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Conformational Changes of S-1 Related to its Dissociation from Actin*

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The peptide pattern obtained after proteolysis of S-1 with trypsin was different in the absence or presence of anions. The affinity of tryptic and undigested S-1 for anions (CN^-, SCN^- or HCO_3^-) was different, as reflected by the altered values of Ki or Ka obtained from ATPase activity measurements. Anions CN^-, SCN^-, HCO_3^-, or PPi induced dissociation of actomyosin when added to acto-S-1 or acto-heavy-meromyosin. Among nucleoside di- and triphosphates, only triphosphates were effective with regard to the dissociation. The results suggest the existence of a regulatory site of cationic nature on S-1, which might be involved in the dissociation of actin from myosin.

Key words: S-1, Myosin, Actin, Dissociation, Proteolysis, Regulatory site.

The contractile protein myosin, the major component of the thick filament, consists of two high molecular weight subunits, the myosin heavy chains (200 KDa), and four low molecular weight subunits, the light chains (15 and 27 KDa) (48).

Two structurally and functionally distinct regions of the heavy chain can be identified and isolated after the treatment of myosin with chimotrypsin (49). These two subfragments are the NH_2-terminal portion of myosin, called subfragment 1 (S-1, 95 KDa) and the COOH-terminal half, known as the rod. The S-1 fragment contains the sites responsible for its ATPase activity and the binding of actin (35).

The interaction of actin with myosin heads, and the actin dependent activation of the myosin Mg^{2+}-ATPase are essential events of the mechanochemical transduction in muscle contraction. The local changes in the S-1 conformation due to nucleotide or actin binding results in a change in actin or nucleotide affinity (20, 42), altough the manner in which their

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sites of binding interact remains obscure. Limited treatment of isolated S-1 with tripsin produce mainly three peptide fragments of 25, 50 and 20 KDa (4, 32) which remain associated under non denaturing conditions (18).

Tryptic cleavage of S-1 does not have a significant effect on some of the activities (55), but it lowers its affinity for actin (8) and decreases the actin activated ATPase activity (19).

Alterations in the tryptic fragmentation pattern of the S-1 or heavy meromyosin in the presence of actin, nucleotide or metal have been shown by several investigators (1-3, 29, 32, 36, 53, 54) and indicate that structural changes take place in the myosin head upon its interaction with those compounds. Also, conformational changes that occur in the 50 KDa region or S-1 as a consequence of mild thermal treatment affect the peptide pattern after limited tryptic proteolysis (39).

Several compounds of anionic nature (CN^-, SCN^- or HCO_3^-) also interact with myosin, affecting its structural and functional properties. The activating effect of dinitrophenol on myosin Mg^{2+}-ATPase activity (11, 17) could be due to conformational changes of the protein (25). It has been suggested that dinitrophenol acts by competing with ATP for a site different from the ATPase active site and from the binding site for actin (26, 51).

SCN^- modifies the ATPase activity of myosin (7) competing with ATP for the same non-catalytic binding sites of myosin (23). BALISH and DREIZEN (5) have reported that SCN^- and ATP occupy the same allosteric sites in S-1, with specific inhibition of the S-1 ATPase.

Several authors have investigated the effect of polyanions, such as heparin, and have found that the binding of this compound to myosin or S-1 inhibits the activation by actin of the Mg^{2+}-ATPase without affecting the Mg^{2+}-or Ca^{2+}-dependent ATPases (6, 22). LABBÉ *et al.* (22) have reported that heparin causes specific changes in the structure of the 50 KDa region of S-1.

The results presented in this report suggest that the dissociation of the actomyosin complex could be induced through the interaction of ATP with a regulatory sites different from the active sites.

Materials and Methods

Chicken skeletal myosin and actin were prepared by the methods of RICHARD *et al.* (38) and SPUDICH and WATTS (41) respectively. S-1 was obtained by digestion of myosin with chymotrypsin following the technique of WEEDS and TAYLOR (49).

