Nucleotide-Induced Changes in the Proteolytically Sensitive Regions of Myosin Subfragment 1†

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ABSTRACT: Limited proteolytic digestions of myosin subfragment 1 (S-1) with elastase, subtilisin, papain, and thermolysin yield fragments that correspond within 1–2K daltons to the 25K, 50K, and 20K fragments produced by trypsin. While papain and thermolysin cut preferentially at the 26K/70K junction, elastase and subtilisin cleave both the 26K/70K and the 75K/22K junctions in S-1. Using the above proteases as conformational probes, we have previously demonstrated that the binding of actin is sensed at both the 26K/50K and the 50K/22K junctions [Applegate, D., & Reisler, E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7109–7112]. We report here that the binding of nucleotides at the active site is also sensed at both junctions. Both 2 mM MgADP and 5 mM MgATP slow the rate of elastase and subtilisin cleavage of the 95K heavy chain. With elastase, the 3-fold decrease in the rate of cleavage induced by nucleotides is evidenced at both the 26K/50K and the 50K/22K junctions. The analysis of subtilisin digestions is complicated by Mg nucleotide induced cleavage at a new site to produce a 91K fragment. Using *N*-methyl-6-anilinonaphthalene-2-sulfonyl chloride (MnsCl) to fluorescently label the 26K peptide, we demonstrate that the additional cleavage site is approximately 4K daltons from the N-terminal portion of the 95K heavy chain. We further demonstrate that, despite the overall 2-fold decrease in the rate of cleavage of the 95K heavy chain, the rate of cleavage at the 50K/22K junction is increased 2-fold in the presence of nucleotides, and consequently the rate of cleavage at the 26K/50K junction must be decreased by a factor of 4. The binding of either 2 mM MgADP or 5 mM MgATP to S-1 caused a 2-fold increase in the rate of the thermolysin cleavage of S-1 at the 26K/70K junction but inhibited its cleavage by papain. The above results demonstrate that the binding of MgADP and MgATP to S-1 is sensed at both the 26K/50K and the 50K/22K junctions of the 95K heavy chain.

A large body of well-documented evidence suggests that the binding of ATP and nucleotide analogues to myosin subfragment 1 (S-1)[1] induces conformational changes in this protein. Nucleotide-induced perturbations of spectroscopic probes at various sites and changes in the reactivity of certain residues on the protein indicate that the binding is sensed over broad regions of S-1 (Morales et al., 1982). To mention just a few examples, Mg nucleotides induce changes in the intrinsic tryptophan fluorescence of S-1 (Werber et al., 1972), in the reactivities of SH_1 and SH_2 thiol groups to alkylation (Watterson & Schaub, 1973; Reisler et al., 1977), in the absorption spectrum of the trinitrophenyl moiety attached to the reactive lysyl residue (Muhlrad, 1977), and in the spectral properties of light chains (Marsh et al., 1982). The most recent addition to the optical and chemical tools of S-1 analysis is the limited tryptic digestion of this protein. Trypsin cleaves the 95K heavy chain of S-1 to produce three discrete fragments (25K, 50K, 20K) that remain associated under nondenaturing conditions. Various lines of evidence suggest that the 25K peptide contains the active site (Szilagyi et al., 1979; Okamoto & Yount, 1983), whereas two separate actin binding sites are located on the other two peptides, one on the 20K peptide and the other on the 50K peptide (Mornet et al., 1981; Yamamoto & Sekine, 1979a; Sutoh, 1983). Tryptic attack at the 50K/20K junction is slowed by actin (Mornet et al., 1979; Yamamoto & Sekine, 1979b), and the binding of nucleotides is sensed at the junction between the 25K and 50K fragments (Hozumi & Muhlrad, 1981; Muhlrad & Hozumi, 1982).

Recently, we initiated the use of proteases other than trypsin to further study the substructure of S-1. We have shown that cleavage of S-1 by a variety of proteases including elastase, subtilisin, papain, and thermolysin results in the production of fragments that correspond within 1–2K daltons to the tryptic fragments (Applegate & Reisler, 1983). While papain and thermolysin cut preferentially at the 26K/70K junction, elastase and subtilisin cleave both the 26K/50K and 50K/22K junctions in S-1. The various proteases proved useful as conformational probes to explore the intersite communication on S-1. In particular, we showed that the binding of actin to the binding sites on the 20K and 50K peptides is sensed at both the 26K/50K and 50K/22K junctions.

