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The interaction of skeletal myosin subfragment 1 with the polyanion, heparin

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The association between chymotryptic skeletal muscle myosin subfragment 1 (S1) and the polyanion, heparin, was investigated as an experimental approach in probing the functional importance of the cationic sites on S1 and their involvement in ionic interactions within the myosin head during energy transduction. The direct binding of heparin, used at micromolar concentrations, and its influence on the structural and functional properties of S1 were followed by gel chromatography, electron microscopy, chemical cross-linking techniques and limited digestion studies.

1. The limited tryptic digestion of S1 showed that the presence of heparin, as well as of the homopolymer, poly-(L-glutamic acid) causes a specific structural change in the 50-kDa heavy chain region of S1 and accelerates the breakdown of this segment into a 45-kDa species by a proteolytic cleavage restricted to its COOH-terminal portion. Under similar experimental conditions, the binding of MgATP and MgADP to S1 led also to the 50-kDa → 45-kDa conversion, suggesting that the S1-nucleotide interactions exhibit some resemblances to the polyanion-S1 interactions; a distortion of the COOH-terminal peptide stretch of the 50-kDa fragment seems to result from the binding of polyanionic ligands to S1. This particular area is adjacent to the actin site containing the 45-kDa and 20-kDa segments of the S1 heavy chain. On the other hand, the polyanions as well as nucleotides induced changes in the interface between the heavy chain and the alkali light chains.

2. Moreover, the binding of heparin to S1 resulted in the self-association of the enzyme and the production of stable small S1 oligomers, most likely dimers, which were demonstrated by the alteration of the size of the S1 particles examined by electron microscopy and their freezing by chemical cross-linking agents. These findings are relevant to the recently reported property of skeletal chymotryptic S1 to form dimers under convenient ionic conditions, in particular in the presence of Mg-nucleotides. The interaction of cationic sites on S1 and possibly on the 50-kDa region of the heavy chain with polyanions promotes the dimerization of the S1 molecules. The binding of S1 to F-actin abolished S1 aggregation.

The association of polyanions, such as RNA, to myosin in the isolated state and in myofibrils has long been recognized [1–4]. Two specific features of the protein were found to be markedly affected by this interaction: these are the aggregation state of the myosin molecule and its actin-activated ATPase activity. Thus, the myosins from skeletal muscle and from tissues other than muscle combine and coprecipitate with polyribosomes at low salt concentration [5]. RNA, DNA and heparin induce the extensive linear aggregation of myosin from *Dictostelium amoebae* [6]. The effect of heparin is of particular interest as the binding of this polyanion to skeletal muscle myosin was first reported to inhibit the actin-stimulated ATPase activity without changing the Mg^{2+}- and Ca^{2+}-dependent ATPases of myosin alone; this inhibition is reversible and is not due to an increase in the ionic strength [7]. Additionally, synthetic polyanionic copolymers containing styrene, maleate, methylacrylate and acrylate were reported to bind to the myosin heads and to act as strong inhibitors of the activation of the myosin ATPase by actin; the inhibition effect was dependent on the number of carboxyl groups present within the polyanion [8]. It is likely that the interactions are largely electrostatic between the polyanions and clusters of cationic sites on the myosin head molecule.

Abbreviations. S1, myosin subfragment 1; 1,5-IAEDANS, *N*(iodoacetyl)-*N'*-(5-sulfo-1-napthyl)ethylenediamine.

Enzyme. ATPase, adenosine-5'-triphosphatase (EC 3.6.1.3).

As an energy tranducer enzyme, the S1 region of skeletal myosin is known to have specific ionic interactions at the interface with F-actin, involving negatively charged sites on the actin monomer and positively charged lysine side-chains on the 20-kDa and 50-kDa segments of the S1 heavy chain [9]. Furthermore, investigations with proton magnetic resonance spectroscopy have shown the notable contribution of surface-accessible lysine residues to the structure of the mobile domains within the myosin-S1 whose flexibility is decreases upon binding to actin; these residues are also part of anion binding sites located on the protein [10]. As judged from these observations, it is conceivable that electrostatic forces could be implicated in the transduction mechanism related to muscle contraction, by allowing conformational transitions to take place within the actomyosin ATPase and/or by favoring the intersite communication between the actin and nucleotide binding sites of S1 [11].

The study reported here was initiated to probe the influence of polyanions on the structure and function of the isolated chymotryptic S1 from rabbit skeletal muscle myosin. On using heparin, a naturally occurring polyanion, we found that it inhibits the actin-dependent activation of the Mg^{2+}-ATPase, at micromolar concentrations; it promotes specific changes in the structure of the actin-binding 50-kDa region of S1 heavy chain; it induces the self-association of S1 and its conversion into small oligomers.

EXPERIMENTAL PROCEDURE

Materials

Rabbit skeletal myosin was prepared according to Offer et al. [12]; subfragment 1 was prepared by chymotryptic digestion of myosin filaments [13] purified by gel filtration over Sephacryl S-200 and eluted with 50 mM Tris/HCl buffer pH 8.2. S1 solutions were dialyzed overnight at 4 °C against the appropriate buffer and were used within 2 days of preparation.

Rabbit skeletal muscle F-actin was obtained by the method of Spudich and Watt [14] as midified by Eisenberg and Kielly [15]. The protein pellet was resuspended in 10 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM $CaCl_2$ and 2 mM Tris/HCl buffer pH 8.0 containing 1 mM NaN3.

The trypsin derivative (27 + 50 + 20 kDa) S1 was prepared essentially as described by Mornet et al. [16]. Protein concentrations were determined by absorbance using $A^{1\%}_{280\,nm} = 7.5\ cm^{-1}$ for S1 [17] and $11.0\ cm^{-1}$ for actin [18]. Calculations were based on M_r of 115000 for [17] and 16000 for heparin [19,20].