Protein concentration was determined as described by LOWRY *et al.* 28. Proteolysis of S-1 was carried out in a reaction mixture containing: 40 mM NaCl, 25 mM imidazol pH 7, S-1 (2 mg/ml). The weight ratio of trypsin to S-1 was 1:100. Digestion was made at room temperature and terminated by the addition of soybean trypsin inhibitor at twice (w/w) the trypsin concentration.

At certain intervals samples were withdrawn and prepared for gel electrophoresis by adding an equal volume of 62.5 mM Tris-HCl, pH 6.8, 2 % Na-SDS, 10 % glicerol, 50 mM dithiothreitol, 0.001 % bromphenol blue, and heating in boiling water for 2 minutes. SDS/PAGE was carried out according to LAEMMLI and FAVRE (24). The relative intensity of the Coomassie bluestained bands were assessed scanning the gels in a densitometer. Molecular weights of the fragments were estimated by comparing their electrophoretic mobilities to those of marker proteins.

Ca^{2+}-ATPase activity of S-1 was calculated from the inorganic phosphate released (14) in 0.1 M Tris-acetate, pH 6.5 and 2 mM Ca-ATP (27).

Binding of S-1 to actin was measured, at room temperature, in a reaction mixture (1 ml) containing: 3 mM imidazol HCl

(pH 7), 3.3. mM $MgCl_2$, 1 mM KCl, 3.0 mM ATP, 8.5 μM S-1, 11 μM actin. Turbidity was followed by 340 nm in a spectrophotometer Perkin Elmer 650-40 (44).

Results and Discussion

Contraction of skeletal muscle is generally accepted to be caused by the relative sliding of the thin filaments past the thick filaments, coupled to the cyclic interaction of S-1 with actin and ATP. It is known that the binding of actin affects ATPase activities of myosin (13), and also that under certain conditions ATP prevents the binding of actin to myosin (43). Several reports have pointed out the presence of regulatory ATP binding sites on the myosin head (10, 15, 21, 34, 45-47), but it is not yet clear whether the ATPase sites and the nucleotide binding sites controlling the relaxation are the same. Adenylyl imidodiphosphate, a nonhydrolyzable analog of ATP, relaxes myofibrils and dissociates actomyosin (37, 57); this seems to indicate that the cause of relaxation is simply the binding of ATP and not its hydrolysis. In the present study we have attempted to examine this point further.

The ATP binding to myosin, and its hydrolysis, as well as the interaction of myosin with actin induce significant localized changes in the secondary and tertiary structures of S-1. An approach to elucidate the relationship between actin and the nucleotide sites of myosin has been the limited tryptic hydrolysis of S-1. The results suggest a three-domain structure for the myosin head where the 25, 50 and 20 K peptides are covalently connected by two protease sensitive regions (9, 31, 33).

Figure 1 shows the distribution of 95, 75, 50, 25 and 20 KDa fragments during the tryptic digestion. It can be seen that the S-1 fragment of 95 KDa is first split into 20 KDa and 75 KDa peptides, and then the 75 KDa peptide is further split into 25 KDa and 50 KDa peptides.

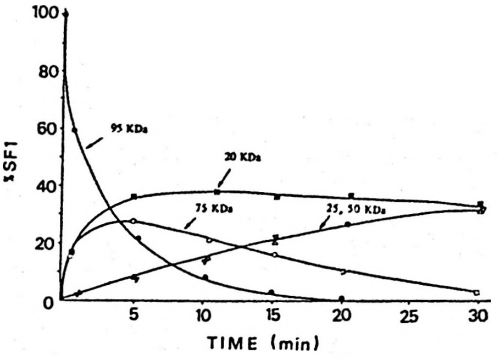
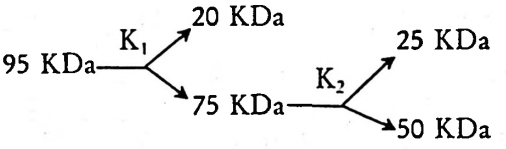


Fig. 1. *Distribution of different peptides during the tryptic digestion of S-1.*
Proteolysis was carried out in a reaction mixture containing, in a final volume of 1 ml: 40 mM NaCl, 25 mM imidazol pH 7. S-1 (2 mg), trypsin (200 mg), made at room temperature.

