Reversible helix–coil transition of pig stomach myosin rod

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Studies using differential scanning calorimetry indicated that the isolated rod domain of the pig stomach myosin undergoes a single reversible helix–coil transition with a temperature midpoint of 325 K at physiological pH and ionic strength. Similar behaviour was observed for both the isolated LMM and SF-2 fragments of the rod. The results suggest that the smooth myosin rod has rather uniform structural stability along its length, and is thus unlikely to contain a specialised region which undergoes a substantial force-generating helix–coil transition under physiological conditions.

Smooth muscle myosin *Myosin rod* *Differential scanning calorimetry* *Helix–coil transition* *Muscle contraction*

1. INTRODUCTION

Smooth muscle myosin, like striated myosin, contains twin heavy chains, which wrap together over much of their length to form a rod-like α-helical coiled coil, but which separate at their N-termini to fold into a pair of pseudoglobular heads. The rod-like tail section of the myosin molecule may be separated from the heads by limited papain proteolysis [1,2]. Subsequent digestion of the rod with either trypsin or chymotrypsin cleaves it into two subfragments: SF-2, the soluble section immediately adjacent to the heads; and LMM, the distal section. It is thought that the cleavage site may correspond to the site of a hinge in the rod, between SF-2 and LMM [3].

Myosin is able to assemble into filaments composed of several hundred individual myosin molecules [4]. Since isolated LMM self-assembles, but SF-2 does not, it is supposed that the LMM section of the rods forms the thick filament backbone, whilst the hinge permits the SF-2, carrying the myosin heads to swing free of this backbone [5]. Such a movement may be necessary to accommodate changes in the attitude of the myosin heads to the actin filaments during the contractile cycle [6]. The SF-2 section of the rod may contain a springlike force-generating element for muscle contraction [7]. At physiological temperatures, the SF-2 section of the rod contains a region of unstable conformation, whose thermal melting would tend to shorten the rod and hence provide contractile force [7]. In support of this idea, it was shown that the rabbit skeletal long SF-2 fragment is indeed partially melted in solution at 37°C [8]. Nonetheless, physical studies [9] and sequence information [10] suggest that the SF-2 has rather uniform structural character along its length.

Whilst the conformational properties of the rabbit skeletal myosin rod have been studied by a variety of methods, other myosins have received little attention. Here, the rod, LMM and SF-2 subfragments of a smooth muscle myosin from pig

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Abbreviations: HMM, heavy meromyosin; LMM, light meromyosin; SF-2, subfragment 2; DSC, differential scanning calorimetry; T_m, temperature midpoint of melting transition

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stomach were isolated, and their thermal melting behaviour characterised using differential scanning calorimetry.

2. MATERIALS AND METHODS

Low ionic strength-insoluble residues of papain and chymotrypsin digestions [1] of pig stomach myosin were used as sources of rod and LMM. A digest of pig stomach HMM rather than rod was used as a source of SF-2. HMM was digested at 10 mg/ml with 0.03 μg papain/ml (Sigma) for 10 min at 25°C. Rod, LMM and SF-2 fragments were purified from these respective digests using ethanol denaturation, ammonium sulphate fractionation and isoelectric precipitation. Exact procedures will be described elsewhere. The preparations appeared homogeneous on Coomassie blue-stained SDS gels [10] (fig.1). The lyophilised fragments were redissolved in 0.6 M KCl, 10 mM Na-phosphate (pH 7.0) and dialysed extensively against the buffer of choice before use. Samples were briefly centrifuged in a Beckman microfuge to achieve the 30–50 mg protein/ml necessary to obtain an acceptable signal on the calorimeter. Thermal melting was observed using a Perkin Elmer DSC 11 Differential Scanning Calorimeter. Samples (15 μl) were sealed in aluminium sample pans and scanned at a heating rate of 5 $K.min^{-1}$ over 305–350 K.