The hydrochloride of dimethyl suberimidate was purchased from Pierce Chemical Company. Trypsin [treated with L-1-(tosylamido)-2-phenylethyl chloromethane] was obtained from Worthington Biochemical Corporation. Heparin, sodium salt, with anticoagulant activity of 140 IU/mg and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide were supplied by Fluka A.G. (Switzerland). Sephacryl S-300 was bought from Pharmacia (Uppsala) and poly(L-glutamic acid) M_r – 15000) was from Sigma Chemical Company (St. Louis, MO, USA). All other reagents were of analytical grade.

Cross-linking reactions on the S1-heparin complex

Native S1 was dialyzed overnight against 100 mM triethanolamine/HCl buffer, pH 8.3, and was reacted at 2 mg/ml, in the absence and presence of 1 μM heparin, with dimethyl suberimidate under the conditions described previously [21]; the heparin was dissolved just prior to use in the same buffer at a concentration of 15 mg/ml. At appropriate times, samples containing 0.030 – 0.050 mg were analyzed by gel electrophoresis carried out in 5 – 18% polyacrylamide slab gels in the presence of 0.1% sodium dodecyl sulfate [22]. Gels were stained with Coomassie brillant blue R-250 and destained according to Weber and Osborn [23]. The proportions of the protein in the different bands observed were determined by scanning the gels at 600 nm in a Joyce-Loebel densitometer. The areas under each peak of the scan, proportional to the amount of protein present, were traced for each component and the areas determined by planimetry.

Native S1 was also dialyzed overnight against 100 mM Mes buffer pH 6.0. It was incubated at 2 mg/ml with 5 mM 1-ethyl-3-(3-dimethylamino)propyl carbodiimide, added as solid, for 10 min at 20 °C, in the absence and presence of 1 μM heparin. The reaction was quenched by the addition of 2-mercapoethanol to a final concentration of 15 mM; the micture was immediately subjected to gel electrophoresis.

Tryptic digestion of the S1-heparin complex

The tryptic digestion of S1 (2 mg/ml) was carried out in the absence and presence of various concentrations of heparin, at a weight ratio of protease to S1 of 1:100 in 50 mM Tris/HCl, pH 7.5 at 20 °C. At suitable time intervals protein samples were withdrawn and analyzed by gel electrophoresis. After 30 min of digestion, the reaction was quenched by the addition of soybean trypsin inhibitor (twice the weight of trypsin). Carbodiimide-activated F-actin was prepared as described previously [9] and subsequently mixed with 5 vol of the S1 digest (actin:S1 molar ratio = 2); after 10 min at 20 °C, the cross-linking reaction was terminated by the addition of 2-mercaptoethanol to 15 mM. The mixture was immediately submitted to gel electrophoresis.

Elution of the S1-heparin complex over Sephacryl S-300

The molecular mass of the heparin-induced S1 aggregates was estimated by gel filtration of S1 and heparin mixtures on a column of Sephacryl S-300 (2.8 × 150 cm) equilibrated with 50 mM Tris/HCl pH 7.5 and calibrated with the following markers: albumin (M_r 64000), catalase (232000), thyroglobulin (660000) and dextran blue.

Turbidity measurement

The influence of heparin on S1 binding to F-actin, in the absence and presence of ATP, was monitored by turbidity at 400 nm, according to Wagner and Weeds [24]. The experiments were made at 20 °C, in a final volume of 2.5 ml, with a Turner model 430 spectrofluorimeter. S1 (0.250 mg/ml, 2 μM) was mixed with F-actin (0.250 mg/ml) in 50 mM Tris/HCl, 10 mM KCl, 1.5 mM $MgCl_2$, pH 8.0. Heparin was used in the concentration range of 1 – 10 μM. When present, ATP was added at 0.1 mM.

ATPase measurements

The steady-state actin-activated Mg^{2+}-ATPase was determined in a medium (1 ml) containing 10 mM KCl, 50 mM Tris/HCl (pH 8.0), 2.5 mM $MgCl_2$, 2.5 mM ATP [25]. The concentration of F-actin and heparin were varied as indicated. The addition of S1 (0.050 mg) was used to start the activity assay. The Mg^{2+}- and Ca^{2+}-ATPase activities were measured as specified earlier [25].

Preparation for electron microscopy

Myosin S1 and heparin S1 complex were visualized indirectly by the mica replication technique [26] in the presence of 50% glycerol or directly by the freeze-drying technique [27] after unidirectional or rotary shadowing with Ta/W at low elevation angle.

RESULTS

Heparin-induced change in the structure of the actin-S1 complex and inhibition of the acto-S1 Mg^{2+}-ATPase

The addition of S1 to F-actin in the absence of nucleotide induces a typical rise of the turbidity of the solution to a plateau representing the associated state of S1 with actin. When the acto-S1 solution was supplemented with Mg-ATP, there was an immediate decrease in turbidity to a plateau, the duration of which is dependent on the rate of ATP hydrolysis at fixed S1 and actin concentrations. When the ATP was completely hydrolyzed, the turbidity rose quickly to a plateau corresponding to the reformation of the actin-S1 complex. Upon adding heparin (10 μM), this turbidity dropped steeply