The kinetics of trypsinolysis of the S-1 fragment (95 KDa) fit into the following model



Proteolytic enzymes have been used to detect the structural changes induced by actin or nucleotide binding, since the access to the S-1 bond to be cut is modulated by the conformation of the vulnerable S-1 region (29, 36, 53). Whether the presence of anions affects the proteolytic pattern of S-1 has been further investigated. Figure 2 shows the time course of tryptic digestion of the S-1 in the presence of different anions. Comparison of the proteolytic patterns obtained indicates that CN^-, SCN^- or HCO_3^- did not affect the location of the cleavage sites but there were differences in the relative rates of cleavage at various sites. The presence of anions led to an increase of the relative rates of hydrolysis of protein 95 KDa (K_1) and of protein 75 KDa (K_2) (table I). The modification of the tryptic fragmentation

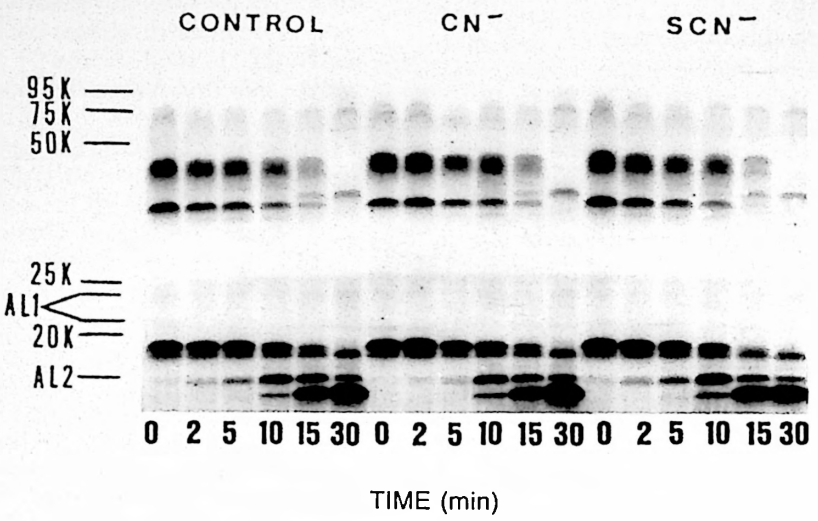


Fig. 2. *Effect of anions on the time course of tryptic digestion of S-1.*
Electrophoresis was on 14 % acrylamide slab gel. When present CN^- or SCN^- was 20 mM. The numbers shown beside each protein band are the relative molecular weights. AL1, alkali light chain 1; AL2, alkali light chain 2.

pattern by anions suggests that conformational changes occur upon their interaction.

The effect of anions on the ATPase activity of the untreated and trypsin-treated S-1 has also been studied. CN^- and HCO_3^- caused an inhibition and SCN^- an activation of Ca^{2+} —ATPase of both tryptic and undigested S-1 at the concentration used. Figure 3 shows the double reciprocals diagrams in which the values of $1/v$-v_o or $1/v_o$-v (v, initial velocity in the presence of anion and v_o in its absence) are represented on the y-axis, whereas the values of 1/[anion] are given on the x-axis. It may be seen that the straight lines obtained for undigested and tryptic S-1 intersect at the same point on the y-axis, thus indicating that the maximal effect was unchanged by tryptic cleavage. The constants of activation or inhibition for anions were calculated from the diagram described by EBEL and LARDY (12). The following Ki (Ka) values were obtained for undigested S-1: HCO_3^- (Ki, 12.8 mM) CN^- (Ki, 12.8 mM) SCN (Ka, 9.1 mM). The corresponding values obtained for tryptic S-1 were: HCO_3^- (Ki, 24.4 mM); CN^- (Ki, 25.0 mM); SCN^- (Ka, 16.7 mM). These results indicate that the affinity for anions decreased with tryptic

