of all myosins. With a few exceptions [7], studies of the myosin rod have been limited to skeletal muscle myosin.

This report describes a study of the flexibility of the rod part of myosin from a vertebrate smooth muscle. The flexibility of this particle is of particular interest in view of the recent finding that smooth muscle myosin can adopt a folded configuration [6], in which the tail is apparently bent in two places.

*Abbreviations:* LMM, light meromyosin; SF 2, heavy meromyosin subfragment 2; PAGE, polyacrylamide gel electrophoresis

## 2. MATERIALS AND METHODS

Myosin rods and LMM fragments were prepared from purified pig stomach myosin as previously described [8]. Lyophilised fragments were reconstituted in 0.6 M KCl, 10 mM imidazole, pH 7.0. For electron microscopy, aliquots were diluted into 0.6 M ammonium formate, 30% glycerol, 10 mM Bistris, 10 mM Tris, 10 mM glycine, (pH 6.5, 7.5, 8.5 or 9.5), to a final concentration of 20 $\mu g \cdot ml^{-1}$, sprayed onto freshly cleaved mica, and rotary shadowed with platinum. Micrographs were taken at a nominal magnification of 25 000 in a Zeiss

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EM 10 electron microscope operated at 80 kV. For length measurements, negatives were reduced to slide format and projected onto a screen, using the 400 Å repeat of tropomyosin paracrystals as a internal calibration standard. Viscosity measurements were made in an Ostwald-type viscometer having an outflow time for water of 23 s at 25°C. Peptide mapping was carried out at 25°C on a 5 mg · ml$^{-1}$ solution of rod, using 0.05 mg · ml$^{-1}$ chymotrypsin, and the digests subsequently analysed by SDS–PAGE [9,10] using an 11–20% linear gradient separating gel.

## 3. RESULTS AND DISCUSSION

Fig.1 shows an SDS gel of the preparations used in this study. Myosin rod had an apparent $M_r$ of 130000. LMM had an apparent $M_r$ of 85000 and contained a small amount of lower $M_r$ (70000–80000) material. There was no detectable cross-contamination between the preparations.

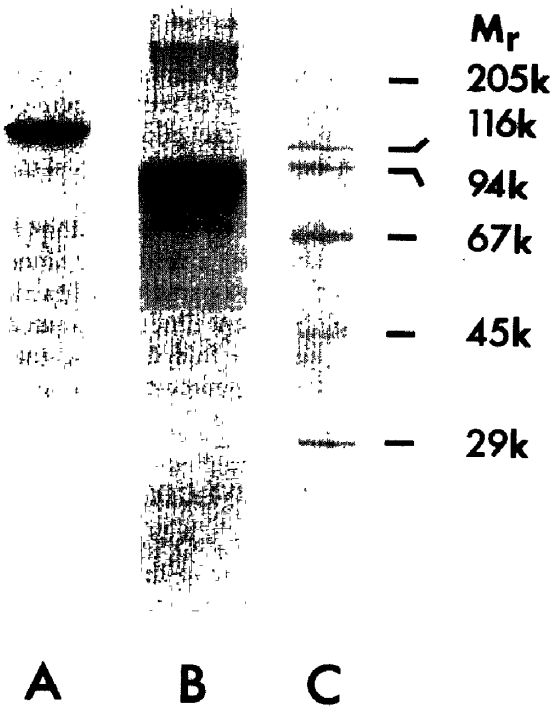


Fig.1. SDS gels of the preparations used. A, Pig stomach myosin rod; B, pig stomach LMM; C, markers: Myosin heavy chain ($M_r$ 205000), $\beta$-galactosidase ($M_r$ 116000), phosphorylase *b* ($M_r$ 94000), bovine serum albumin ($M_r$ 67000), ovalbumin ($M_r$ 45000), carbonic anhydrase ($M_r$ 29000).

