Substructure of Skeletal Myosin Subfragment 1 Revealed by Thermal Denaturation†

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ABSTRACT: The stability of myosin subfragment 1 (S1) to thermal denaturation has been followed by limited tryptic proteolysis. Digestions done during the thermal denaturation show that at temperatures at and above 37 °C there is a marked increase in the susceptibility of S1 to tryptic degradation, as evidenced by the loss of all bands corresponding to the normally trypsin-resistant fragments of 50, 27, and 21 kDa of the heavy chain and to the light chain. The enhanced digestion of S1 appears to be due to a general unfolding of all segments of S1, although the 50-kDa segment appears to unfold at a lower temperature than the remainder of the S1 structure. Digestions done after 30-min exposure to higher temperatures or after subsequent cooling to 25 °C show marked differences in the susceptibility of the S1 to trypsin. This suggests that, on cooling, a substantial portion of the S1, but not the 50-kDa segment, is capable of refolding to a state corresponding closely to that in the native S1. These data indicate that in terms of thermal denaturation the S1 behaves as though it is comprised of two domains—an unstable 50-kDa domain and a more stable domain comprised of the 27- and 21-kDa segments of the heavy chain interacting with the light chain, as proposed recently by Setton and Muhlrad [Setton, A., & Muhlrad, A. (1984) *Arch. Biochem. Biophys. 235*, 411–417]. The rates of thermal inactivation of the ATPase of S1 are found to correspond closely to the decay rates for the 50-kDa fragment, suggesting that this segment in S1 is closely associated with the ATPase function of the protein.

In recent years the concept that the heavy chain of S1[1] may be folded into separate domains has gained some support. The main evidence for this view is that trypsin (Balint et al., 1975; Mornet et al., 1979; Yamamoto & Sekine, 1979a; Muhlrad & Hozumi, 1982) and a number of other proteases (Chaussepied et al., 1983; Applegate & Reisler, 1983; Mornet et al., 1984) cleave the native structure of S1 at one or both of two narrow stretches located at about 27–29 and 73–75 kDa from the amino terminus of the heavy subunit to produce, in the case of trypsin, an active tryptic S1 comprised of the light chain and three protease-resistant heavy chain fragments of 27, 50, and 21 kDa arranged in this order in the linear sequence (Lu et al., 1978). Further support for this concept lies in the recent observations that the isolated 21-kDa fragment, and, to a lesser extent, the 50-kDa fragment, can be renatured such that they show moderate to weak affinity for actin (Mulhrad & Morales, 1984; Muhlrad et al., 1986).

From studies involving both cross-linking and limited proteolysis approaches, it has been possible to demonstrate that both the 21- and the 50-kDa segments in S1, or in their isolated renatured forms, do contain contact surfaces for actin (Mornet et al., 1981a,b; Yamamoto & Sekine, 1979b; Sutoh, 1983; Muhlrad & Morales, 1984; Muhlrad et al., 1986). The 27- and 50-kDa regions appear to be associated with nucleotide binding since either or both are labeled with a variety of photoaffinity analogues (Nakamaye et al., 1985; Mahmood & Yount, 1984). Proximity of regions of these three fragments to one another has also been confirmed by cross-linking (Yamamoto & Sekine, 1979b; Hiratsuka, 1986a), suggesting a more intimate interaction of the three heavy chain segments than is conveyed by the separate domain view.

Recent studies have suggested that the 50-kDa segment of the heavy chain could be preferentially unfolded during thermal denaturation at 35 °C (Setton & Muhlrad, 1984). This observation would lend strong credence to the domain hypothesis since it demonstrates that a segment of the S1 (50 kDa) is capable of independent unfolding. However, this finding appears to be contradicted by the observations of Mocz et al. (1984), who showed that digestion of S1 by trypsin at 37 °C in the absence of MgATP resulted in the loss of all three of the normally resistant heavy chain fragments, thereby suggesting a more general unfolding of the entire S1 structure. Because of this uncertainty, a more detailed analysis of the effect of temperature on the structure of S1 seemed warranted.