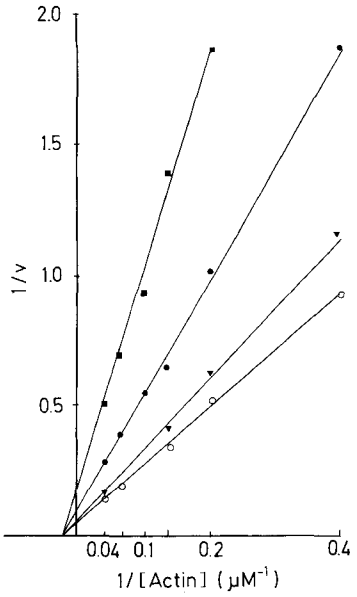

Fig. 1. *Double-reciprocal plots of acto-S1 MgATPase activity at various heparin concentrations.* The conditions were as described in Experimental Procedure. The assays were carried out in the absence (0) and presence of heparin: 0.5 μM (▼), 1 μM (●) and 1.5 μM (■). $1/v$ in arbitrary units

to a constant value equal to about 50% of the initial value. In the range of heparin concentrations investigated (1 – 10 μM) the extent of turbidity decrease was dependent on the concentration of heparin. When the acto-S1 – heparin mixture was supplemented with MgATP, the turbidity declined further to a plateau, the amplutude of which was similar to that of the plateau produced by the ATP-dissociated acto-S1 solution without heparin; however, the duration of this second plateau was significantly larger. After exhaustion of the ATP, the turbidity increased to a final plateau similar to that observed before addition of the nucleotide. In order to assess whether the heparin-induced decrease of the turbidity of the acto-S1 complex was the result of S1 release from F-actin, chemical cross-linking studies [9] and sedimentation experiments were carried on actin-S1 in the presence of heparin. They indicated that practically all the S1 remained bound to actin in the presence of heparin. On the other hand, examination of the actin-activated Mg-ATPase activity of S1, obtained as a function of increasing F-actin concentration, in the presence of micromolar concentrations of heparin (0.5 – 2 μM) showed that the polyanion acts as a strong uncompetitive inhibitor of the acto-S1 ATPase by decreasing the maximal turnover rate (V) without changing significantly the apparent affinity of S1 for actin (K_{app}) (Fig. 1). A K_i value of 0.25×10^6 M^{-1} was calculated. Under the same conditions of ATPase activity measurements, heparin had no effect on the elevated Mg-ATPase of the carbodiimide-cross-linked acto-S1 complex [9] nor on the Mg-ATPase of S1 alone.

Influence of heparin on the structure of the S1 heavy chain

To assess the interaction of heparin with S1 and its impact on the structure of the myosin head, we investigated the tryptic susceptibility of S1 in the presence of micromolar concentrations of the polyanion. Fig. 2A shows that heparin did not alter the specific scission of the heavy chain at the two connector segments linking the three fragments of 27 kDa, 50 kDa and 20 kDa which make up the S1 heavy chain polypeptide [22,28]. Also, the proteolytic sensitivity of the two alkali light chains was not changed except that the tryptic degradation of the L1 light chain was somewhat accelerated in the presence of heparin. However, while the 27-kDa and 20-kDa peptides remained fairly stable, the band intensity of the 50-kDa fragment decreased progressively with the concomitant appearance of a smaller entity of 45 kDa when heparin was present in the reaction medium. Only a trace amount of the latter species was formed in the digest without heparin. The addition of Mg^{2+} decreased but did not abolish the 50-kDa→45-kDa conversion. Interestingly, Fig. 2B shows that the 45-kDa species was also produced upon tryptic digestion, under similar conditions, of the binary S1-ATP and S1-ADP complexes as recently reported by Hozumi [29]. A major differential feature of the nucleotide-containing digests was the specific breakdown of the 27-kDa fragment into a 22-kDa derivative. The 45-kDa peptide band was formed not only in the presence of heparin but also in the presence of a micromolar concentration of poly(glutamic acid) (Fig. 2C). This polyanion also induced the production of two other fragments of 31 kDa and 29 kDa. The observed amount of the latter peptides was smaller than that corresponding to the major 45-kDa entity.

All the new tryptic peptides generated by the two polyanions employed were issued from the 50-kDa region of the S1 heavy chain and could be cross-linked to actin in the presence of carbodiimide similarly to the residual 50-kDa fragment (Fig. 3A). The remain associated to the F-actin pellet upon treatment of the cross-linking reaction mixture with Magnesium pyrophosphate (Fig. 3A, lanes c and d). They did not result from a new proteolytic cleavage at the 20-kDa-fragment-containing COOH-terminal portion of the S1 heavy chain as they incorporated no fluorescence when the S1 was labeled with 1.5-IAEDANS at the SH1 thiol group of the 20-kDa fragment [28]. Like the 45-kDa peptide produced by the addition of the polyanions to S1, the 45-kDa material formed in the presence of nucleotide was also readily cross-linked to actin giving rise to a new band corresponding to the actin – 45-kDa-peptide species (Fig. 3B). Furthermore, in a parallel study, we have isolated the ATP-induced 45-kDa peptide by gel filtration under denaturing conditions [30] and we have analyzed its NH_2-terminal amino acid sequence as far as the 20th residue; the peptide was initiated by the sequence Met-Gln-Gly-Thr-Leu which was identical to that recently reported from this laboratory for the parent 50-kDa fragment [31]. Therefore, the 45-kDa fragment resulted from a tryptic cleavage occurring at the COOH-terminus of the 50-kDa segment. These findings suggest that the binding of the nucleotide substrates and the tested polyanions to S1 induces conformational changes in the 50-kDa heavy-chain segment with a special alteration of the tryptic sensitivity of its COOH-terminal portion.