### 3.1. Electron microscopy

Representative appearances obtained from glycerol-sprayed, rotary shadowed preparations of pig stomach myosin rods and LMM are shown in fig.2. Since the orientation of the molecules appeared more or less random, their alignment was apparently not influenced by the retracting glycerol droplet, but rather reflected to some extent their conformation in solution. Only 13 of the 159 rods measured showed sharp (>90°) bends. Such bends, when they occurred, were only single and appeared consistently at a position 45 ± 4 nm (SD) from one end of the molecule. The total length of the rod was measured as 153 + 8 nm (SD) (fig.3) in good agreement with ~156 nm obtained from negatively-stained segments of chicken gizzard myosin rod [8], and with a recent measurement of 154 ± 0.6 nm (SE) for skeletal myosin rod [11]. No sharp bends were observed in LMM preparations. The length of the longer section of rod away from the hinge (107 ± 9 nm) was consistent with the measured length for isolated LMM of 99 ± 10 nm (SD). On this basis, the region showing limited flexibility was taken to be the LMM–(SF 2) junction of the rod. No obvious differences in the flexibilities of the LMM and SF 2 domains on either side of the hinge were noted, consistent with these domains having identical thermal stability [7]. Raising the pH of the rod solution from 6.5 to 9.5 did not induce greater flexibility in the hinge region. This suggests that any pH-induced changes in crossbridge disposition, such as 'swing-out' [12] from the thick filament, are not due to a major conformational transition in the hinge. The observed limited flexibility of the hinge is consistent with sequence information available for this region in other myosins [13], which suggests that the hinge is not random coil. Inclusion of glycerol in the sprayed solutions might have affected the structural properties of the rod [4], but the fact that some localised bending did occur argues otherwise. The data thus indicate the presence in this rod of a single hinge, of limited flexibility. In the folded (10 s [6]) form of smooth muscle myosin, the tail is bent in two places, once close to the hinge identified here, and once where the heads contact the tail, about halfway along LMM. The