Table I. *Effect of anions on the rate constants K_1 and K_2 of tryptic digestion.*
The values of constants corresponding to the control were calculated from data shown in fig. 1. The values in the presence of anions were calculated from data obtained in similar experiments

Conditions	Rate constants ($\times 10^3\ s^{-1}$)	
	K_1	K_2
Control	2.5	1.3
HCO_3^- (5 mM)	3.3	1.6
CN^- (20 mM)	3.3	2.5
SCN^- (20 mM)	3.3	2.5

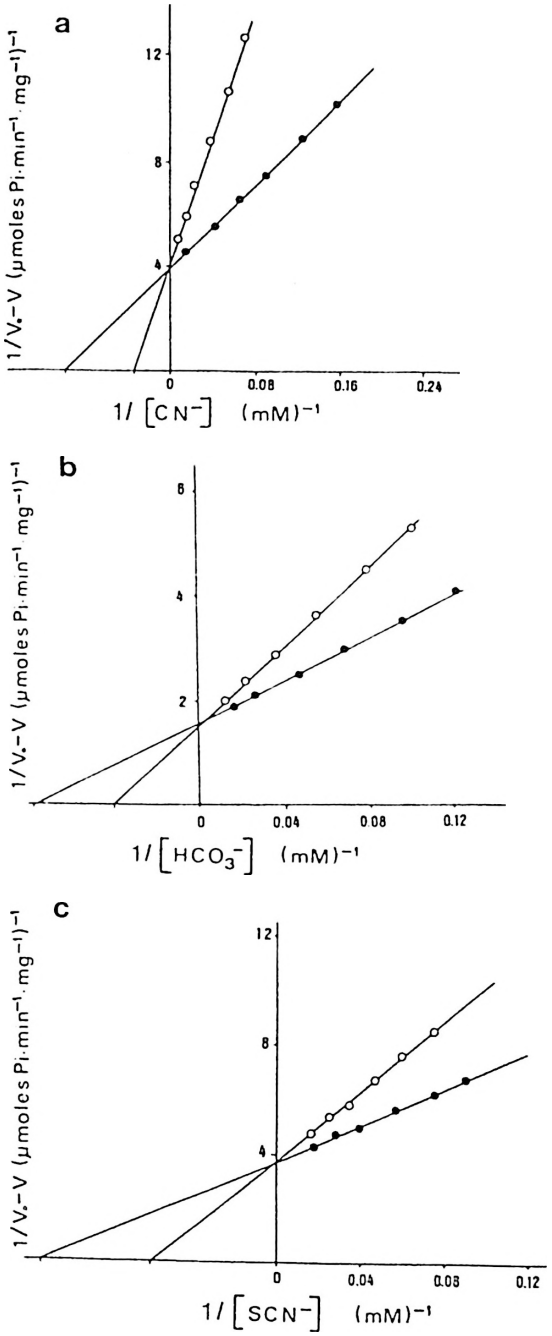


Fig. 3. *Effect of CN^- (a), HCO_3^- (b) and SCN^- (c) on Ca^{2+}-ATPase activity of tryptic (○) and undigested (●) S-1.*

cleavage. Probably, the anions bind to S-1 through specific sites, inducing selective conformational changes. Previous reports about the possible interrelation between nucleotide binding sites and anion binding sites indicate that anions bind to S-1 at sites different from those corresponding to the catalytic sites; moreover ATP, but not ADP, occupies the same regulatory sites as the anions (5, 22, 23, 27) whereas ADP binds to the active site.

Binding of ATP to S-1 dissociates actin from S-1. ATP-induced structural change (30, 50, 52, 56) alters the actin binding site and facilitates the dissociation of actin from its binding site.