In this study we have employed the limited tryptic proteolysis approach to examine the thermal denaturation of S1 in the temperature range 25–40 °C. If the proteins are digested at these temperatures, it is observed that at temperatures above 31 °C the protein exhibits a marked increase in its susceptibility to tryptic attack. At 37 °C or higher temperatures there is a loss of the normal digestion pattern as evidenced by decreased intensity of the bands corresponding to the heavy chain fragments of 50, 27, and 21 kDa and to the light chain.

Similar data are obtained if the protein is first incubated for 30 min and subsequently digested for an additional 30 min at the same temperature. Conversely, if the protein is cooled after being heated for 30 min and then digested at 25 °C, substantial amounts of 27-kDa fragment, 21-kDa fragment, and light chain are present in the digests but no 50-kDa fragment is observed. These results suggest that the loss of the 50-kDa fragment in thermally denatured S1 may reflect the inability of this segment to refold on cooling to 25 °C, in contrast to the other segments of the heavy chain and the light

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[1] Abbreviations: S1, subfragment 1 of myosin; S1A2 and S1A1, subfragment 1 with associated A2 and A1 light chains, respectively; BSA, bovine serum albumin; TAME, (*p*-toluenesulfonyl)-L-arginine methyl ester; kDa, kilodalton(s).

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chain, which appear to refold with a high degree of fidelity.

By examining the decay of the heavy chain fragments when tryptic S1 is digested at higher temperatures, it is found that the 50-kDa fragment is apparently unfolded at a much higher rate than the other regions of the heavy chain. Thus it appears that while all segments of the heavy chain are unfolded by exposure to high temperatures (37 °C), the 50-kDa segment is the most labile to thermal denaturation, in agreement with the observations of Setton and Muhlrad (1984). Additionally, there appears to be a reasonable correlation between the rate of thermal inactivation of S1 and the rate of decay of the 50-kDa segment measured at the different temperatures used in this study. This result extends the work and supports the conclusion of Setton and Muhlrad (1984), based on their study of S1 inactivated by heating at 35 °C, that the 50-kDa segment may be directly involved in the ATPase function of S1.

The present data also indicate two distinct structural domains for S1 with regard to thermal denaturation and renaturation. The 50-kDa segment appears to be labile to heat denaturation and unfolds at a much faster rate than the remaining structure. Although the 27-kDa segment, 21-kDa segment, and light chain constituents of S1 eventually unfold at elevated temperatures, on cooling they appear to be able to refold to a state very closely resembling their structure in the native protein, suggesting that they are interacting in S1.

Materials and Methods

Distilled water was converted to reagent grade by a Millipore QTM system. N^{α}-*p*-Tosyllysine chloromethyl ketone treated trypsin and soybean trypsin inhibitor were purchased from Sigma. All other reagents were analytical grade.

Proteins. Myosin was prepared from male albino New Zealand rabbits by the procedure of Godfrey and Harrington (1970). S1 was prepared as described by Weeds and Taylor (1975) and separated into the light chain based isozymes by (diethylaminoethyl)cellulose as described by these workers. Protein concentrations were obtained by absorbance, using $E^{1\%}_{280nm}$ of 5.5 and 7.5 for myosin and S1, respectively.

Digestions with Trypsin. In general, S1 at 1.0 mg/mL in 0.05 M imidazole, pH 7.0, in the presence of 0.12 M NaCl was digested with trypsin as indicated in the text, using ratios of 50:1 and 100:1 (w/w, S1:trypsin). Aliquots were removed at required times and placed on ice after mixing with a 2-fold excess of soybean trypsin inhibitor over trypsin. For the studies on the thermal inactivation, S1 at 1.0 mg/mL was first digested for 30 min with 1:50 trypsin to S1 at 25 °C to convert it to tryptic S1. Tryptic S1 without any inhibitor was then immediately placed in a water bath at the desired temperature, and at different times aliquots were removed and diluted to 0.2 mg/mL for measurements of the Ca^{2+}-ATPase activity according to the procedure of Kielley and Bradley (1956). The remainder of the samples were then examined by gel electrophoresis as described below.