In this study, we have utilized the above proteases to examine the effect of the binding of MgADP and MgATP on the substructure of S-1. We have found that the binding of nucleotides at the active site is sensed at both the 26K/50K and 50K/22K junctions. We also present evidence that an additional cleavage site approximately 4K daltons from the N-terminal portion of the 95K heavy chain is accessible to subtilisin in the presence of MgADP or MgATP.

Materials and Methods

Chemicals. Trypsin (crystallized, bovine type XI), soybean trypsin inhibitor, α-chymotrypsin, elastase, Pronase subtilisin, papain, Pronase thermolysin, catalase, phenylmethanesulfonyl fluoride (PMSF), ATP, and ADP were purchased from Sigma (St. Louis, MO). *N*-(Iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)-ethylenediamine (IAEDANS) and *N*-methyl-6-anilinonaphthalene-2-sulfonyl chloride (MnsCl) were obtained from Molecular Probes (Plano, TX). All other chemicals were reagent grade.

Proteins. Myosin from back and leg muscle of rabbits was prepared according to Godfrey & Harrington (1970). S-1 was

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[1] Abbreviations: S-1, myosin subfragment 1; PMSF, phenylmethanesulfonyl fluoride; IAEDANS, *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine; MnsCl, *N*-methyl-6-anilinonaphthalene-2-sulfonyl chloride; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

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prepared by digestion of myosin filaments with α-chymotrypsin (Weeds & Pope, 1977). The concentration of S-1 was calculated from its absorbance at 280 nm, assuming an absorption coefficient $A^{1\%}$ of 7.5 cm^{-1}.

Proteolysis. For all digestions, the reaction mixture included 40 mM NaCl/25 mM imidazole, pH 7.0, with a final S-1 concentration of 2 mg/mL. All reactions were carried out at 19–20 °C. Digestions were started with the addition of the appropriate proteases. The weight ratios of enzyme to S-1 were as follows: trypsin, 1:100; elastase, 1:10; subtilisin, 1:100; papain, 1:100; thermolysin, 1:20. To follow the proteolytic reaction, 250-μL aliquots were removed at the given time interval, and the appropriate inhibitor at 3× the enzyme concentration was added to stop the reaction. The inhibitors were as follows: soybean trypsin inhibitor for trypsin, PMSF for elastase and subtilisin, EDTA for thermolysin, and iodoacetic acid for papain. The samples were denatured and run on $NaDodSO_4$/polyacrylamide gels. Gel electrophoresis was carried out according to Laemmli (1970), using discontinuous (10% and 15% w/v) polyacrylamide gels. The optical densities of Coomassie blue stained protein bands and the respective mass distributions were determined with a Helena Laboratories (Beaumont, TX) Quick Scan R & D gel scanner equipped with an integrator. Molecular sizes of fragments were estimated by comparing their electrophoretic mobilities to those of marker proteins.

In order to examine the time course of production of the various fragments during proteolytic digestions of S-1, we quantitated their formation relative to the extent of heavy chain digestion; i.e., the normalized amount of each fragment formed at a given time was divided by the theoretical maximum amount of this fragment that could be produced from the cleaved fraction of the heavy chain. Digestion rates were obtained by monitoring the decay in the intensity of the respective bands as a function of digestion time. All decay curves were fitted by single exponentials and yielded the first-order rate constants for cleavage of S-1 (Reisler & Liu, 1982).

Fluorescent Labeling of S-1 with IAEDANS and MnsCl. The SH_1 groups of S-1 were labeled with the fluorescent dye IAEDANS essentially as described by Duke et al. (1976). The 25K fragment of intact S-1 was labeled by a 45-min incubation of S-1 at 0 °C with a 10× molar excess of MnsCl, a lysine-directed reagent. The modification was stopped by passing the reaction mixture through two Penefsky columns (Penefsky, 1977). The extent of labeling (0.8–1.2 mol of MnsCl per mole of S-1) was determined spectrophotometrically; an extinction coefficient of 2.0×10^4 M^{-1} cm^{-1} at 330 nm was used (Hiratsuka & Uchida, 1977). The fluorescent S-1 was then subjected to proteolytic digestion by subtilisin. Fluorescent bands were located on gels by illumination with long-wave ultraviolet light before staining with Coomassie blue. Most of the label was attached to the 26K fragment of S-1. Although we have not identified the modified residue, it is likely the reaction proceeds at the reactive lysine residue (Lys-83) of S-1.