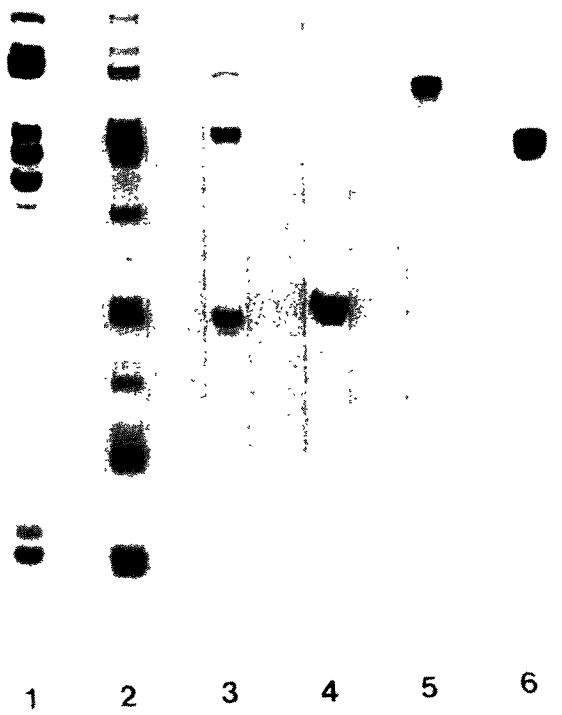

Fig.1. SDS–slab gel electrophoresis (as in [2]) of subfragment preparations: (1) pig stomach HMM; (2) papain digest of pig stomach HMM; (3) ethanol-resistant fraction of papain digest; (4) purified pig stomach SF-2; (5) purified pig stomach rod; (6) purified pig stomach LMM. Gels 1–3 and 4–6 were run on separate occasions.

3. RESULTS AND DISCUSSION

DSC thermograms were obtained at different pH-values for pig stomach rod in 0.6 M KCl, when the rod is soluble, or 0.12 M KCl, when it self-associates into short filaments as checked by electron microscopy (not shown). At high salt concentrations, the rods were seen to melt in an apparently monophasic process (fig.2a), with a temperature midpoint (T_m) for the transition of about 325 K (fig.3). Lowering the salt concentration to 0.12 M KCl did not substantially affect this behaviour (fig.3) indicating that the formation of rod aggregates had little effect on the structural stabilities of the component rods as represented by their melting characteristics. In addition, the simple melting pattern observed suggested that there were no regions of conformational instability within the rod, that is, the SF-2 and LMM domains of the rod had very similar structural stabilities.

The behaviour of the isolated LMM and SF-2 domains were examined (fig.2b,c). The two fragments had similar thermostabilities, SF-2 being 2 K less stable and tending to melt over a broader temperature range. Such effects would be expected for decreasing chain length [12]. A rather broad melting profile has been reported for the rabbit skeletal SF-2 [13]. Increasing pH caused some destabilisation of the rod, LMM and SF-2 (fig.3), whilst increasing KCl from 0.12–0.6 M had a slight stabilising effect on the intact rod. These salt and pH effects indicate that electrostatic effects within and between chains are involved to some extent in stabilising the coiled coil of the rods. However, the absence of major effects on T_m

of changing salt concentration and pH suggests that hydrophobic interactions and hydrogen bonds are the dominant stabilising influences [12]. Recent sequence data [10] indicated that myosin rod, in common with other coiled coil proteins, is stabilised by a seam of hydrophobic contacts between the two chains [12]. In some proteins, for example tropomyosin, interchain electrostatic interactions are thought additionally to stabilise the structure [12]. The present results, while not permitting a quantitative assessment of the relative importance of these two factors in smooth myosin rod do suggest that electrostatic effects are relatively weak.

Significantly, the melting of all 3 fragments was substantially reversible under all the conditions tested, as indicated by cooling and rescanning melted samples (fig.2). This indicates that the thermally separated chains of the rod can reanneal (in register) on cooling. The reversibility was preserved over several cycles of heating and cooling. This behaviour is in complete contrast to that of skeletal myosin rods (not shown), which undergo an irreversible gelation reaction upon melting [14]. The basis for this gelation reaction is thought to be the formation of hydrophobic bonds between nonpolar residues which are normally internal to the structure, but which are exposed as the rod melts to random coil [14]. In smooth rods the coiled coil structure is apparently regained without the formation of such bonds. The structural source of this difference in behaviour between smooth and skeletal rods is unclear at present. One possibility is that sulphydryl residues, present in striated rods