Heparin-induced self-association of S1

A simple approach for illustrating the S1-heparin interaction was provided by the elution profiles of the enzyme obtained upon chromatography of S1-heparin mixtures over a calibrated Sephacryl S-300 column (Fig. 4). When an S1 sample was mixed with heparin just before gel filtration, its elution profile changed as compared to S1 without heparin, used as control. At a molar ratio of heparin:S1 = 1:8, most

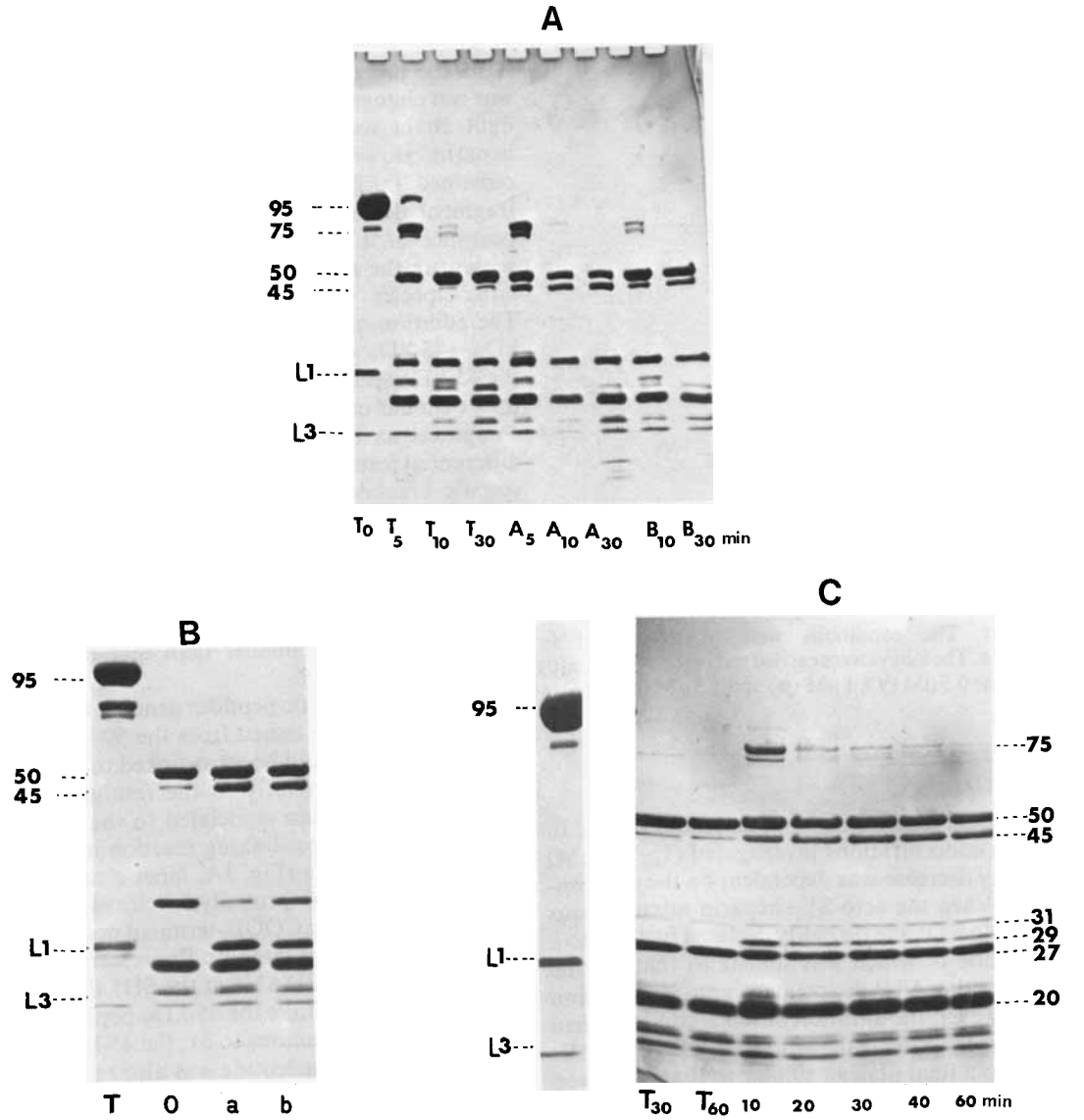

Fig. 2. *Comparison of the influence of polyanions and nucleotides on the cleavage of S1 heavy chain with trypsin.* (A) Time course of the digestion of S1 in the absence (T) and presence of heparin at concentrations of 1 μM (A) and 0.1 μM (B). S1 (2 mg/ml) was treated with a trypsin/S1 weight ratio of 1:100 in 50 mM Tris/HCl, pH 7.5, 20 °C. (B) S1 digested by trypsin at pH 7.5, for 15 min, in the absence (0) and presence of 5 mM MgATP (a) and 5 mM MgADP (b); a protease/enzyme weight ratio of 1:20 was used. T = native S1. (C) Time course of the tryptic digestion of S1 in the presence of poly(L-glutamic acid). The concentration of the polyanion was 1 μM and the proteolytic conditions were the same as those used with heparin; digestion controls (T) were performed at the interval times of 0, 30 and 60 min. The digests were analyzed by electrophoresis on a 5 −18% polyacrylamide gradient slab gel. Numbers at the sides represent molecular mass in kDa

of the S1 emerged prior to the control and about 20% of the protein material was excluded in an area suggesting a molecular mass of approximately 280 kDa. When the molar ratio of heparin:S1 was increased to 1:4, a large proportion of the S1 eluted as a 280-kDa species. Thus, heparin seems to remain tightly bound to S1 during gel filtration and alters its conformation in a way which induces the aggregation of S1 to higher-molecular-mass forms.