Hydrolysis of Synthetic Substrate. The proteolytic cleavage of *p*-nitrophenyl acetate in the presence and absence of nucleotides was followed spectrophotometrically by monitoring its absorbance at 400 nm. These experiments were done to determine the effect of nucleotides on the intrinsic rate of hydrolysis of elastase, subtilisin, and papain.

Results

Effect of Nucleotide Binding to S-1 on Its Digestion at the 26K/70K Junction. Reactions with Papain and Thermolysin. As shown previously, the digestion of the 95K heavy chain by both papain and thermolysin produces only two fragments, the

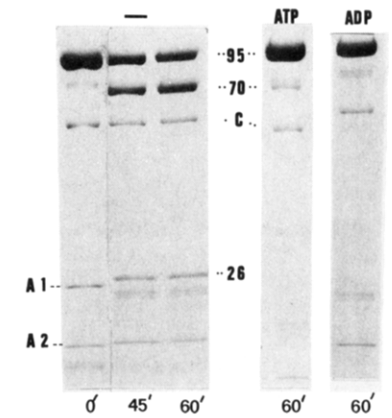

FIGURE 1: Effect of MgATP and MgADP on the time course of papain digestion of S-1. Representative electrophoretic pattern of papain digestion of S-1 in the absence (–) and presence of nucleotides (5 mM MgATP and 2 mM MgADP). The molecular sizes of the fragments are given in kilodaltons in the middle lane. Catalase, denoted as C, was added as an internal standard. Light chain bands (A1 and A2) are indicated. Digestion times in minutes are indicated below each line.

70K and the 26K peptides (Applegate & Reisler, 1983). The latter peptide, corresponding to the 25K produced by trypsin, is believed to contain the active site of S-1 (Szilagyi et al., 1979). Addition of either 5 mM MgATP or 2 mM MgADP to the papain digestion medium reduced the cleavage of S-1 to a negligible rate (Figure 1). This decrease in the rate of proteolysis could not be attributed to an effect of nucleotides on the protease, since cleavage of the synthetic substrate *p*-nitrophenyl acetate was decreased by only 20% in the presence of 5 mM MgATP or 2 mM MgADP. Moreover, the rate of digestion of heat-denatured S-1 by papain was the same in the presence and absence of nucleotides. We conclude that the binding of MgATP or MgADP protects the 26K/70K junction in S-1 from cleavage by papain.

The nucleotide-induced protection of the 26K/70K junction in S-1 against papain cleavage has a practical application. The digestion of myosin by papain to form MgS-1 is frequently plagued by the unwanted cleavage at the 26K/70K junction. We have noted that addition of MgATP to myosin in the standard papain digestion medium (0.2 M ammonium acetate, pH 7.2) protects this junction from cleavage by papain and yields significantly less fragmented S-1.

Despite the fact that thermolysin cuts S-1 at the same 26K/70K junction as papain, the binding of nucleotides to S-1 has a very different effect on the cleavage by these enzymes (Figure 2). By monitoring the disappearance of the 95K heavy chain band vs. time (not shown), we find that addition of either 5 mM MgATP or 2 mM MgADP activates the thermolysin cleavage of S-1 by a factor of 2, whereas as discussed above the reaction with papain is greatly inhibited by nucleotides. It should be noted that thermolysin digestion of denatured S-1 was unaffected by the presence of nucleotides.

Thus, it is apparent that the binding of nucleotides at the active site is sensed at the 26K/70K junction. However, the kinetic rates of cleavage by the two enzyme probes, papain and thermolysin, change in the opposite sense due to nucleotide binding to S-1.

Effect of Nucleotide Binding on Digestion of S-1 by Elastase. Limited digestion of the 95K heavy chain of S-1 by elastase produces five fragments: the 75K, 70K, 50K, 26K, and 22K peptides (Figure 3). We have previously shown that the 50K, 26K, and 22K fragments correspond to the 50K, 25K, and 20K fragments produced by tryptic cleavage of S-1 (Applegate & Reisler, 1983). While the presence of 5 mM

