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# Domain structure of myosin subfragment-1

## Selective denaturation of the 50 kDa segment

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The structure of the myosin subfragment-1 (S1) from rabbit skeletal muscle was studied using differential scanning microcalorimetry. Three independently melting regions (domains) were revealed in S1. Selective denaturation of the middle 50 kDa segment of the S1 heavy chain resulted in the disappearance of the heat sorption peak corresponding to the melting of the first, the most thermolabile domain without any effect on the thermally induced blue shift of the intrinsic tryptophan fluorescence spectrum which occurs within the temperature region of melting of the second domain. It is concluded that the most thermolabile domain seems to correspond to the N-terminal part of the 50 kDa segment devoid of tryptophan residues.

Myosin subfragment-1; Domain structure; Scanning microcalorimetry; Tryptophan fluorescence; Rabbit skeletal muscle

## 1. INTRODUCTION

Myosin subfragment 1 (S1) is an isolated myosin head, i.e. the segment of myosin molecule containing the sites responsible for ATPase activity and actin binding. It is well known that trypsin [1,2] and many other proteinases [3,4] cleave the S1 heavy chain into 3 fragments, 25 kDa, 50 kDa and 20 kDa, which sometimes are referred to as 'domains'. All of the fragments can be isolated in partially renatured form [5,6].

Recently it has been found that the most labile part of the S1 molecule is the middle 50 kDa segment of the heavy chain which can be selectively and irreversibly denatured by rather mild treatments [7–10]. These results suggest that the S1 molecule is composed of at least two domains with different stabilities: an unstable domain corresponding to the 50 kDa segment and a more stable domain comprising the 25 kDa and 20 kDa interacting segments and the light chain [9,10]. The data on the segmental motion of S1 in solution [11] are in favour of this proposal.

To obtain direct evidence for the existence of independently folded cooperative parts (domains) in the S1 molecule we have studied the thermal denaturation of S1 by means of differential scanning microcalorimetry using the 'successive annealing' method introduced by one of us earlier [12]. The method allows one to reveal separate domains in a protein molecule, i.e. the regions which melt independently from each other. Recently we have found 3 such domains in the S1 molecule [13]. In the present work we show that the most thermolabile domain corresponds to the N-terminal part of the 50 kDa segment of the S1 heavy chain.

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## 2. MATERIALS AND METHODS

S1 from rabbit skeletal muscle myosin was prepared by digestion of myosin filaments with chymotrypsin [14]. Selective degradation of the 50 kDa segment in S1 was carried out by digestion of S1 (1–2 mg/ml) with trypsin at a trypsin/S1 ratio of 1:50 (w/w) in 50 mM imidazole, pH 7.0, either after incubation of S1 in 22% methanol for 20–30 min [10] or after incubation of S1 at 40°C for 20 min [9]. The preparations were purified by ion-exchange chromatography on a Whatman DE-52 column. Selective denaturation of the 50 kDa segment in S1 without further proteolytic degradation was carried out by incubation of S1 (2 mg/ml) in a mixture containing 0.2 M sucrose, 30 mM NaCl and 10 mM Hepes, pH 7.7, at 35°C for 3 h [8]. The preparations were examined by SDS-12.5% polyacrylamide slab gel electrophoresis [2].

Concentration of S1 was determined spectrophotometrically using $E^{1\%}_{280nm} = 7.5$; concentrations of the S1 derivatives were measured by the Bradford method [15] using S1 as a standard.

All temperature-dependent measurements for S1 and its derivatives except some cases described below were carried out in a solution of 10 mM Hepes, pH 7.3, containing 1 mM $MgCl_2$ at protein concentrations from 0.5 to 2.0 mg/ml and a heating rate of 1°C/min.

Calorimetric measurements were carried out on a DASM-4 differential adiabatic scanning microcalorimeter (USSR) with a 0.47 ml cell. The special construction of the cell avoids artifacts caused by protein aggregation. Decomposition of the total heat sorption curves into elementary components was done by means of the 'successive annealing' method as described earlier [12,13].

Fluorescence spectra were measured by a laboratory-built instrument with monochromatic excitation from a mercury lamp (280.4 nm). Fluorescence light was collected from the front surface of the



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thermostated cell. All spectra were corrected for spectral sensitivity of the instrument. Intensity of the light of the mercury line at 365 nm scattered by the front surface of the sample was used as a measure of protein aggregation which occurs at high temperatures.

## 3. RESULTS AND DISCUSSION

Fig. 1a shows the total heat sorption curve for S1 and its resolution into peaks performed by the 'successive annealing' method. Each peak corresponds to the melting of a separate cooperative region (domain) in the S1 molecule [13]. It is seen that S1 contains 3 domains melting with maxima at about 39°C, 47°C and 51°C (domains 1, 2 and 3, respectively).

Trypsin cleaves the S1 heavy chain into 50 kDa, 25 kDa and 20 kDa fragments (Fig. 2b). The fragmentation does not cause significant changes in the S1 heat sorption curve (data not shown). Treatment of S1 by methanol or mild heating (up to 40°C) followed by the tryptic proteolysis results in complete selective degradation of the 50 kDa segment (Fig. 2c,d). Fig. 1b demonstrates the total heat sorption curve and results of the 'successive annealing' of the S1 preparation in which the 50 kDa segment was degraded by means of the methanol and trypsin treatment (see Fig. 2c). It can be seen that this preparation retains domains 2 and 3 but the peak corresponding to domain 1 disappears completely. The same effect was observed for the S1 preparation in which the 50 kDa segment was degraded by mild heating followed by the tryptic proteolysis (data not shown).

