Selective cleavage of the connector segments within the myosin-S1 heavy chain by staphylococcal protease

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The existence of the two connector segments linking the tryptic 50 kDa fragment of skeletal S1 heavy chain to the adjacent 27 kDa and 20 kDa peptides was ascertained by digestion of S1 with staphylococcal protease which was found to act specifically at these particular regions. Three new peptides of M_r 28000, 48000 and 22000 were produced and the novel S1 derivative formed had an intact actin-activated ATPase activity. Amino acid sequence analyses indicated that the 48 kDa and 22 kDa peptides overlap the two connector elements.

Myosin-S1 heavy chain *Connector segment* *Staphylococcal protease* *Peptide structure*

1. INTRODUCTION

The limited tryptic cleavage of the skeletal myosin S1 heavy chain produces the 3 major fragments of 27 kDa, 50 kDa and 20 kDa [1,2]. These are also formed upon tryptic digestion of the head part of other different vertebrate myosins [3–5]. This property has led to the suggestion that the S1 heavy chain can be considered, in general, as a complex of these 3 peptide units which are covalently linked by two lysine-containing and vulnerable connector segments located at the surface of the protein [6]. The fragments could generate through their primary and/or tertiary structure, hinged functional domains within the myosin head [7]. The 27 kDa fragment is thought to contribute to nucleotide binding and bears in its C-terminal portion sequence homologies with other ATP-dependent enzymes [8]; the 20 kDa and 50 kDa peptides contain sites for actin attachment to S1 [9]. The two connector regions could serve as flexible joints involved in segmental movements of the head during myosin activity. The NH_2-terminal connector segment between the 27 kDa and 50 kDa peptides is specifically sensitive to the binding of nucleotides and to changes in ambiant conditions [10] whereas the C-terminal segment between the 50 kDa and 20 kDa regions is particularly sensitive to actin interaction. Actin prevents its cleavage by trypsin [2,11] and the 50 kDa–20 kDa cut inhibits the acto-S1 Mg^{2+}-ATPase [2] by reducing the actin affinity for the S1–ADP.P complex [12].

In order to assess the extent of the two boundaries formed by these connector segments and to further understand their role in heavy chain functions and conformation, we here investigated the fragmentation of rabbit skeletal myosin S1 with *Staphylococcus aureus* V8 protease which specifically splits peptide bonds on the COOH-terminal side of dicarboxylic amino acids [13]. The protease is shown to promote a highly selective cleavage of the heavy chain at the two connector areas producing new proteolytic fragments and S1 derivatives which can be useful for future studies on the structure and activity of the S1 molecule.

2. MATERIALS AND METHODS

Subfragment-1 from rabbit skeletal muscle

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myosin was isolated as in [14]. F-actin was prepared as in [15]. Digestion of S1 (5 mg/ml) with staphylococcal protease (Miles) was carried out at a weight ratio of protease to S1 of 1:50 in 50 mM Tris–HCl (pH 7.8) or in 100 mM potassium phosphate (pH 7.8), at 25°C. The reaction was quenched by mixing 25 μl aliquots of the digestion mixture with 175 μl boiling 5% β-mercaptoethanol–4% SDS solution. The fragmentation of S1 with trypsin was performed as in [2]. SDS–polyacrylamide gel electrophoresis was done in 5–18% polyacrylamide slabs [6]. The actin-activated Mg^{2+}-ATPase activity was measured as in [6]. The chemical cross-linking between F-actin and fragmented S1 was conducted in the presence of 1-ethyl-3-(3-dimethylamino)propyl) carbodiimide (EDC) as in [2,9].

The proteolytic fragments of S1 heavy chain were purified by gel filtration over Sephacryl S-300 under the dissociating conditions reported in [16]. Automated analysis of peptides was performed in a liquid phase Sequencer (Socosi). Phenylthiohydantoins were identified by high pressure liquid chromatography [17]; the peptides (100–150 nmol) were sequenced up to the twentieth residue.

3. RESULTS AND DISCUSSION

3.1. *Fragmentation of the S1 heavy chain with staphylococcal protease*

As illustrated in fig.1, the reaction of the bacterial protease with native chymotryptic S1 (A1 + A2) produces 3 heavy chain fragments with M_r 28000, 48000 and 22000. These peptides were issued from a specific hydrolysis of the S1 heavy chain by the protease and not from the action of a trypsin or trypsin-like contaminant within the protease preparation. The cleavage pattern was not affected by the presence of soybean trypsin inhibitor in the digestion medium. The electrophoretic mobilities of the protease-produced peptides are different from those corresponding to the 3 heavy chain tryptic peptides of M_r 27000, 50000 and 20000, used as markers.