The association of the S1 molecules caused by heparin was further directly demonstrated by chemical cross-linking experiments and elctron microscopy. Fig. 5 shows the time course of the reaction of S1 with the cross-linking agent, dimethyl suberimidate, in the absence and in the presence of 1 μM heparin. In addition to the production of the 120-kDa band corresponding to the intramolecular cross-linking of the 95-kDa heavy chain to the alkali A1 and A2 light chains [32], there was formation of a new entity with an apparent mass of 275 kDa when heparin was added to the reaction medium. Furthermore, the presence of heparin markedly influenced the extent of cross-linking between the heavy and alkali light chains: as illustrated in Fig. 6A, the amount of the 120-kDa band formed in the presence of the polyanion was reduced to about 50% of the original value measured in the absence of heparin. The comparative estimation of the amount of residual A1 and A2 light chains suggested that this decrease could be accounted for by a preferential reduction of the cross-linking of the A1 light chain (Fig. 6A, inset). For comparison, the influence of nucleotides on the production of the 120-kDa species is shown also in Fig. 6B. Mg-ADP and Mg-ATP decreased the extent of the heavy-light chain cross-linking to 50% and 70% of the control, respectively; Magnesium pyrophosphate and Mg^{2+} alone did not change the cross-

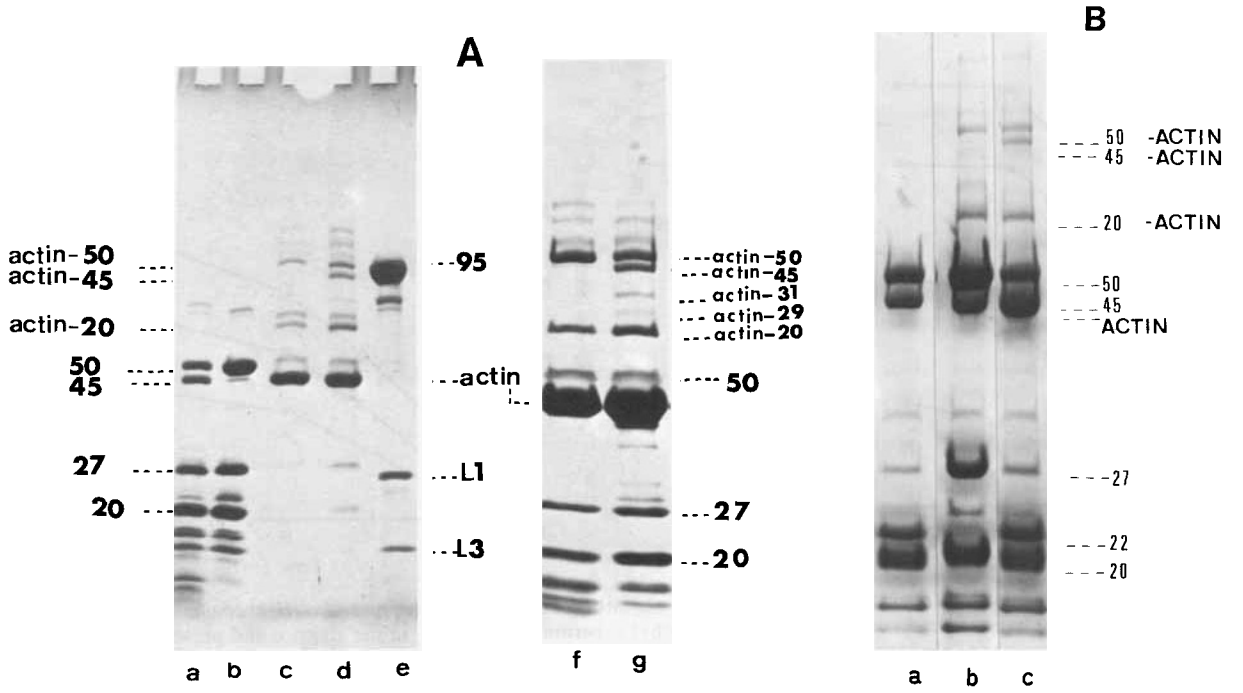

Fig. 3. *Cross-linking of F-actin to the tryptic peptides originated from the 50 -kDa segment of S1 heavy chain.* (A) Carbodiimide-activated actin (2(mg/ml) was cross-linked to S1 (2 mg/ml) fragmented in the absence (c, f) and presence of 1 μM heparin (d) or 1 μM poly(L-glutamic acid) (g); (a) and (b) were the starting digests of the S1-heparin complex and native S1 respectively, before addition of actin; (e) = native S1. The reaction mixtures corresponding to samples c and d were treated with magnesium pyrophosphate [9] before electrophoresis. The band just above 'actin-20' is, most likely, actin LCl-cross-linked product [38]. (B) Carbodiimide-activated actin was cross-linked to S1 fragmented in the presence of 5 mM MgATP (c); (a) starting digest of S1 obtained in the presence of nucleotide as described in Fig. 2; (b) digest of native S1 cross-linked to F-actin. The cross-linking reactions were carried out as reported in Experimental Procedure and were analyzed by electrophoresis on 5–18% polyacrylamide gradient slab gel. Numbers on the sides represent molecular mass in kDa