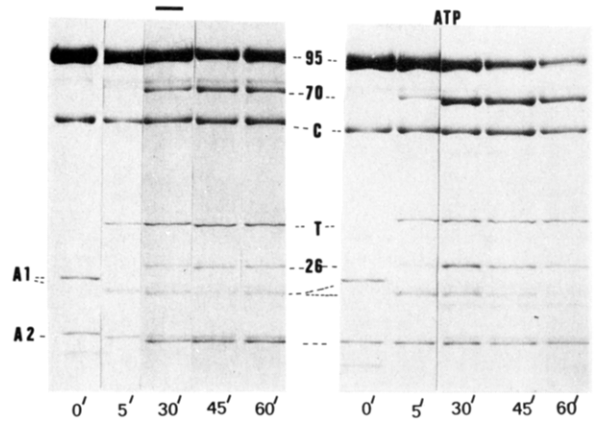

FIGURE 2: Effect of MgATP on the time course of thermolysin digestion of S-1. Representative electrophoretic patterns of thermolysin digestion of S-1 in the absence (–) and presence of 5 mM MgATP. Numbers in the middle lane are in kilodaltons and refer to the size of the fragments. Catalase (C), thermolysin (T), and light chain bands (A1 and A2) are indicated. Times of digestion in minutes are indicated below each lane.

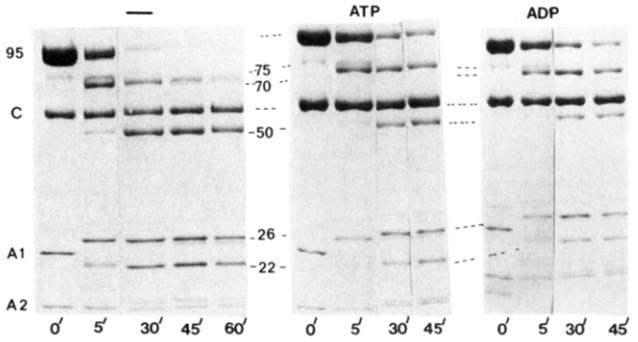

FIGURE 3: Representative electrophoretic patterns of elastase digestion of S-1 in the absence (–) and presence of nucleotides (5 mM MgATP and 2 mM MgADP). Numbers beside bands are in kilodaltons. Catalase (C) and light chain bands (A1 and A2) are as indicated. Digestion times in minutes are indicated under each lane.

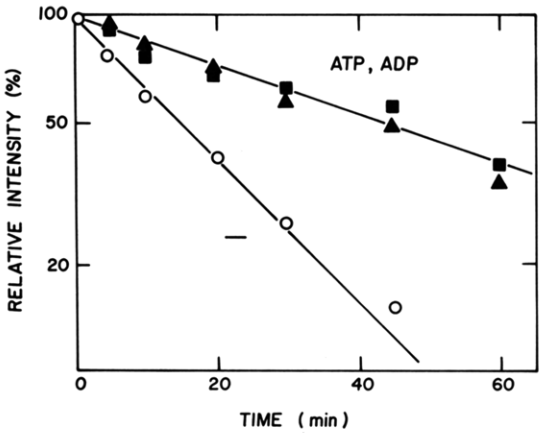

FIGURE 4: Effect of MgATP and MgADP on elastase digestion of S-1. Relative intensities of the 95K heavy chain band in the absence (○) and presence of nucleotides [5 mM MgATP (▲) and 2 mM MgADP (■)] are plotted vs. digestion times.

MgATP or 2 mM MgADP in the elastase digestion medium does not alter the S-1 digestion pattern, there is a 2.8-fold decrease in the rate of the digestion as monitored by the disappearance of the 95K heavy chain band vs. time (Figure 4). Plots of the relative intensity of the 22K and 26K protein bands vs. time (Figure 5) indicate that both fragments are produced at a slower rate in the presence of nucleotides than in their absence, with a stronger inhibition noted for the 22K