Similar disappearance of domain 1 was found in the case of the selective denaturation of the 50 kDa segment without further degradation by trypsin. In this experiment S1 was incubated at 35°C in 10 mM Hepes, pH 7.7, 30 mM NaCl in the presence of 0.2 M sucrose to prevent the protein aggregation [8]. The change of the solvent composition does not significantly influence the positions of the heat sorption peaks and the ratio between their areas. In accord with data of Setton et al. [8], incubation of the protein under such conditions caused inactivation of S1 ATPase and denaturation of the 50 kDa segment monitored by disappearance of the electrophoretic 50 kDa band in trypsin-treated samples. The incubation for 3 h resulted in 85–90% denaturation

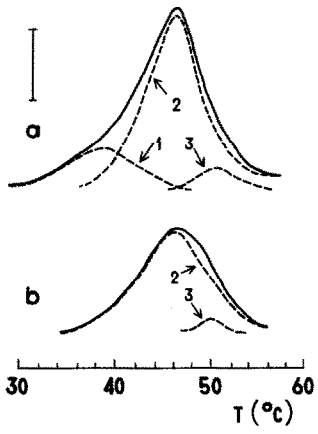


Fig. 1. Heat sorption curves (solid lines) and their resolution into elementary peaks corresponding to domains 1, 2 and 3 (dotted lines) for intact S1 (a) and for S1 digested by trypsin after methanol treatment (b). Protein concentration 2 mg/ml. Conditions: 10 mM Hepes, pH 7.3; 1 mM $MgCl_2$. Heating rate 1 K/min. Vertical bar corresponds to 500 J/K · kg.

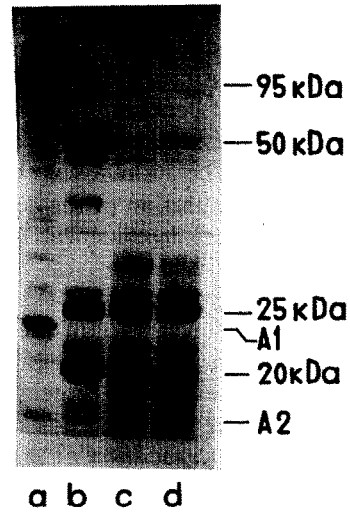


Fig. 2. Representative SDS-gel electrophoretic patterns for S1 preparations used in the experiments: (a) intact S1; (b) tryptic S1; (c) S1 cleaved with trypsin after the methanol treatment; (d) S1 cleaved with trypsin after the heat treatment. A1 and A2 are alkali light chains.

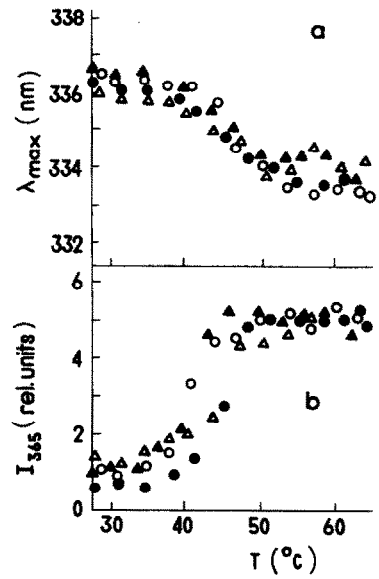


Fig. 3. Temperature dependences of the spectrum position of tryptophan fluorescence (a) and intensity of scattered light at 365 nm (b) for intact S1 (○), tryptic S1 (●), S1 cleaved by trypsin after the methanol treatment (△), and S1 cleaved by trypsin after the mild heat treatment (▲). Protein concentration 0.5 mg/ml. Fluorescence was excited at 280.4 nm. Heating rate was 1°C/min.

of the 50 kDa segment accompanied by 85–90% inactivation of S1 ATPase and in 80% decrease of the area under the domain 1 calorimetric peak.

Summing up, one can conclude that the most thermolabile domain 1 in S1 is a separate cooperative region localized somewhere in the 50 kDa segment.

For more detailed localization of the domain 1 in S1 we studied the temperature dependencies of tryptophan fluorescence of S1 and its derivatives with the denatured and digested (by trypsin 50 kDa) segment. Fig. 3a shows the temperature dependence of the fluorescence spectrum position, the parameter which is least sensitive to possible artifacts caused by the protein aggregation. It can be seen that neither fragmentation of S1 by trypsin nor selective tryptic degradation of the 50 kDa segment resulting in disappearance of domain 1 change essentially the temperature dependence of the fluorescence spectrum position for S1. For all preparations studied the spectral shift towards shorter wavelengths occurs within the temperature region from 40°C to 50°C, i.e. in the region of domain 2 melting (see Fig. 1). Since the spectral shift is accompanied by an increase in intensity of the scattered light at 365 nm (Fig. 3b), it is reasonable to assume that it is caused by transfer of some tryptophan residues to a more hydrophobic environment in the course of the protein aggregation induced by domain 2 denaturation. From these data one can assume that the most thermolabile domain 1 in S1 does not contain tryptophan residues.

It is known that S1 has 5 tryptophan residues all of them being in the heavy chain: 2 residues are located in the 25 kDa segment and 3 residues are in the C-terminal half of the 50 kDa segment [16]. Thus the most thermolabile domain 1 in S1 seems to be formed by the N-terminal region of the 50 kDa segment which does not contain tryptophan residues. It may form the tip of the myosin head which binds antibodies specific to the 50 kDa segment [17,18]. Experiments directed to the identification of another two domains are in progress now.

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