Gel Electrophoresis. The samples were denatured and then subjected to electrophoresis on 12.5% acrylamide gels, using a 4% stacking gel as described by Laemmli (1970). In general, 7- and 3-μg loads of native S1 and tryptic S1 were loaded on each well. The gels were then stained with Coomassie Brilliant Blue R 250 and subsequently destained by diffusion. For quantitative densitometric analyses, internal standards of BSA or actin were added to the samples prior to the addition of the denaturant. The densitometric measurements were done with a computerized Shimadzu CS-930 thin-layer scanner in the absorption mode at 550 nm. Normalization of the amount of fragments present was done by dividing the amount measured by the molecular weight of the fragment.

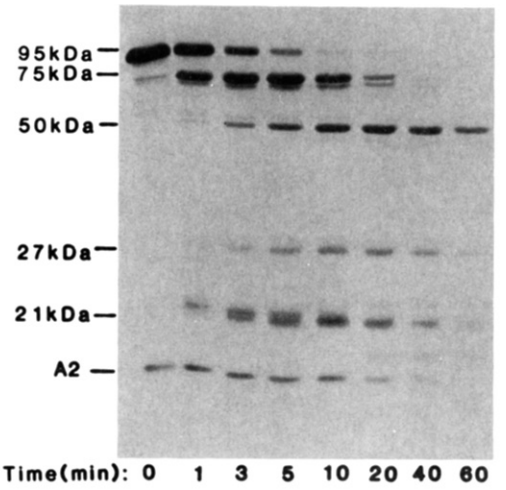

FIGURE 1: Sodium dodecyl sulfate gel electrophoretograms showing the time course of cleavage of S1A2 (1.0 mg/mL) by trypsin at 37 °C in the presence of 10 mM MgATP and 0.05 M imidazole, pH 7.0, with 1:50 by weight trypsin to S1.

Results

Standardization of Conditions To Examine Thermal Unfolding of S1. Before examining the unfolding of the S1 structure by heat, it was necessary to define proteolytic conditions that would detect the unfolding of the S1 structure. It has been reported that S1 is stable to denaturation at 37 °C for prolonged periods, provided there is an adequate supply of MgATP (Dreizen & Richards, 1972; Burke & Sivaramakrishnan, 1982). This stability has been confirmed by recent studies on the tryptic susceptibility of S1, where cleavage is restricted to the two "linker" regions located at about 27 and 75 kDa from the N terminus, as found by digestion of the protein at 25 °C (Mocz et al., 1984). The failure to detect other proteolytic products indicates that S1 does not unfold appreciably in the presence of substrate. Figure 1 shows the course and extent of such a digestion at 37 °C in the presence of MgATP at 1:50 trypsin to S1. An inspection of this electrophoretogram indicates that, in agreement with previous studies (Mocz et al., 1984), trypsin initially cleaves at the two linker sites. However, the resulting tryptic S1 is slowly degraded, as evidenced by a gradual decrease in the intensity of staining of the normal heavy chain fragments. Additionally, the loss in the intensity of the A2 light chain on prolonged digestion indicates that there is some subunit dissociation since only in the undissociated form does the light chain become susceptible to trypsin (unpublished results). For digestion times of 30 min with 1:50 or 1:100 (data not shown) trypsin to S1, the S1 appears to be converted to tryptic S1 without significant further degradation. For this reason these digestion conditions were chosen to examine the effect of heat on the structure of S1 in the absence of MgATP.

