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Effect of Tryptic Cleavage on the Stability of Myosin Subfragment 1. Isolation and Properties of the Severed Heavy-Chain Subunit†

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ABSTRACT: The procedure of thermal ion-exchange chromatography has been used to examine the effect of prior tryptic cleavage on the stability of myosin subfragment 1 (SF1). Although it is found that digestion does destabilize the subunit interactions at physiological temperatures, the heavy-chain subunit can be isolated either as an equimolar complex comprised of 50K, 27K, and 21K fragments or as one comprised of 50K, 27K, and 18K peptides. Thus, the interactions within the heavy chain are considerably more stable than those between the two subunits. Both forms of the free severed heavy chain exhibit ATPase properties similar to those of the parent tryptic SF1. The V_{max} for the actin-activated MgATPase of the free severed heavy chain is the same as that for both undigested and tryptic SF1(A2). Since its K_m for actin is similar to that of tryptic SF1(A2), it may be concluded that changes in the affinity of SF1 for actin induced by trypsin [Botts, J., Muhlrad, A., Takashi, R., & Morales, M. F. (1982) *Biochemistry 21*, 6903-6905] are not dependent on the presence of the associated alkali light chain. Furthermore, the communication between the SH1 site and the ATPase site is also shown to be independent of the associated alkali light chain, and it persists despite the cleavages present in the free heavy chain. Studies on the ability of these severed heavy chains to reassociate with free A1 and A2 chains indicate that the binding site is retained in the 21K-severed heavy chain but is lost in the 18K form.

The subfragment 1 (SF1)[1] cross-bridge region of the myosin molecule can be isolated as a stable complex comprised of a 95-kdalton polypeptide, representing the amino-terminal half of a myosin heavy chain, together with a single alkali light chain which exists in two chemically related forms called A1 and A2 (Yagi & Otani, 1974; Weeds & Taylor, 1975). Both the 95K chain and its associated light chain are folded into a very compact pear-shaped head which is approximately 190 Å long and about 40 Å at its widest point (Elliott & Offer, 1978). Within this head reside two distinct sites—an ATPase site and an actin binding site—whose coupling appears to be essential for contraction. The manner in which these sites communicate with one another remains obscure in the absence of detailed knowledge of the folded structure of the head, but in recent years, significant gains in characterizing the topology of SF1 have been made possible by the application of limited proteolysis together with photoaffinity labeling and with limited cross-linking approaches.

Limited tryptic digestion of SF1 is confined primarily within two small stretches of between 1 and 2 kilodaltons located at about 75K (T1) and 27K (T2) from the amino-terminal end of the heavy chain, and it gives rise to a stable tryptic SF1 complex comprised of the light chain and a severed heavy chain consisting of three protease-insensitive peptides of 27K, 50K, and 21K arranged in this order in the linear sequence (Balint et al., 1975). Although the ATPase site has not been unequivocally characterized, it appears from photoaffinity labeling that regions of the 27K (Szilagyi et al., 1979; Okamoto & Yount, 1983) and 50K (Mahmood et al., 1984) segments are involved here. The actin binding sites in the rigor acto-SF1 complex have been characterized by chemical cross-linking and are located at the 21K and 50K segments, each of which can bind to actin (Mornet et al., 1981a; Yamamoto & Sekine, 1979; Sutoh, 1982, 1983).

Functionally, cleavages at the T1 and T2 sites do not have a significant effect on the ATPase properties of SF1 (Mornet

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[1] Abbreviations: DEAE, diethylaminoethyl; SF1, SF1(A1), and SF1(A2), myosin subfragment 1 and the A1- and A2-containing isoenzymes, respectively; A1 and A2, two alkali light chains; T1 and T2, two regions located at about 75 and 27 kilodaltons, respectively, from the amino terminus of the heavy chain and which are vulnerable to tryptic attack; HC, heavy chain of SF1; 18K-severed heavy chain and 21K-severed heavy chain, 27K, 50K, and 18K and 27K, 50K, and 21K forms of the fragmented heavy chain, respectively; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

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et al., 1979), but they do lower the protein's affinity for actin in the presence of MgATP (Botts et al., 1982). This decrease in affinity for actin appears to be related to the cleavage within the T1 site (Mornet et al., 1981b). Whether this is a local effect restricted to changes solely in the heavy-chain subunit or whether it involves changes in the manner in which the two subunits interact is not presently known.

In the present study, this point has been examined by studying the stability of native and tryptic SF1 by the procedure of thermal ion-exchange chromatography. It is found that tryptic cleavage markedly destabilizes the subunit interactions in this protein. The dissociated severed heavy chain, on the other hand, can be isolated either as a 50K, 27K, and 21K complex or as a 50K, 27K, and 18K complex depending on the conditions of the thermal chromatography.