In order to check whether the dissociation of actomyosin induced by ATP could be due to its interaction with the myosin head through the regulatory sites capable of binding nucleotides or anions, the effect of ATP, ADP and anions upon actin S-1 complex has been studied. Figure 4a, shows that addition of 1 mM ATP to actin S-1 complex caused a rapid drop in turbidity due to dissociation of the protein complex, followed by a recovery as the ATP is exhausted and the protein complex is reformed. The same effect was obtained with anions (5 mM HCO_3^-, 5 mM PPi, 20 mM CN^-, or 20 mM SCN^-), although no recovery was observed (fig. 4b), this being consistent with the fact that these anions remain unchanged during the experiment. One mM ADP did not induce dissociation (fig. 4c) in agreement with data of GREENE AND EISENBERG (16) and SLEEP and GLYN (40) in what refers to the binding constants of actin to S-1.

Based on the observations of our group and those of others (see above) indicating that ATP and anions, which occupy the same regulatory sites on S-1, are capable of causing dissociation, whereas ADP which binds to the catalityc sites is not, we suggest that the dissociation of the actomyosin complex could be induced through regulatory sites different from the active sites.

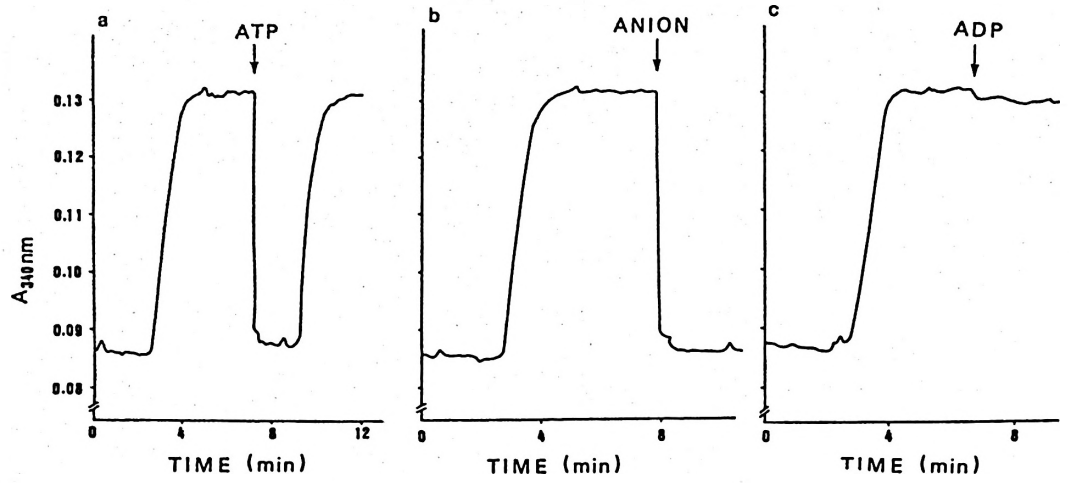


Fig. 4. *Time courses of the change in turbidity of S-1 induced by addition of ATP (a), anions (b) and ADP (c).*
Turbidity measurements were monitored continuously.

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Resumen

La unión de aniones a S-1 origina cambios conformacionales que modifican su sensibilidad a la proteolisis limitada por tripsina. La afinidad de S-1 sometida a hidrólisis por tripsina por los aniones (CN^-, SCN^- o HCO_3^-) es diferente a la que presenta en su estado nativo, como se refleja en los distintos valores de Ki o Ka, obtenidos en las medidas de actividad ATPasa. Los aniones CN^-, SCN^-, HCO_3^- o PPi inducen la disociación de actomiosina cuando se añaden a acto-S-1 o actomeromiosina pesada. Entre los nucleósidos di- y trifosfato sólo los trifosfatos son efectivos, en lo que se refiere a la disociación. Los resultados sugieren la existencia en S-1 de un sitio regulador de naturaleza catiónica, involucrado en la disociación de actomiosina.

Palabras clave: S-1, Miosina, Actina, Disociación, Proteolisis, Sitio regulador.

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