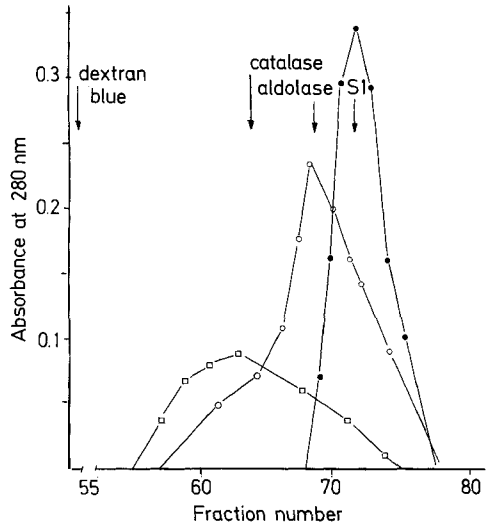

Fig. 4. *Elution pattern of S1-heparin mixtures from Sephacryl S-300.* The column (2.8 × 150 cm) was loaded with 2 ml of S1 (10 mg/ml) containing 10 μM heparin (○) and with 2 ml of S1 (7 mg/ml) containing 14 μM heparin (□); 2 ml of native S1 (10 mg/ml) were also placed on the same column (●). Elution was carried out with 50 mM Tris/HCl, pH 7.5 at 4 °C; the fractions (4.0 ml) were monitored at 280 nm

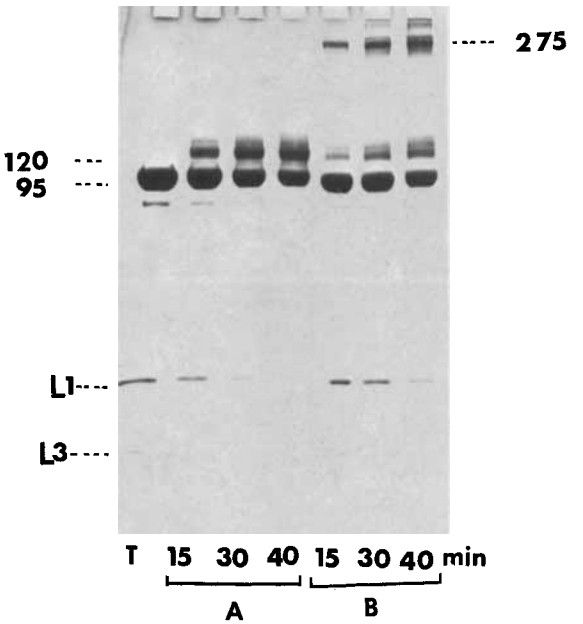

Fig. 5. *Cross-linking of associated S1 molecules with dimethyl suberimidate.* S1 (1.6 μM) was reacted in 100mM triethanolamine/HCl, pH 8.3, at 20 °C, with 75-fold molar excess of dimethyl suberimidate, in the absence (A) and presence (B) of 1 μM heparin. At the times indicated, the reaction was quenched by the addition of glycine to 100 mM. Samples were subjected to electrophoretic analysis on a 5–18% gradient acrylamide slab gel. T = native S1. Numbers on the sides represent molecular mass in kDa

linking. We did not determine whether the cross-linking of the two light chains was differently affected by the nucleotides. The cross-linking of the S1-heparin complex was performed under conditions which avoid the production of non-specific protein aggregates; therefore, the extent of the 275-kDa band

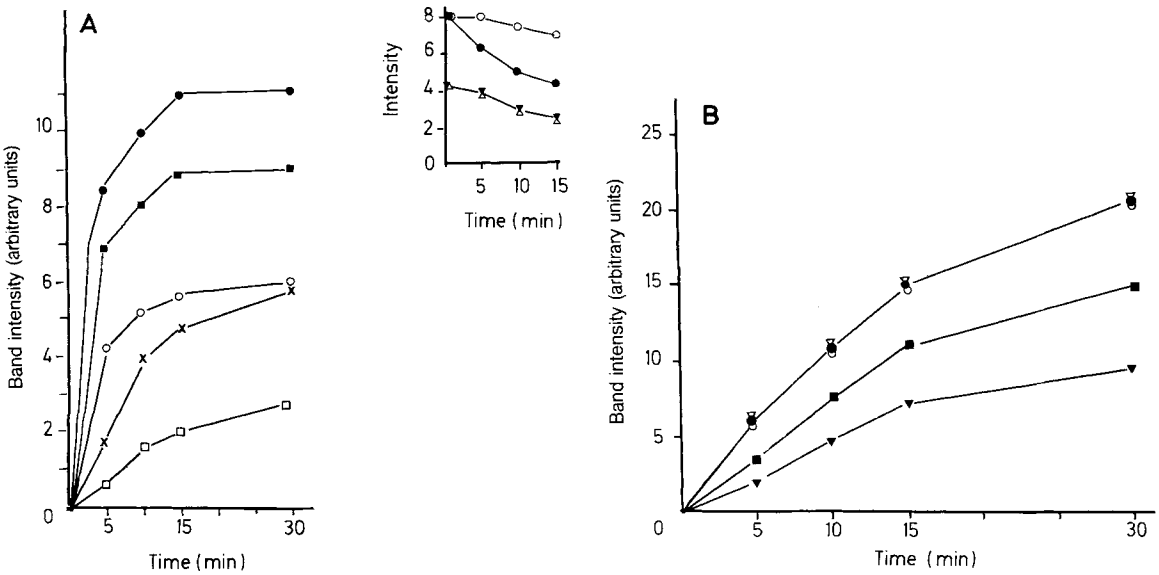

Fig. 6. *Influence of the polyanions and nucleotides on the extent of cross-linking between the heavy and alkali light chains of S1.* (A) The intensities of the 120-kDa and 275-kDa bands formed during reaction of dimethyl suberimidate with S1, in the absence and presence of polyanions (1 μM), were estimated by densitometry of electrophoretic slab gels (5 – 18% gradient acrylamide). (●) 120-kDa band concentrations in the absence of polyanions; (O,X) 120-kDa band concentrations in the presence of heparin and poly(L-glutamic acid), respectively, (■, □) 275-kDa band concentrations in the presence of heparin and poly(L-glutamic acid) respectively. Inset: estimation of the A1 light-chain band intensities during reaction of the cross-linker with S1 in the presence (O) and absence (●) of heparin; the corresponding A2 light-chain band concentrations were also measured (△, ▲). (B) The intensities of the 120-kDa band formed during reaction of dimethyl suberimidate with S1, in the absence (●) and presence of 5 mM Mg-ATP (■), 5 mM MgADP (▼), 5 mM $MgCl_2$ (O) and 5 mM magnesium/pyrophosphate (▽).