Also, while the S1 heavy chain was entirely converted into these fragments by trypsin, a small fraction of the heavy chain was consistently observed to remain refractory to the staphylococcal protease even after a prolonged digestion time (2 h) or further treatment of the digest with fresh

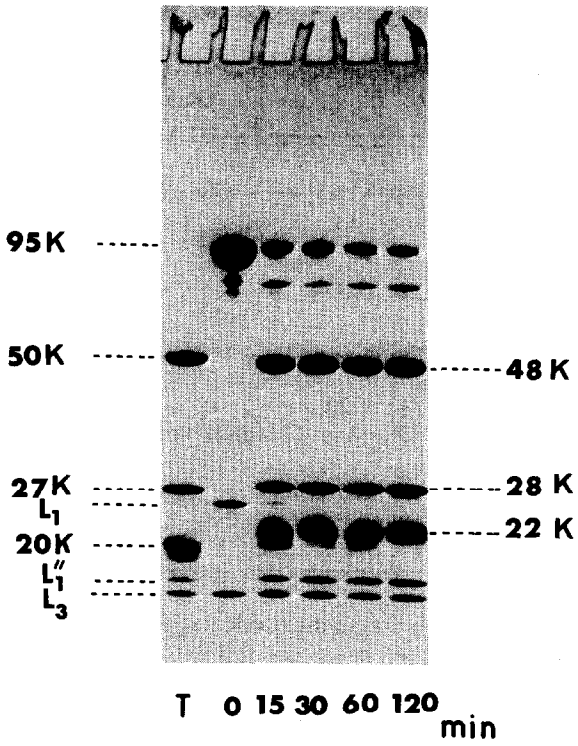

Fig.1. Time-course of the fragmentation of skeletal myosin–S1 with staphylococcal protease. Conditions: 45 μM S1 in 50 mM Tris–HCl, pH 7.8, 25°C; protease to S1 = 1:50 (w/w); T = trypsin-digested S1.

protease (final weight ratio of the protease to S1 = 1:25). This residual S1 could be in a different conformation and thus has a much lower susceptibility towards the bacterial protease. Finally the A_1 light chain was degraded into an apparently single product of 17 kDa; interestingly, most of the light chain remained intact when the reaction was conducted on the parent myosin.

The 28 kDa, 48 kDa and 22 kDa fragments were generated by a proteolytic cleavage at the two connector segments and not at other different regions of the heavy chain. A fluorescent 22 kDa band was produced when the digested S1 was labeled with 1.5 IAEDANS at the SH1 thiol group present within the C-terminal portion of the heavy chain [1]. The site of cleavage between the NH_2-terminal 28 kDa and 48 kDa peptides was established by analysis of the amino acid sequence of the NH_2-terminal 20 residues within the isolated 48 kDa peptide. This sequence was compared with the one we obtained in parallel for the isolated

Table 1

The NH_2-terminal sequences of the 48 kDa and 50 kDa fragments derived from the cleavage of rabbit skeletal S1 heavy chain with staphylococcal protease and trypsin

200 210
Ile–Thr–Gly–Asp–Lys———Glu–Ala–Thr–Ser–Gly–Lys–Met–Gln-
Val Asp
|← .....
.....27 kDa—→|
_____ 28 kDa _____ —→|← _____ 48 kDa _____
220 230
Gly–Thr–Leu–Glu–Asp–Gln–Ileu–Ileu–Ser–Ala–Asn–Pro–Leu–Leu–Glu–Ala–Phe–Gly-
.....50 kDa.....
_____ 48 kDa _____

tryptic 50 kDa fragment (table 1). The numbering of the sequenced segments follows that given for the complete sequence of the NH_2-terminal tryptic 27 kDa peptide (Elzinga, personal communication); the positioning of the analysed segments was made possible by the sequence homologies we observed between these segments and the corresponding regions within the nematode myosin heavy chain (Karn, personal communication).

The 27 kDa and 50 kDa fragments are joined by a peptide stretch of about 9 amino acids spanning residues 205–213. The COOH-terminal 5 residues of this connector segment are included in the NH_2-terminal part of the protease-produced 48 kDa peptide whereas it seems likely that the remaining N-terminal 4 residues make up the C-terminal portion of the 28 kDa fragment. The analysis of this region should allow the easy determination of the entire structure of the 27–50 kDa connector element of the skeletal S1 heavy chain. These data suggest that the 22 kDa fragment produced by the protease would necessarily overlap the tryptic 20 kDa peptide and the connector segment between the 48 dDa and 20 kDa components.

