Volume 303, number 2,3, 255–257 FEBS 11106 June 1992

Thermal denaturation of the alkali light chain–20 kDa fragment complex obtained from myosin subfragment 1

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Received 6 March 1992

The thermal denaturation of the myosin subfragment 1 (S1) from rabbit skeletal muscle and of its derivatives obtained by tryptic digestion has been studied by means of differential scanning calorimetry. Two distinct thermal transitions were revealed in the isolated complex of the C-terminal 20 kDa fragment of the S1 heavy chain with the alkali light chain. These transitions were identified by means of a thermal gel analysis method. It has been shown that the thermal denaturation of the 20 kDa fragment of the S1 heavy chain correlates with the melting of the most thermostable domain in the S1 molecule. It is concluded that this domain is located in the C-terminal 20 kDa segment of the S1 heavy chain.

Myosin subfragment 1; Domain structure; Scanning microcalorimetry; Rabbit skeletal muscle

1. INTRODUCTION

Myosin subfragment 1 (S1), obtained by myosin proteolysis with chymotrypsin, is an isolated myosin head retaining intact alkali light chains and the main properties of myosin such as ATPase activity and ability to interact with actin. The problem of elucidation of domain structure in the S1 molecule is very important since interdomain interactions in myosin head might play an essential role in the mechanism of energy transduction by the actomyosin complex. The recently published data of electron microscopy of myosin heads [1], X-ray diffraction on crystallized S1 [2] and the data on segmental motion of S1 in solution [3] indicate that S1 may consist of separate structural domains.

It is well known that trypsin and many other proteinases cleave the S1 heavy chain into 3 fragments of 25, 50 and 20 kDa [4,5], which are often designated as 'domains' of S1. It is most likely that the C-terminal 20 kDa segment of the S1 heavy chain interacts with the alkali light chains [6]. This was confirmed by the isolation of a soluble complex formed by alkali light chain and the 20 kDa fragment [7].

Recently we have studied the thermal denaturation of S1 by means of differential scanning microcalorimetry using the successive annealing method [8,9]. The method allows separate structural domains in a protein molecule to be revealed, i.e. the regions which melt independently from each other. We have found 3 such domains in the S1 molecule [8,9]. The most thermolabile calorimetric domain was identified as a region in the N-terminal part of the 50 kDa segment [8]. The thermal stability of this domain was different in the S1 isoforms containing alkali light chains A1 or A2 [9]. In the present work we have investigated the thermal denaturation of the isolated complex of the 20 kDa fragment of the S1 heavy chain with alkali light chain.

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2. MATERIALS AND METHODS

S1 from rabbit skeletal muscle myosin was prepared by digestion of myosin filaments with chymotrypsin [10]. Split (25 kDa-50 kDa-20 kDa)-S1 was obtained by tryptic digestion of S1 [11]. The isolated complex of the 20 kDa fragment with alkali light chain was obtained as described by Chaussepied et al. [7]. Light chains were dissociated from myosin by the method of Perrie and Perry [12], and the largest part of the DTNB light chain was removed by ethanol fractionation [13]. The preparations were examined by SDS-gradient 6–20% polyacrylamide slab gel electrophoresis [11]. Concentration of S1 was determined spectrophotometrically by using $E^{1\%}_{280\,nm} = 7.5$; concentrations of other preparations were measured by the Bradford method [14].

All temperature-dependent measurements were carried out in 10 mM HEPES, pH 7.3, containing 1 mM $MgCl_2$ at protein concentrations of 1.0–4.0 mg/ml and a constant heating rate 1°C/min.

Calorimetric measurements were carried out on a DASM-4 differential scanning microcalorimeter (USSR) with platinum 0.47 ml cells of special construction preventing artifacts caused by protein aggregation. Decomposition of the total heat-sorption curve into elementary peaks was performed by the successive annealing method as described earlier [9].

Identification of the thermal transitions was done by the thermal gel analysis method [15,16]. After heating at a constant rate (1°C/min) to appropriate temperatures the aliquots (50 μl) of protein samples were cooled to room temperature and stored at 4–6°C for 10–12 h until they were solubilized by addition of equal volume of the buffer 20 mM Tris,

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pH 8.0, containing 2 mM EDTA, 10% glycerol, 0.2% SDS, 0.001% Bromophenol blue. The samples were subjected to SDS-polyacrylamide gel electrophoresis. After staining with Coomassie blue R-250 and destaining the gels were scanned on a DSA-1 scanning densitometer (USSR). The peak areas for each protein band were determined and normalized with respect to the unheated sample.