Tryptic Digestion of S1 as a Function of Temperature. The effect of heat on the extent and pattern of tryptic cleavage is shown in Figure 2A. These electrophoretograms show the fragments remaining after subjecting S1 to trypsin at the indicated temperatures for 30 min. Above 34 °C there is a rapid loss of all of the heavy chain fragments as well as of the A2 light chain. Since digestions in the presence of MgATP at 37 °C show little if any loss of the normal heavy chain fragments or of the light chain, it is possible to conclude that, in the absence of nucleotide, the entire S1 structure unfolds above 34 °C. Similar results were obtained when the S1 was preincubated for 30 min prior to the addition of the trypsin as shown in Figure 2B, although in this case there is clearly

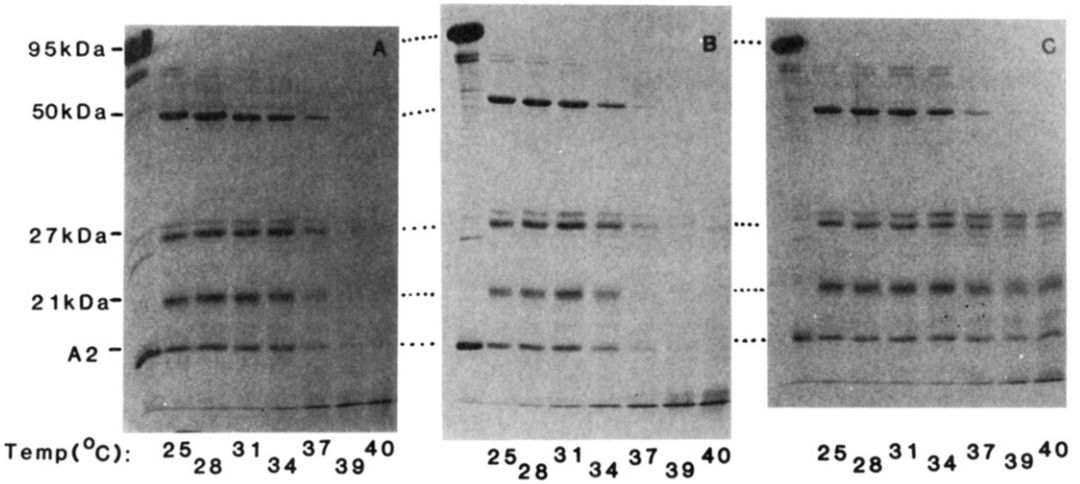

FIGURE 2: Sodium dodecyl sulfate gel electrophoretograms of 30-min digests of S1A2 (1.0 mg/mL) in the absence of MgATP with 1:50 trypsin to S1 by weight: (A) trypsin added at the same time S1 was placed in water bath at the indicated temperatures; (B) trypsin added to S1 after a 30-min preincubation at the indicated temperature; (C) digestions carried out at 25 °C for 30 min following a 30-min incubation at the indicated temperatures.

more unfolding as indicated by the more rapid digestion of the heavy chain. These results are in general agreement with those of Mocz et al. (1984), but they are in apparent disagreement with the results of Setton and Muhlrad (1984), who found that S1 heat denatured at 35 °C and subsequently digested at 25 °C with trypsin resulted in the loss of the 50-kDa fragment from an otherwise normal pattern of fragments.

Tryptic Digestions at 25 °C following Incubations at Varying Temperatures for 30 min. The results of the experiment approaching the conditions employed by Setton and Muhlrad (1984) are shown in Figure 2C. In this situation there appear to be significant amounts of the 27-kDa fragment, 21-kDa fragment, and light chain present even in the samples incubated at 40 °C for 30 min. Again there are very small amounts of the 50-kDa fragment, and the results in this case are similar to those reported by Setton and Muhlrad (1984). Since digestions that are performed at temperatures above 37 °C indicate that there is a loss of all heavy chain fragments as well as of the light chain, the presence of substantial amounts of the 27-kDa fragment, 21-kDa fragment, and light chain peptides suggests that, on cooling to 25 °C, an appreciable portion of the S1 structure is able to reassociate and refold to a structure resembling that corresponding to the native protein. There are also present appreciable amounts of 29-kDa fragment in the electrophoretograms of S1's heated at 35 °C or higher. It is likely that this species represents a portion of the 50-kDa fragment.