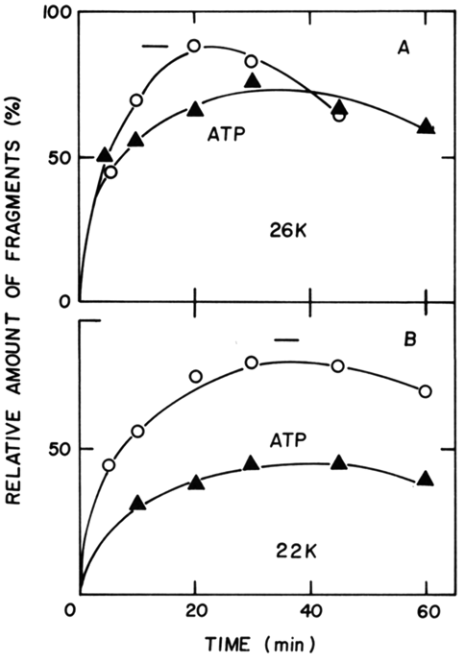

FIGURE 5: Effect of MgATP on production of the 26K and 22K peptide fragments by elastase digestion of S-1. (A) Relative amount of the 26K peptide and (B) relative amount of the 22K peptide formed during elastase digestion of S-1 in the absence (○) and presence (▲) of 5 mM MgATP. The amounts of the fragments produced were calculated as described under Materials and Methods.

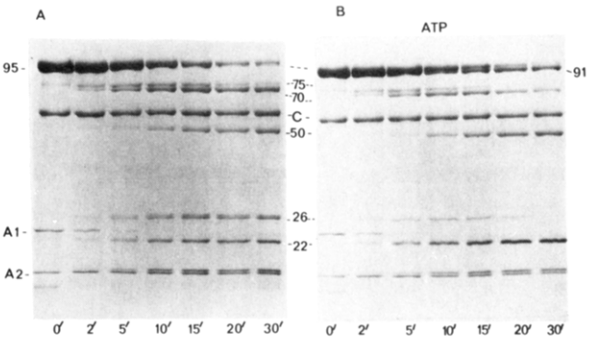

FIGURE 6: Subtilisin digestion of S-1 in the absence (A) and presence (B) of 5 mM MgATP. Numbers beside bands are in kilodaltons. Catalase (C) and light chain bands (A1 and A2) are as indicated. Digestion times in minutes are indicated.

peptide production. The rate of cleavage of the synthetic substrate, *p*-nitrophenyl acetate, by elastase is unchanged by the presence of nucleotides. We thus conclude that both the 26K/50K and 50K/22K junctions are affected by the binding of nucleotides at the active site of S-1.

Effect of Nucleotide Binding on Digestion of S-1 by Subtilisin. (A) A Nucleotide-Induced Subtilisin Cleavage Site. Limited digestion of S-1 by subtilisin produces three peptide fragments similar to those produced by elastase (26K, 50K, 22K). However, in contrast to elastase digestions, the binding of nucleotides to S-1 alters the reaction of S-1 with subtilisin (Figure 6). Examination of the electrophoretic pattern in Figure 6 shows that the 95K heavy chain is cleaved to a 91K fragment in the presence of 5 mM MgATP (or 2 mM MgADP, not shown). It is also evident that in the presence of nucleotides the 26K fragment disappears shortly after its initial formation, whereas the band corresponding to the 22K peptide accumulates at a rapid rate. This is shown quantitatively, in parts A and B of Figure 7, by plotting the relative intensities of the 22K and the 26K protein bands vs. time in the presence and absence of nucleotides. Note that the 22K peptide ac-

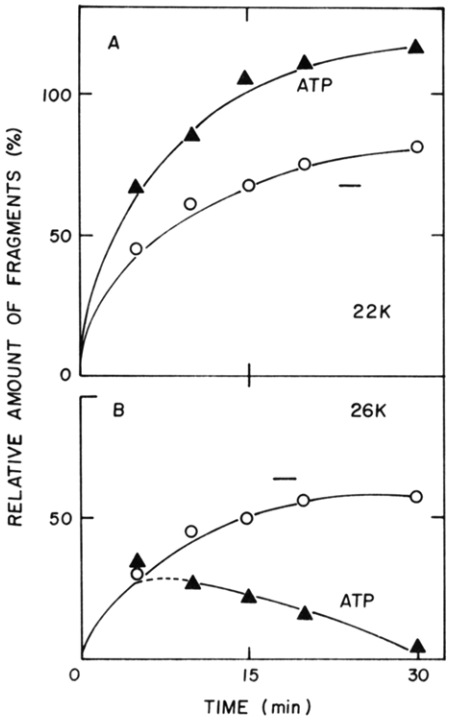

FIGURE 7: Effect of 5 mM MgATP on production of the 22K and 26K peptide fragments by subtilisin digestion of S-1. Relative amounts of the 22K (A) and 26K (B) peptides obtained during subtilisin digestion of S-1 in the absence (○) and presence (▲) of 5 mM MgATP. The amounts of fragments produced were calculated as described under Materials and Methods.