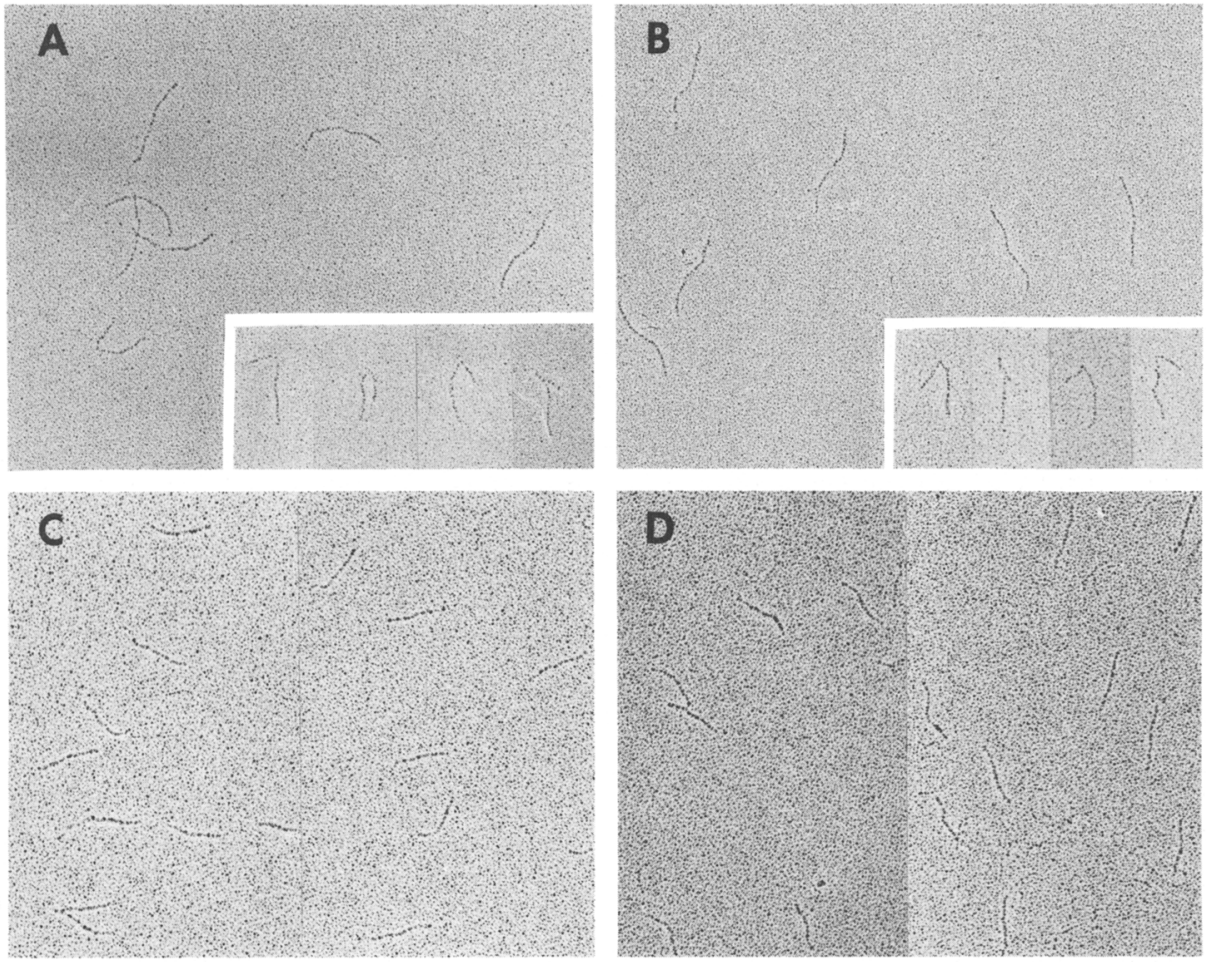


Fig.2. Electron micrographs of single rod and LMM molecules. (A) Rods, pH 6.5; (B) rods, pH 8.5; (C) LMM, pH 6.5; (D) LMM, pH 8.5. Insets: examples of sharply bent rod molecules.

finding that only the first of these sites is especially flexible in the isolated tail suggests that the second bend is induced by a heads–tails interaction, perhaps in a similar way to that in which antibody binding can induce bends in the myosin tail [14].

### 3.2. *Viscosity studies*

The EM study described above was carried out on rod solutions sprayed at room temperature. The possibility was considered that the flexibility of the hinge might be greater at physiological temperature. Accordingly, the flexibility of the rods in solution was examined by comparing the viscosity of LMM solutions with those of rod, at various temperatures. Viscosity measurements are highly sensitive to changes in molecular shape [15], and an increase in flexibility with temperature might be expected to result in a fall in intrinsic viscosity. Fig.4 shows, however, that no change in intrinsic viscosity occurred for either LMM or rods over the range 5–40°C, indicating that neither fragment detectably changed its flexibility in this range, and suggesting therefore that the degree of flexibility estimated by EM at ~20°C also applies at more physiological temperatures.

### 3.3. *Peptide mapping of rods*

Chymotryptic digestion of pig stomach myosin rod yields resistant ~40 kDa SF 2 and ~85 kDa LMM fragments directly (fig.5), in contrast to

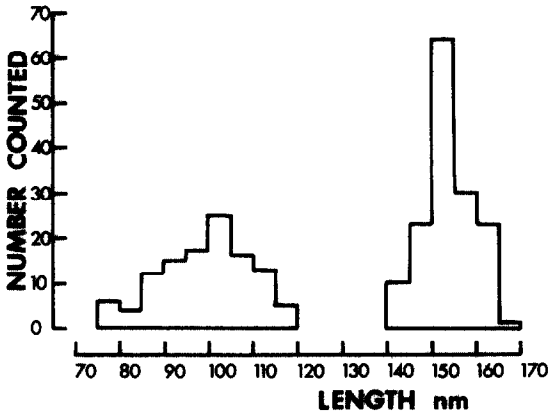


Fig.3. Histograms of length distributions for (left) LMM, (right) rods.

skeletal muscle myosin rod which is initially cleaved to a ~60 kDa SF 2 and a ~70 kDa LMM [16,17], and subsequently over a broad zone [18]. With the smooth muscle myosin rod examined here, a close correspondence was observed between the sites of bending and of cleavage. It remained nonetheless possible that the susceptibility of the SF 2–LMM junction of this rod was due simply to a clustering of susceptible residues, rather than the presence of locally different higher-order structure