3.2. *Influence of heavy chain cleavage with staphylococcal protease on the acto–S1 ATPase activity*

As observed earlier with trypsin [2], we found that the bacterial protease, employed at a mild

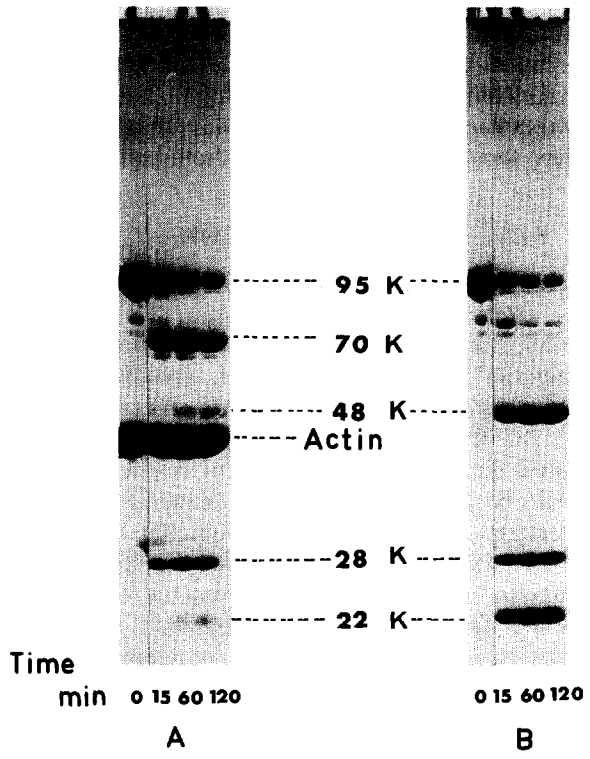

Fig.2. Actin protection against the cleavage of the 48–22 kDa connector segment by staphylococcal protease. Conditions: 45 μM S1 was digested in the presence (A) and absence (B) of F-actin (molar ratio actin/S1 = 2).

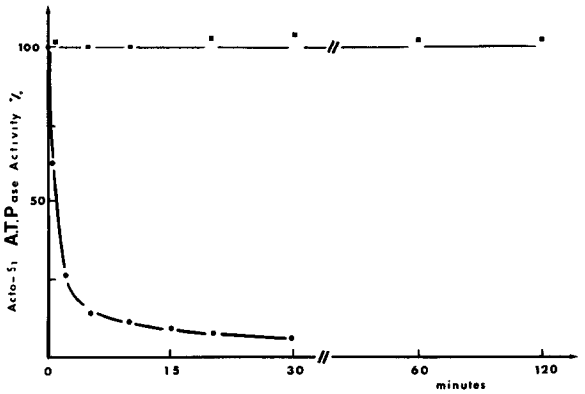

Fig.3. Comparison of the effects of S1 digestion with staphylococcal protease and trypsin on the actin-activated Mg^{2+}-ATPase. S1 was treated with the bacterial protease (■) and trypsin (●) as in fig.1.

concentration ($E/S = 1:50$), does not significantly hydrolyse the heavy chain between the 48 kDa and 22 kDa peptides when the S1 is complex to F-actin; only the 28 kDa–70 kDa joint is cleaved within the acto–S1 complex (fig.2). In addition, fig.3 shows that the 48–22 kDa cut does not affect the acto–S1 Mg^{2+} ATPase, in contrast to the tryptic scission of the 50–20 kDa region which induces the loss of this activity. As this inhibition is due to a decrease of the actin affinity for the S1–ADP.P complex [12] and since actin has lysine residues as binding sites in the NH_2-terminal region of the 20 kDa peptide [9,18], the 22 kDa fragment appears to contain intact, positively charged actin site and connector segment. As illustrated in fig.4, the chemical cross-linking between actin and the 22 kDa region occurs with a much higher efficiency than between actin and the 20 kDa segment. Thus, for the studies employing the fragmented S1 species, the protease-generated (28–48–22 kDa)–S1 seems to be a better derivative than the tryptic (27–50–20 kDa)–S1.

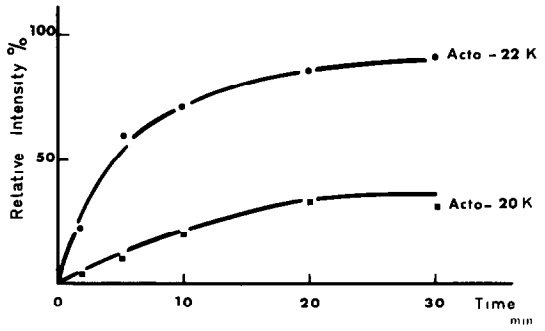

Fig.4. Time-course of the chemical cross-linking of actin to the 22 kDa and 20 kDa regions of the S1 heavy-chain. F-actin was cross-linked to the protease-derivative (28–48–22 kDa)–S1 and to the tryptic (27–50–20 kDa)–S1, in the presence of carbodiimide [9]; the amount of cross-linked actin-20 kDa and actin-22 kDa entities present on electrophoretic gels was measured by densitometry.

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