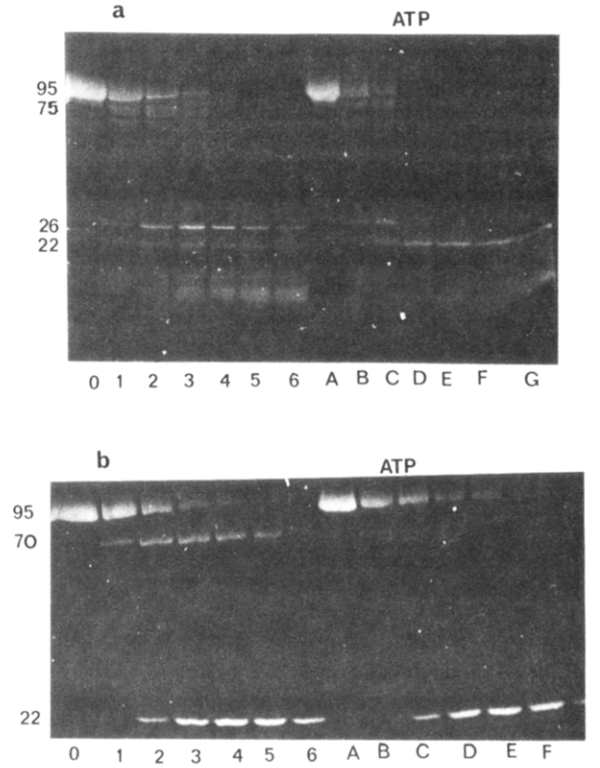

FIGURE 8: Subtilisin digestion of fluorescently labeled S-1. (a) Digestion of MnsCl-labeled S-1 in the absence (lanes 0–6) and presence (lanes A–G) of 5 mM MgATP. Times of digestion are 0, 2, 5, 10, 15, 20, and 30 min, respectively. MnsCl labeling of S-1 was carried out as described under Materials and Methods. Numbers beside bands are in kilodaltons. (b) Subtilisin digestion of IAEDANS-labeled S-1 in the absence (lanes 0–6) and presence (lanes A–F) of 5 mM MgATP. Digestion times are as in (a). Note that the 30-min digestion time point in the presence of 5 mM MgATP is not shown. Numbers beside bands are in kilodaltons.

cumulates beyond 100% in the presence of MgATP.

(*B*) *Identification of the Nucleotide-Induced Subtilisin Cleavage Site.* An explanation which could account for these observations is that an additional subtilisin cut approximately 4K daltons from the N-terminal portion of the 95K heavy chain is introduced in the presence of nucleotides. Such cleavage would cause the production of the 91K fragment and its subsequent degradation into a 50K fragment and two distinct 22K fragments, which would fortuitously comigrate on our gels. This, and parallel reactions shown in the digestion scheme presented in Figure 9, would account for the pattern and products of S-1 digestion by subtilisin in the presence of nucleotides. According to such a scheme, the two 22K fragments would correspond to the C-terminal portion of S-1 and the degraded 26K fragment. In order to determine that the 26K fragment is indeed converted into a 22K peptide, we attempted to fluorescently label this fragment. The modification was carried out with MnsCl, a fluorescent probe that most likely modified the reactive lysine on the 26K fragment. The fluorescently labeled S-1 was subjected to subtilisin digestion in the presence and absence of nucleotides. The digestion carried out in the absence of nucleotides provides an indication of the specificity of labeling (Figure 8a, lanes 0–6). While the predominantly labeled band is the 26K peptide, some of the label also appears on the A-1 light chain, on the 22K fragment, and to some extent on the 50K fragment. In spite of this limitation, it is apparent that in the absence of nucleotides the fluorescence of the 26K band increases with time of subtilisin digestion. In contrast, in the presence of nucleotides the fluorescence of the 26K band decreases after the second time point, with a simultaneous increase in the fluorescence of the 22K band. We conclude that in the presence of nucleotides the 26K band is cleaved into a fragment that comigrates with the 22K band. In order to account also for the formation of the 91K fragment, the cleavage site must be 4K daltons from the N-terminal portion of the 95K heavy chain (i.e., 4K from the N-terminal of the 26K peptide). In the fluorescent gel shown in Figure 8a, the 91K and 95K bands cannot be resolved because of the heavy overloading of the samples. However, the Coomassie blue stained gel with the normal loading of the same samples clearly shows the 95K/91K doublet in the subtilisin digestion in the presence of nucleotides.