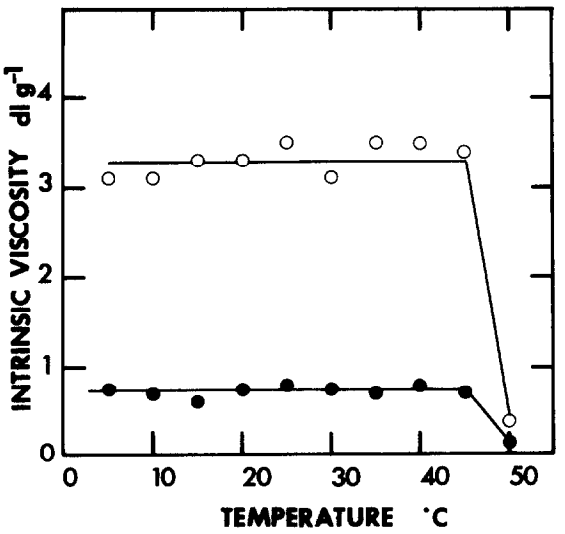


Fig.4. Intrinsic viscosity *vs* temperature plots for (○) Rod, (●) LMM. Buffer: 0.6 M KCl, 10 mM Na phosphate, 0.5 mM EDTA, 0.5 mM dithioerythritol, pH 7.6.

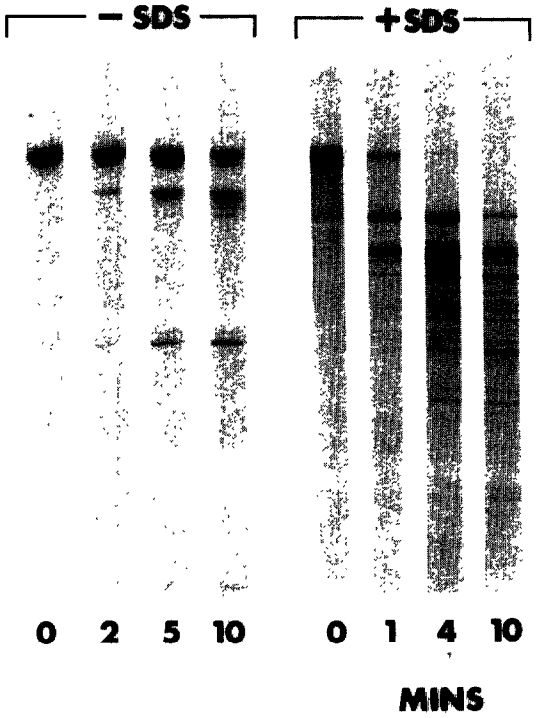


Fig.5. Native (left) and SDS-denatured (right) peptide maps of pig stomach myosin rod. Conditions: left, rods, 5 mg·ml$^{-1}$, chymotrypsin 0.05 mg·ml$^{-1}$, 0.6 M KCl, 10 mM imidazole, 1 mM $MgCl_2$, 1 mM cysteine, pH 6.8. Right, 0.125 M Tris–HCl, 0.5% SDS, 1 mM EDTA, pH 6.8 [19]. Same protein concentrations. Both incubations at 25°C.

at the hinge site. Fig.5 shows, however, that for this pig stomach myosin rod in the presence of denaturing amounts of SDS, chymotryptic chain cleavage occurred in quite different positions to that of native rod. The susceptibility of the native SF 2–LMM junction must therefore reflect the presence there of a distinctly more proteolytically accessible structure, and it seems very likely that this corresponds to the hinge identified above by EM.

In conclusion, the present results indicate that the smooth muscle myosin tail contains a single discrete hinge of locally flexible structure, ~45 nm from the head-tail junction. Since only a single hinge site is observed in the isolated tail, the second bend in the tail characteristic of the folded 10 s form of smooth muscle myosin must be induced by head–tail binding.

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