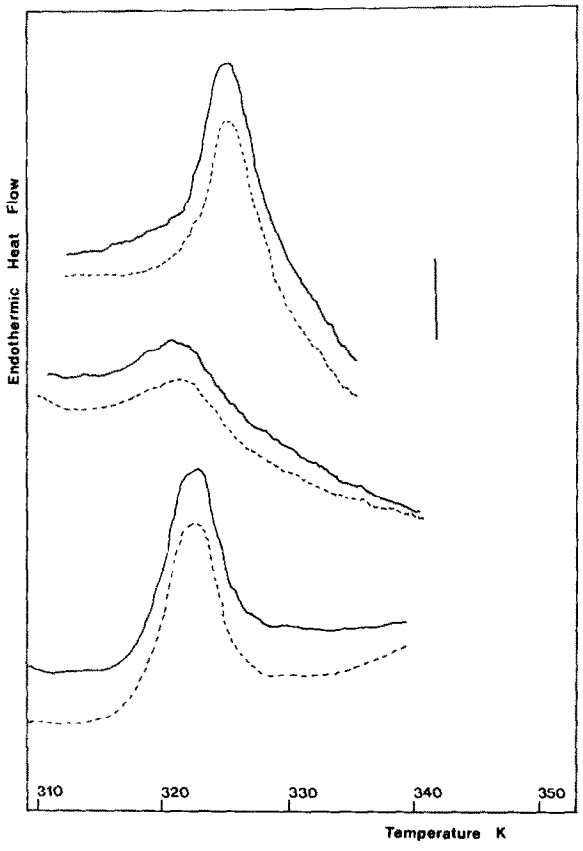

Fig.2. Representative DSC thermograms obtained for: (top) pig stomach rod; (centre) pig stomach SF-2; (bottom) pig stomach LMM; (——) initial scan; (– – –) repeat scan after cooling; condition, 0.12 M KCl, 10 mM sodium phosphate (pH 7.6). The vertical axis is endothermic heat flow, the bar representing 0.2 mcal.s^{-1}.

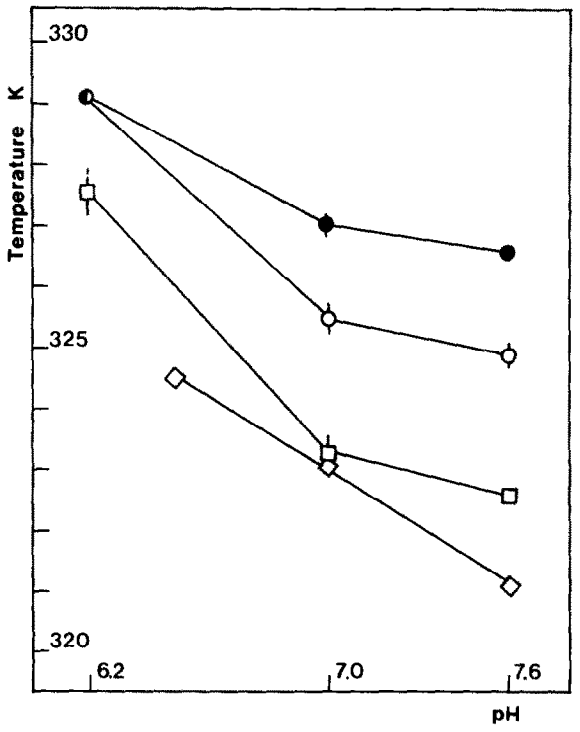

Fig.3. Variation with pH and [KCl] of T_m for myosin subfragments: (open symbols) 0.12 M KCl, 10 mM sodium phosphate; (filled symbols) 0.6 M KCl, 10 mM sodium phosphate; (○,●) pig stomach rod; (□) pig stomach LMM; (◇) pig stomach SF-2. Error bars indicate the standard deviation of the mean of 2–4 determinations.

[9] but absent in smooth rods [15], may play a role in gelation.

These findings suggest that this smooth myosin rod has rather uniform structural stability along its length, as was indicated for a nematode myosin rod [10]. Whilst the presence of short stretches of low thermal stability within the smooth myosin rod cannot be excluded, contraction theories postulating a substantial region of easily-melted structure within SF-2 [7] are unlikely to apply to this smooth muscle myosin.

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