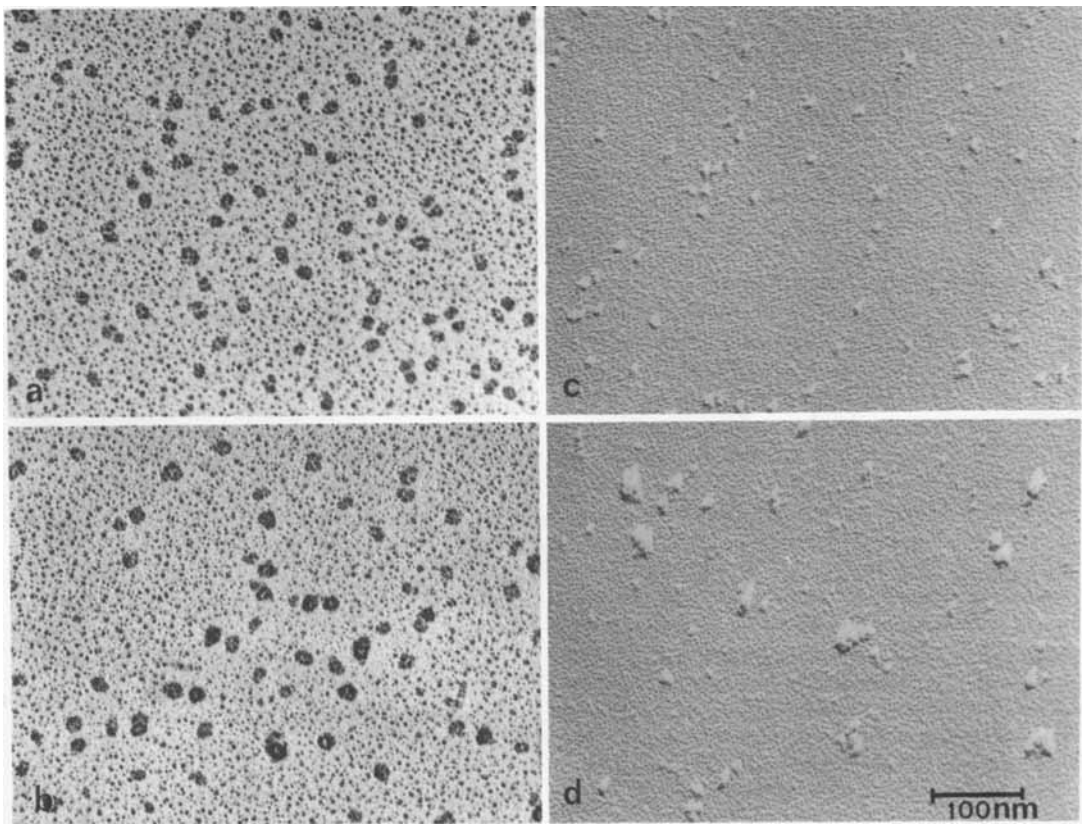

Fig. 7. *Electron micrographs of native S1 and S1-heparin complex.* Samples of S1 (2 mg/ml) (a,c) and S1 mixed with 4 μM heparin (b,d) in 50 mM Tris/HCl, pH 7.5 were diluted to 10 – 25 μg/ml with 40 mM ammonium acetate containing 50% glycerol (a, b) or with 40 mM ammonium acetate without glycerol (c, d). Samples a and b were sprayed on freshly cleaved mica, dried *in vacuo* and rotary shadowed with Ta/W at a 6° elevation angle. Samples c and d were sprayed directly onto carbon-coated electron microscope grids, washed with water, quick-frozen in supercooled liquid nitrogen and then freeze-dried *in vacuo*. The grids were unidirectionally shadowed with Ta/W at a 15° elevation angle. Magnification × 130000

leveled off after 15–20 min of reaction, being limited by the original concentration of reagent. This is supported by the fact that the production of the 120-kDa band, due to the intramolecular cross-linking between the heavy and alkali light chains, followed the same course. The 275-kDa species comprised the intact 95-kDa heavy chain as no such band was observed with the tryptically digested (27 + 50 + 20 kDa) S1. The 275-kDa band was due, most probably, to an intermolecular cross-linking between two or three associated S1 molecules and not to the cross-linking between S1 and a number of heparin molecules, as the polyanion is known to contain very few free amino groups [33]; the 275-kDa species is also formed when the cross-linking reaction is catalyzed by another protein-protein cross-linker, 1-ethyl-3(3-dimethylamino-propyl)carbodiimide, or when the S1 was supplemented with the other polyanion tested, poly(glutamic acid). However, much less of the 275-kDa material was formed in the presence of the latter ligand whereas it inhibited the heavy-chain–light-chain cross-linking to the same extent as heparin (Fig. 6A). The addition of F-actin suppressed the production of the 275-kDa band.