A more quantitative estimate of the decay of these fragments under the two experimental protocols was next done by repeating these experiments adding known amounts of BSA or actin as internal standards for subsequent densitometric analysis of the gel electrophoretograms. The normalized amounts of each fragment formed by digestion with trypsin at increased temperature or upon cooling to 25 °C are shown in parts A and B of Figure 3, respectively. Despite the scatter in the data, these plots show that the 50-kDa fragment is degraded at a much faster rate than the remainder of the S1 structure. At 35 °C or lower it appears that the 50-kDa segment unfolds preferentially in agreement with the observations of Setton and Muhlrad (1984) for their study at 35 °C.

Thermal Inactivation of S1 ATPase and Its Possible Relation to 50-kDa Segment Unfolding. The previous work of Setton and Muhlrad (1984) led to the suggestion that the loss of ATPase activity induced by heat may be related to the unfolding of the 50-kDa segment of the heavy chain of S1. To examine this suggestion in more detail, we have examined the rates of thermal inactivation and the extent of 50-kDa segment decay in the temperature range 34–40 °C. These results are shown in Figure 4. Both the inactivation and the 50-kDa segment loss obey first-order kinetics. The apparent first-order rate constants obtained from these plots are shown in Table I and exhibit a reasonable correlation with the rates of decay for both processes.

Table I: Rates of Thermal Inactivation of S1 and of 50-kDa Segment Decay

temp (°C)	inactivation[a] k (min^{-1})	50-kDa segment decay[b] k (min^{-1})
34	0.018	0.017
37	0.053	0.037
39	0.096	0.088
40	0.146	0.128

[a] Inactivation data are accurate to ±2%. [b] 50-kDa segment decay data are accurate to ±10%.

Discussion

This work was undertaken to obtain information about the substructure of the S1 region of myosin and, in particular, to determine its compatibility with the domain hypothesis for the structure of the associated heavy chain subunit (Mornet et al., 1981b, 1984; Applegate & Reisler, 1983). This latter proposal suggests that the three protease-resistant fragments of the heavy chain, formed when native S1 is digested by trypsin, correspond to unique structural domains. Such a model requires that each domain be stabilized by intradomain forces, and since it is likely that the number of different types of forces present in these domains will differ, it is not unreasonable to expect that these domains may show differential stability to structural perturbations.

Evidence for such differential stability was the recent observation that tryptic digestion of heat-denatured S1 resulted in the selective loss of only the 50-kDa heavy chain fragment from the digestion pattern, consistent with the selective unfolding of this region (Setton & Muhlrad, 1984). However, in another study on the effect of heat on S1 structure, it appeared that there was a loss of all of the heavy chain fragments and of the light chain, suggesting a more general unfolding of all of the heavy chain segments (Mocz et al., 1984). The present data indicate that this apparent difference