3. RESULTS AND DISCUSSION

Fig. 1A shows the total heat-sorption curve for S1 fragmented with trypsin into (25 kDa-50 kDa-20 kDa)-S1 and its decomposition into peaks corresponding to the melting of separate domains. Fragmented S1 contains 3 domains melting with maxima at about 42, 47 and 51°C (domains 1, 2 and 3, respectively). The temperatures of domain melting do not differ significantly from that of intact S1 described earlier [8].

The preparation of the isolated complex of the 20 kDa fragment with alkali light chain (Fig. 2c) demonstrates two distinct calorimetric peaks with maxima at about 43 and 53.5°C (Fig. 1B). The 53.5°C peak probably corresponds to the melting of the 20 kDa fragment since the isolated alkali light chains melt with the maximum at about 42°C (Fig. 1C). Partial degradation of A1 light chain after trypsin treatment does not significantly change the character of the thermal denaturation of the light chains (data not shown).

For further identification of the thermal transitions we have studied the thermal denaturation of the complex formed by alkali light chain and the 20 kDa heavy chain fragment by means of thermal gel analysis method. The method allows the denaturation of the separate peptides to be revealed. After heating, denatured peptides interact with each other due to the formation of disulfide bonds. This is accompanied by the disappearance of specific protein bands and the appearance of high molecular weight aggregates. This method is quite applicable to the preparation studied since both the 20 kDa heavy chain fragment and each alkali light chain contain SH groups. The data obtained are shown in Fig. 3. It can be seen that the midpoint for the disappearance of both alkali light chains bands is 43.5°C, i.e. it practically coincides with the calorimetric transition at about 43.5°C (Fig. 1B). On the other hand, the disappearance of the 20 kDa fragment from the gel (Fig. 3) corresponds to a calorimetric transition with a maximum at about 53.5°C(Fig. 1B). This transition correlates with the melting of domain 3 in the S1 molecule (Fig. 1A). Summing up, one can conclude that the most thermostable domain, 3, in S1 is the region localized somewhere in the C-terminal 20 kDa segment of the S1 heavy chain.

It is necessary to note that the first peak on the S1 thermogram (Fig. 1A) is a complex one. It consists of the sum of two peaks which melt independently but coincide in their position. One of them represents the melting of alkali light chain associated with S1 heavy chain (Fig. 1B). Its area is about half of that for the whole peak 1 of S1 (Fig. 1A). Another peak corresponds to the melting of a region in the N-terminal part of the 50 kDa segment (domain 1) [8]. This explanation is in

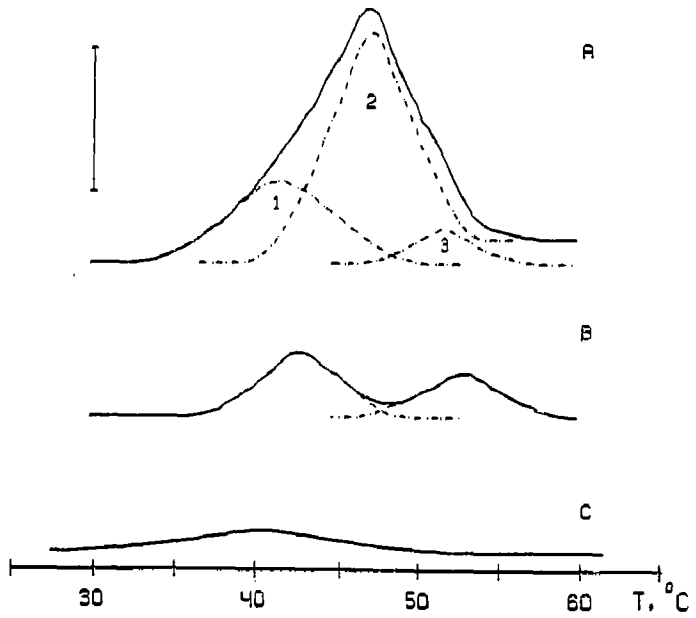

Fig. 1. Heat-sorption curves (solid lines) and their resolution into elementary peaks corresponding to the domains (dotted lines) for (A) tryptic (25 kDa-50 kDa-20 kDa)-S1 (2 mg/ml), (B) isolated complex of the 20 kDa fragment with alkali light chain (1.8 mg/ml), (C) isolated alkali light chains (4 mg/ml). Conditions: 10 mM HEPES, pH 7.3, 1 mM $MgCl_2$. Heating rate 1°C/min. Vertical bar corresponds to 100 kJ/°C · mole.