(*C*) *Effect of Nucleotides on the Rate of Cleavage at 26K/50K and 50K/22K Junctions.* The comparison of the relative rates of disappearance of the heavy chain in the presence and absence of nucleotides, i.e., the decay of the 95K + 91K and 95K bands, respectively, reveals by an analysis similar to the one shown in Figure 4 that the overall rate of subtilisin digestion of the S-1 heavy chain is slowed by a factor of 2 by nucleotides. The nucleotide-induced cleavage site complicates direct attribution of this effect to one or perhaps both junctions on the basis of simple analysis of the Coomassie blue stained gels. To avoid the complicated kinetic analysis (see Figure 9), we selectively labeled with IAEDANS the SH_1 thiol on the 22K peptide of S-1 and subjected the modified protein to subtilisin digestion in the presence and absence of nucleotides (Figure 8b). The only fluorescent products of subtilisin digestion of IAEDANS-labeled S-1 are those which contain the C-terminal 22K peptide (95K, 91K, 70K, and 22K peptides; see cleavage scheme in Figure 9). Solution of the kinetic rate equations describing the disappearance of the fluorescent 95K, 91K, and 70K bands reveals that the rate of cleavage of the 50K/22K junction, k_2, can be determined by monitoring the decay of the combined intensity of the 95K,

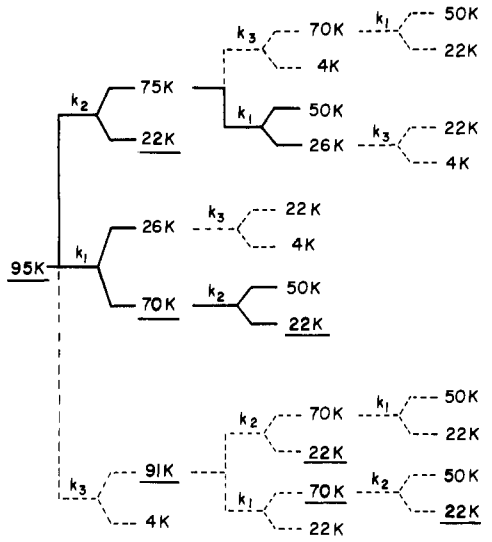

FIGURE 9: Scheme for subtilisin digestion of the 95K heavy chain of S-1. In the absence of nucleotides the digestion proceeds along the solid-line pathway. In the presence of nucleotides the flow occurs through both the solid-line and broken-line branches of the pathway. The underlined fragments denote products that are fluorescently labeled when IAEDANS-labeled S-1 is cleaved by subtilisin (i.e., the C-terminal 22K peptide and the intermediates in its production). Note that trypsin digestion of S-1 proceeds only along the solid-line pathway.

91K, and 70K peptides (not shown). We found that k_2 was increased by a factor of 2 in the presence of nucleotides. Thus, while there is an overall slowing of the cleavage of the 95K heavy chain in the presence of nucleotides, cleavage of the 50K/22K junction proceeds at a faster rate. We may conclude, then, that the cleavage at the 26K/50K junction must be slowed 4-fold by the binding of nucleotides to S-1.

(D) Subtilisin Digestion of the MgATP–Acto–S-1 Ternary Complex. We have previously shown that actin binding slows the subtilisin digestion of S-1 by a factor of 2, with both the 26K/50K and 50K/22K junctions sensing the actin binding (Applegate & Reisler, 1983). The 95K/91K cut exposed to subtilisin by nucleotides is not accessible to the protease in the acto–S-1 complex. We found that this site remains inaccessible even upon addition of 2 mM MgADP to the acto–S-1 complex. In fact, we have found that, with the four proteases mentioned above, the digestions of the MgADP–acto–S-1 ternary complex resembled very closely the digestions of acto–S-1.

Discussion

In this study, we probed the effect of nucleotide binding on the substructure of S-1 by using the method of limited proteolysis of S-1. We have previously shown that digestion of the myosin head by the four proteases used in this work (papain, thermolysin, elastase, and subtilisin) results in products that correspond within 1–2K to the peptides produced by tryptic digestion of S-1, i.e., the 25K, 50K, and 20K fragments (Applegate & Reisler, 1983). This is particularly interesting in view of the different substrate specificities of the enzymes used and may be indicative of a unique "open" conformation of the linker peptides that connect the main protease-resistant fragments. In the same study we further showed that the binding of actin to S-1 protects both the 26K/50K and the 50K/22K junctions from digestion by the various proteases.