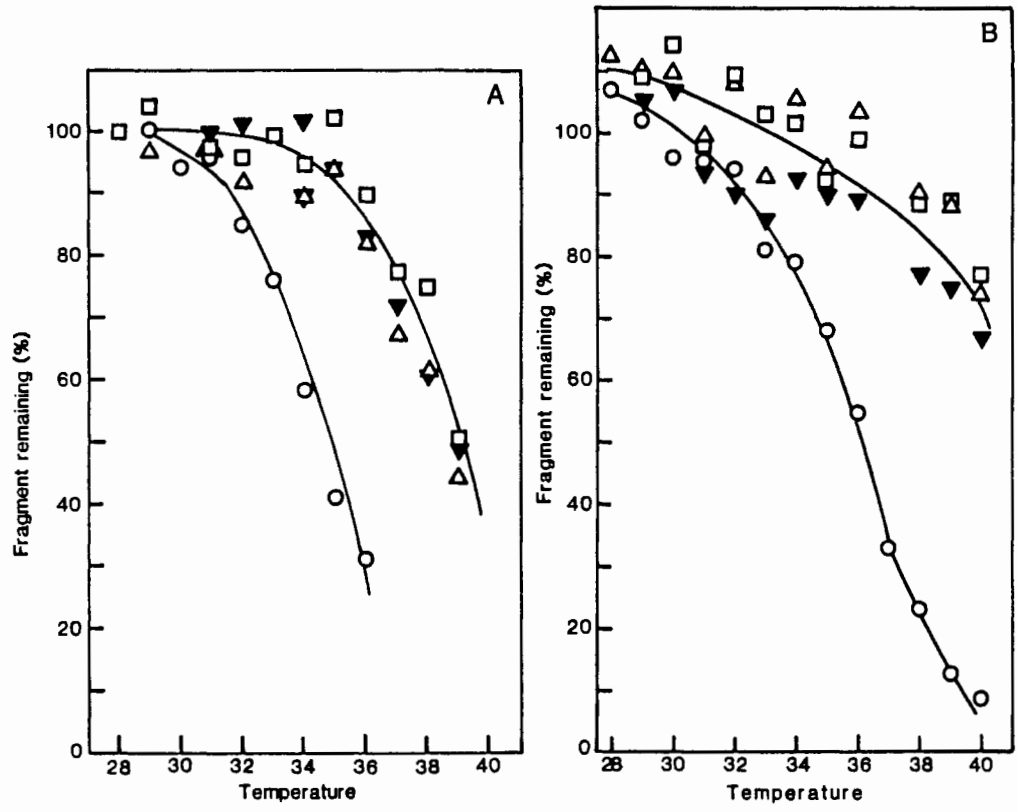

FIGURE 3: Percentage of heavy chain fragments and of A2 light chain remaining after a 30-min tryptic digestion of S1A2 (1 mg/mL): (A) digestion at the indicated temperature by using 1:100 trypsin to S1 by weight; (B) digestion at 25 °C after incubation at the indicated temperatures for 30 min by using 1:50 trypsin to S1 by weight. (○) 50 kDa; (▼) 27 kDa; (△) 21 kDa; (□) A2. The accuracy is 10% for the 50-kDa fragment and 15% for the remaining fragments and light chain. The 100% values correspond to the amounts of these fragments generated at 25 °C.

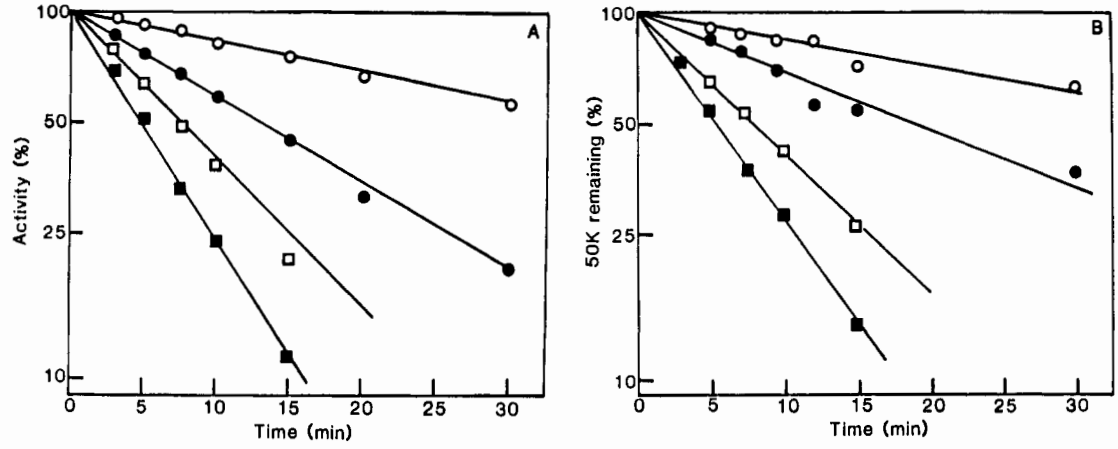

FIGURE 4: Semilogarithmic plots of (A) the Ca^{2+}-ATPase activity remaining when S1A2 (1 mg/mL), after a 30-min digest by trypsin at 25 °C, is incubated at the indicated temperature and (B) the amount of 50-kDa fragment remaining in these samples. The activity data are accurate to 2%, and the amounts of the 50-kDa fragment remaining are accurate to 10%. (○) 34, (●) 37, (□) 39, and (■) 40 °C.

is related to variations in the experimental protocols employed by these two laboratories.