Finally, an electron microscopical analysis of S1, in the absence and presence of heparin, was carried out using rotary and unidirectionally shadowed protein preparations sprayed in the presence of glycerol onto mica or freeze-dried on carbon grids (Fig. 7). S1 alone was uniformly distributed as globular and sometimes pear-shaped molecules. The dimensions of rotary shadowed S1 control were 14.8 ± 1.5 nm long axis and 7.5 ± 0.6 nm small axis (25 molecules measured): they are in accord with those reported previously for S1 without LC2 light chain [34]. The dimensions for the heparin-S1 complex were 22.5 ± 2.9 nm long axis and 8.5 ± 0.7 nm small axis (17 molecules measured). Thus, in the presence of heparin, the S1 particles were larger; clusters of two or three globules were also observed.

DISCUSSION

The experiments described in this paper show a number of interesting features of the direct association of myosin-S1 with heparin. The polyanion apparently interacts with high affinity with the isolated S1 and affects several of its structural and functional properties.

The heparin-S1 complex was resistant to gel chromatography like the complexes described for a number of other heparin-binding enzymes [35,36]. In the latter cases the polyanion was shown to remain bound to the eluted protein fractions [35]. One supposed mechanism by which polyelectrolytes may exert their action on the target protein involves conformational changes which alter the association-dissociation equilibrium of the protein subunits or induce the intermolecular aggregation of the protein [35–37]. Thus since S1 has an oligomeric and multidomain structure, its interaction with the polyanions would also help to assess the conformational transitions in the enzyme.

The addition of heparin or poly(glutamic acid) to S1 markedly decreased the extent of cross-linking of the heavy chain to the alkali light chains, especially A1. A similar effect also resulted from the binding of Mg-nucleotides to S1. These results suggest that changes in the interface between the heavy and light subunits of S1 were promoted by its interaction with the polyanions and nucleotides. The influence of the nucleotides on the cross-linking between the heavy and light chains is understandable as the cross-linking involves the N-terminal 27-kDa heavy-chain segment [32]. This region also participates in the binding of the adenosine moiety of the nucleotides [38–40]. Moreover it is specifically converted by trypsin into a 22-kDa entity upon binding of nucleotides to S1. Heparin did not seem to interact directly with the 27-kDa heavy-chain segment as it neither changes the proteolytic susceptibility of this region nor modifies the ATPase activity of S1 alone. In contrast, the comparison of the data related to the tryptic degestion of S1 in the presence of MgATP or ADP and in the presence of heparin or poly(glutamic acid) further suggest that the myosin-head–nucleotide interactions bear some resemblaces to the polyanion–S1 interactions. The binding of the two kind of ligands had a similar effect on the proteolytic susceptibility of the 50-kDa segment of the S1 heavy chain. The 50-kDa region is involved in the attachment of S1 to F-actin [9,21,41]. A portion of it is also close to the ATP binding site [42]. The COOH-terminal peptide stretch in the 50-kDa peptide, whose tryptic digestion was accelerated in the presence of both nucleotides and polyanions lies adjacent to the actin-binding 45-kDa and 20-kDa regions of the S1 heavy chain [9]. The conformation of this particular part of the 50-kDa fragment appears to be sensitive to the binding of nagatively charged ligands. Therefore, one anticipates that heparin would be able to affect the F-actin–S1 interaction. The addition of heparin to the rigor acto-S1 complex did change the turbidity of the solution but, in contrast to ATP or pyrophosphate, it did not cause the dissociation of the acto-S1 complex, under the conditions we emploeyd. The polyanion appears to affect rather the conformation of the attached S1 particles. In this regard, the interaction of ATP with acto-S1 undoubtedly exhibits an additional factor of specificity. Although heparin did not compete with F-actin for binding to S1 in the presence of ATP, it decreased the turnover rate of the acto-S1 Mg-ATPase. This effect was not produced by an alteration of the structure of the ATPase site since the enzymatic activities of S1 alone and of the covalent avto-S1 complex were unaffected. It could be due to a decrease in the rate of product release from S1-heparin complex reversibly bound to F-actin. The change in this rate-limiting step of the acto-S1 ATPase could be consequent on the ionic binding of the polyanion to the 50-kDa fragment of the S1 heavy chain, which in turn promotes changes in the 50-kDa-fragment–F-actin interface or in the communication pathway between the actin and nucleotide binding sites. Recently, monovalent anions and ATP were found to bind to allosteric sites in S1, different from the hydrolytic site, but linked to the acto-S1 interaction zone, with specific inhibition of the acto-S1 ATPase [43]. These anions affected K_m but not V for actin activation.

Finally, the results of three different approaches, namely Sephadex S-300 gel filtration, chemical cross-linking and electron microscopy, have clearly illustrated the formation of S1 oligomers in the presence of micromolar concentrations of heparin. According to the cross-linking data, most of the S1 material was associated as dimers or trimers. The tendency of native chymotryptic skeletal myosin-S1 to form head-to-head dimers in aqueous medium was recently reported [44]; it was also found that the binding of Mg-nucleotides and other Mg-phosphate compounds strongly favors the dimeric state [44]. The exact mechanism of such an auto-association of S1 is still unknown but it could involve a definitive change in the ionic structure of S1. It is conceivable that heparin shifts the monomer-dimer equilibrium by stabilizing the dimer form. The effect of heparin required a definite charge density on the

polyanion because, as expected, the addition of Mg^{2+} (5 mM) leads to the suppression of S1-S1 cross-linking. It is also noteworthy that under similar conditions, the reaction of dimethyl suberimidate on S1 in the presence of Mg-nucleotides did not result in the formation of apparent S1-S1 cross-linked species. The addition of F-actin, both in the presence and absence of Mg^{2+}, abolished the S1 association. However the interaction of S1 with heparin in the presence of the divalent cation was still effective as indicated by the turbidimetric and acto-S1 ATPase measurements.

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