The main conclusion of the present study is that the binding of nucleotides affects both junctions as well. Yet, in contrast to actin, the nucleotide effect depends on the enzyme used and the position of the proteolytic cut. The results of the different digestion experiments are summarized in Table I. We note

Table I: Effect of MgATP (5 mM) and MgADP (2 mM) on Rates of Proteolytic Cleavage at 26K/50K and 50K/22K Junctions in Myosin Subfragment 1

protease	changes in cleavage rates: 26K/50K junction	changes in cleavage rates: 50K/22K junction	new cuts
papain	decrease		
thermolysin	increase		
elastase	decrease	decrease	
subtilisin	decrease	increase	95K/91K
trypsin[a]	decrease[a]		29.5K/27K[a]

[a] Taken from Muhlrad & Hozumi (1982).

that nucleotides protect the 26K/50K junction from papain and subtilisin digestion but activate its cleavage by thermolysin. Nucleotide binding also protects the 50K/22K junction from elastase digestion but increases the rate of subtilisin cleavage at the same region. Thus, the effect of nucleotides on the substructure of S-1 appears to be more complex than the effect of actin. The binding of actin to S-1 protects both junctions from proteolysis by all enzymes tested (Applegate & Reisler, 1983). This overall protection may be correlated with NMR observations on quenching of the internal motions in S-1 by the bound actin (Highsmith et al., 1979; Prince et al., 1981). It is possible then that the binding of actin to S-1 leads to a partial "tightening" of the open-linker peptide conformation. In contrast, while the binding of either MgATP or MgADP is sensed at both the 26K/50K and the 50K/22K junctions, the detected effect may be either a decrease or an increase in the cleavage at the particular position.

Presently, we can only speculate on how the binding of nucleotides is sensed at the open-linker peptides. It seems unlikely that all effects reported here and previously arise from direct contact of the respective sites and the nucleotide. It is possible that local changes in the environment of the residues susceptible to the particular proteases could cause the effects reported here. One could also envision nucleotide-induced conformational changes in the linker peptides, which would have opposite effects on the binding of the different proteases to their respective cleavage sites. The NMR studies are consistent with such localized nucleotide-induced changes in S-1 (Highsmith et al., 1979). Nucleotide analogues do not have any measurable effect on the S-1 spectrum, suggesting little or no change in the internal motions upon binding of nucleotides. This does not exclude the possibility of local structural changes or motions. Such changes may include the motion of SH_1 or SH_2 residues (Burke & Reisler, 1977; Miller et al., 1982; Dalbey et al., 1983) and may be responsible for the perturbation of linker peptides. Whatever the mechanism of signal transmission from the active site, it is clear that the binding of nucleotides is sensed at many regions of S-1, including the two linker peptides. That the binding of actin is also sensed at the linker peptides argues for a general sensitivity of these structural regions.

A separate and very interesting effect is the nucleotide-induced cleavage by subtilisin at a site approximately 4K daltons from the N-terminal portion of the 95K heavy chain. This identifies another site on S-1 probably analogous to the chymotryptic site detected on smooth muscle myosin head (Okamoto et al., 1980) that can sense the binding of nucleotides and actin to the protein. It is interesting to note that this site is not accessible to subtilisin in acto–S-1 and in the ternary complex of MgADP–acto–S-1. The 95K/91K region may become more flexible upon the binding of ATP and immobilized again in the ternary complex with actin.

In conclusion, our results show that nucleotide binding at the active site of S-1 is sensed at both the 26K/50K and the 50K/22K junctions. We further show that an additional cleavage site 4K daltons "distant" from the N-terminal portion on the 95K heavy chain is accessible to subtilisin in the presence of nucleotides. This position is added to the list of perturbable regions on S-1 that communicate with the active site and the actin binding sites.

After submission of this work for publication we became aware of a similar study carried out by Mornet et al. (1984). These authors reach the same conclusions as presented here and previously (Applegate & Reisler, 1983).

Registry No. MgATP, 1476-84-2; MgADP, 7384-99-8; elastase, 9004-06-2; subtilisin, 9014-01-1; papain, 9001-73-4; thermolysin, 9073-78-3; trypsin, 9002-07-7; proteinase, 9001-92-7.

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