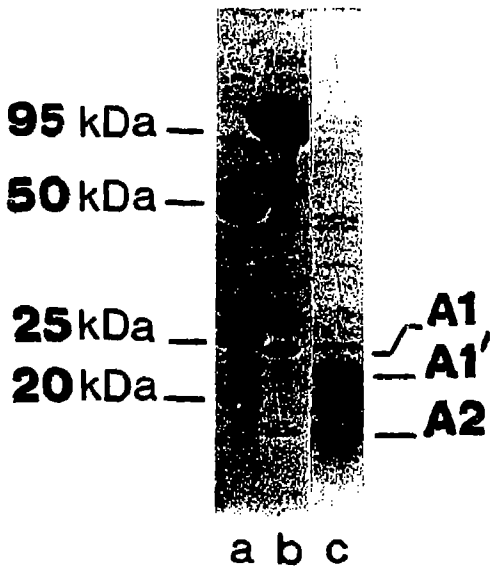

Fig. 2. Representative SDS-gel electrophoretic patterns for the preparations used in the experiments. (a) Tryptic (25 kDa-50 kDa-20 kDa)-S1, (b) intact S1, (c) isolated 20 kDa fragment–alkali light chain complex. A1 and A2 are alkali light chains. A1' denotes the tryptic degradation product of A1.

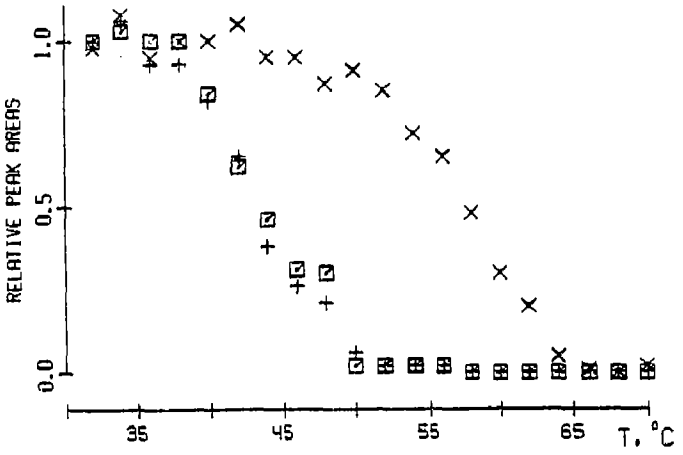

Fig. 3. The thermal denaturation of the isolated complex of the 20 kDa fragment with alkali light chain as studied by the thermal gel analysis method. Normalized relative peak areas for the bands of 20 kDa fragment (×), A1′ (□) and A2 (+) are plotted as a function of the maximum temperature to which the preparation was heated.

good agreement with our data obtained earlier. Indeed, we observed two peaks with maxima at 36 and 40°C for the isoform of S1 containing only A1 light chain [9]. In this case A1 affects the structure of domain 1 and it melts at 36°C, whereas alkali light chain itself melts at about 40°C.

Analysing Fig. 1 we may conclude that the peak at 43°C (Fig. 1B) represents the melting of alkali light chains associated with S1 heavy chain. Alkali light chains complexed with S1 melt cooperatively (Fig. 1B), whereas isolated alkali light chains melt with low cooperativity (Fig. 1C). It is worthwhile to mention that thermal denaturation may induce dissociation of alkali light chains from S1. Hamai and Konno [17] have shown that heating up to 44°C results in dissociation of alkali light chains. If dissociation of alkali light chains occurs after their thermal denaturation then the calorimetric peaks observed at 53.5°C (Fig. 1B) and at 51°C (domain 3, Fig. 1A) represent the melting of a certain region in the 20 kDa segment of S1 heavy chain, which already does not contain associated alkali light chain.

Acknowledgements: We are very grateful to Dr. S.M. Kuznetsova for help with thermal gel analysis experiments and to Prof. N.B. Gusev for stimulating discussions. This work was supported by an International Science Cooperation Grant, DMB 9003692, from the US, NSF and Russian Academy of Sciences.

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