Below temperatures of about 34–35 °C the S1 shows very little evidence of unfolding on the basis of tryptic susceptibility, but at 34–35 °C a marked reduction only in the amount of 50-kDa fragment occurs, consistent with the unfolding of this segment (Figures 2A,B). At 37 °C or higher there is a loss of all of the normal heavy chain fragments and of the light chain and a huge increase in the amount of peptide material running at the tracking dye front (Figure 2A). The latter data are in agreement with the observations of Mocz et al. (1984) and suggest that the entire S1 structure becomes unfolded at 37 °C.

The thermal instability of the 50-kDa segment relative to the other heavy chain fragments and to the light chain is apparent in the results presented in Figure 3. The data indicate that under these conditions the T_m for 50-kDa segment unfolding is about 5 °C lower than that for the other heavy chain segments and the light chain, which appear to melt together as a cooperative unit. These results confirm the conclusions of Setton and Muhlrad (1984) that at 35 °C heat denaturation of S1 leads to the preferential unfolding of the 50-kDa segment.

The tryptic susceptibility at 25 °C of heat-denatured S1 provides evidence that a substantial portion of the S1 structure may be capable of refolding to a conformation similar to that which they enjoy in native S1. This is based on the observation that, while most of the S1 structure is unfolded after a 30-min incubation at 37 °C or above (Figure 2A,B), on cooling to 25 °C a significant fraction is resistant to trypsin (Figures 2C

and 3B). The regions of S1 that appear to be involved here are the 27-kDa segment, 21-kDa segment, the light chain, and a 29-kDa portion of the 50-kDa segment, on the basis of their appearance in the digestion patterns of S1 preincubated at 35 °C or higher for 30 min. Additional evidence for this view is the absence of any significant amount of an 18-kDa fragment which is known to be formed from the 21-kDa segment when light chain is removed from S1 (Burke et al., 1983). The insignificant amount of 50-kDa fragment in samples heated at 37 °C or higher (Figure 2C) indicates that this segment of the heavy chain was unable to refold to the state that is resistant to tryptic attack as in native S1. There is, however, some evidence that part of the 50-kDa segment can refold, based on the presence of 29-kDa fragment in the samples that were heated at 37 °C or higher, since this product was also seen by Setton and Muhlrad (1984) on digestion of S1 heat denatured at 35 °C.

The loss of the light chain and heavy chain fragments, when S1 is preincubated for 30 min and digested at these temperatures, can be attributed to the unfolding and subunit dissociation of the S1 and not to the temperature dependence of the tryptic activity. In control experiments with the synthetic substrate TAME, the data showed that only a 2-fold increase in the trypsin activity occurred over the temperature range 25–40 °C. When allowances were made for this by digesting heat-denatured S1 at 25 and 37 °C with 1:50 and 1:100 (w/w), respectively, trypsin to S1, there was little loss of the 27-kDa fragment, 21-kDa fragment, and light chain bands over a 60-min digest at 25 °C. At 37 °C no material corresponding to the normal fragments or light chain was present after 30 min (data not shown).

The observation that the rate of thermal inactivation of the ATPase of S1 appears to parallel the decay of the 50-kDa fragment (Figure 4) suggests that the integrity of the 50-kDa segment is crucial for this function as suggested by Setton and Muhlrad (1984). A number of recent studies based on chemical modification (Korner et al., 1983; Hiratsuka, 1986b), proteolysis (Chaussepied et al., 1986a,b), and photoaffinity labeling (Mahmood & Yount, 1984) have pointed to the importance of this region to the ATPase function. Moreover, it is known that this segment also provides a contact with actin in both the absence and presence of nucleotide (Yamamoto & Sekine, 1979b; Mornet et al., 1981a,b; Chen et al., 1986).

The fact that thermal denaturation of S1 at moderate temperatures results in the preferential unfolding of the 50-kDa segment without affecting the remainder of the S1 structure (Figures 2C and 3) strongly suggests that the 50-kDa segment is capable of independent unfolding. This is consistent with the interpretation of Setton and Muhlrad (1984) that the 50-kDa fragment is a distinct domain. The loss of all of the remaining S1 structure to about the same relative extent (Figure 3A), when digested at the elevated temperatures, and their presence in the same relative proportions when the heat-treated samples are digested at 25 °C (Figure 3B) suggest that they behave as a cooperative unit. These results are similar in many respects to the effect of methanol on the substructure of S1 (Burke & Sivaramakrishnan, 1986), where it was also found that the 50-kDa segment of S1 could be unfolded independently of the remainder of the structure.

Registry No. ATPase, 9000-83-3; MgATP, 1476-84-2.

References

Applegate, D., & Reisler, E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7109–7112.

Balint, M., Sreter, F., Wolf, I., Nagy, B., & Gergely, J. (1975) *J. Biol. Chem. 250*, 6168–6177.

Burke, M., & Sivaramakrishnan, M. (1981) *Biochemistry 20*, 5908–5913.

Burke, M., & Sivaramakrishnan, M. (1986) *J. Biol. Chem. 261*, 12330–12336.

Burke, M., Sivaramakrishnan, M., & Kamalakannan, V. (1983) *Biochemistry 22*, 3046–3053.

Chaussepied, P., Bertrand, R., Audemard, E., Pantel, P., Derancourt, J., & Kassab, R. (1983) *FEBS Lett. 161*, 84–88.

Chaussepied, P., Mornet, D., Audemard, E., Derancourt, J., & Kassab, R. (1986a) *Biochemistry 25*, 1134–1140.

Chaussepied, P., Mornet, D., Barman, T. E., Travers, F., & Kassab, R. (1986b) *Biochemistry 25*, 1141–1154.

Dreizen, P., & Richards, D. H. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 145–152.

Godfrey, J. E., & Harrington, W. F. (1970) *Biochemistry 9*, 886–893.

Hiratsuka, T. (1986a) *Biochemistry 25*, 2101–2109.

Hiratsuka, T. (1986b) *J. Biol. Chem. 261*, 7294–7299.

Kielley, W. W., & Bradley, W. B. (1956) *J. Biol. Chem. 218*, 653–659.

Korner, M., Thiem, N. V., Cardinaud, R., & Lacombe, G. (1983) *Biochemistry 22*, 5843–5847.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lu, R., Sosinski, J., Balint, M., & Sreter, F. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1695.

Mahmood, R., & Yount, R. G. (1984) *J. Biol. Chem. 259*, 12956–12959.

Mocz, G., Szilagyi, L., Chen Lu, R., Fabian, F., Balint, M., & Gergely, J. (1984) *Eur. J. Biochem. 145*, 221–229.

Mornet, D., Pantel, P., Audemard, E., & Kassab, R. (1979) *Biochem. Biophys. Res. Commun. 89*, 925–932.

Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981a) *Nature (London) 292*, 301–306.

Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981b) *Biochemistry 20*, 2110–2120.

Mornet, D., Ue, K., & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 736–739.

Muhlrad, A., & Hozumi, T. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 958–962.

Muhlrad, A., & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1003–1007.

Muhlrad, A., Kasprzak, A. A., Ue, K., Ajtai, K., & Burghardt, T. P. (1986) *Biochim. Biophys. Acta 869*, 128–140.

Nakamaye, K. L., Wells, J. A., Bridenbaugh, R. L., Okamato, Y., & Yount, R. G. (1985) *Biochemistry 24*, 5226–5235.

Setton, A., & Muhlrad, A. (1984) *Arch. Biochem. Biophys. 235*, 411–417.

Sutoh, K. (1983) *Biochemistry 22*, 1579–1585.

Weeds, A. G., & Taylor, R. S. (1975) *Nature (London) 257*, 54–56.

Yamamoto, K., & Sekine, T. (1979a) *J. Biochem. (Tokyo) 86*, 1869–1881.

Yamamoto, K., & Sekine, T. (1979b) *J. Biochem. (Tokyo) 86*, 1863–1868.