Actin activation studies of the native and tryptic heavy chains demonstrate that the changes in the K_m of SF1 for actin noted previously by Botts et al. (1982) are attributable solely to changes in the heavy-chain subunit and are not dependent on the presence of the associated alkali light chain. The communication between the SH1 site and the ATPase site in SF1, demonstrable from the altered ATPase accompanying SH1 modification (Sekine & Kielley, 1964), is also found to be independent of the bound light chain.

Studies on the ability of the two forms of the heavy chain to reassociate with the free alkali light chains to form reconstituted tryptic SF1 species indicate that the light chain binding potential is retained only for the severed heavy chain containing the 21K fragment.

Materials and Methods

Distilled water was purified to reagent grade by a Millipore QTM system and used throughout. *N*-[^{3}H]Ethylmaleimide was obtained from New England Nuclear (Boston, MA). Trypsin, soybean trypsin inhibitor, insoluble trypsin, and bovine pancreatic trypsin inhibitor were purchased from Sigma Chemical Company (St. Louis, MO). All other reagents were analytical grade.

Preparations of Proteins. Myosin was prepared by the procedure of Godfrey & Harrington (1970). SF1 was prepared by chymotryptic digestion of myosin and separated into its isoenzymes by the method of Weeds & Taylor (1975). Actin was prepared by the procedure of Spudich & Watt (1971). The free alkali light chains were obtained by denaturation of myosin by treatment in 6 M guanidine hydrochloride followed by ethanolic precipitation of the myosin heavy chains as described by Holt & Lowey (1975). The light chains were separated by DEAE-cellulose chromatography as described by these authors. Tryptic SF1(A2) was digested under conditions similar to that of Mornet et al. (1979) except that the solvent conditions were 50 mM imidazole and 0.1 mM dithiothreitol, pH 7.0. The digestion was terminated by the addition of either 2-fold excess of soybean trypsin inhibitor or an equal weight of the bovine pancreatic trypsin inhibitor. Protein concentrations were obtained either by absorption employing $E^{1\%}_{280nm}$ values of 5.5, 7.5, 11.0, and 2.0 for myosin, SF1, actin, and the alkali light chains, respectively, or by the colorimetric method of Lowry et al. (1951) with calibration curves constructed from the respective purified proteins. The calibration curve of SF1(A2) was used for determining the concentration of the SF1 heavy chain.

Thermal Ion-Exchange Chromatography of SF1. This was done essentially as described previously for the heavy-chain preparations (Sivaramakrishnan & Burke, 1982; Burke et al., 1983). The only modifications were that 15-mg loads were applied to the columns (1.6 × 33 cm) and the internal column temperature was regulated to 39.5 °C. The solvent conditions and the manner of eluent collection were as described in these earlier studies. Aliquots (75 μL) were removed from each fraction for protein determination by the absorbance at 595 nm according to the Bradford (1976) procedure.

Studies on the Reassociation of Alkali Light Chains to Native and Severed Heavy Chains. This was done in 0.093 M imidazole, 10 mM ATP, and 14 mM $MgCl_2$, pH 7.0 at 4 °C. The heavy-chain fractions (0.2–0.3 mg/mL) were incubated with a 6-fold molar excess of either the A1 or the A2 light chains at 4 °C and incubated for variable periods from 2 to 16 h, usually the latter, before being examined by gel electrophoresis under nondenaturing conditions. Preparation and isolation of reconstituted tryptic SF1 isoenzymes proceeded in essentially the same way as described previously for reconstituted native SF1 species (Burke et al., 1983).

Gel Electrophoresis under Nondenaturing Conditions and in the Presence of Sodium Dodecyl Sulfate. This was done essentially as described elsewhere (Burke & Sivaramakrishnan, 1981) for the native gel electrophoresis. Sodium dodecyl sulfate gel electrophoreses were done by the procedure of Laemmli (1970) using 12.5% polyacrylamide for the separating gel. The gels run in the presence of sodium dodecyl sulfate were subsequently stained and destained by the procedure of Weeds (1976). Molecular weights were estimated on the basis of the mobilities of the following standard proteins: myosin, phosphorylase *a*, bovine serum albumin, actin, carbonic anhydrase, chymotrypsinogen, soybean trypsin inhibitor, myoglobin, and lysozyme.

Densitometric Analysis of Gel Electrophoretograms. This was done by scanning the electrophoretogram at 550 nm using the linear transport attachment of the Gilford 250 spectrophotometer. The recorded patterns were copied, and the peaks corresponding to each band were carefully excised and weighed.

Estimation of the Degree of Dissociation of SF1. The plotted elution patterns obtained from thermal ion-exchange chromatography were photocopied, and the areas of the peaks were determined by their excision and by subsequent weighing. Since the first two fractions are not completely resolved, estimates of their areas were made by dropping a perpendicular from the minimum between them to the base line.

ATPase Activity Measurements. The Ca^{2+}- and EDTA-activated ATPase activities of the SF1 species were done as described by Kielley & Bradley (1956) and by Kielley et al. (1956). Actin-activated ATPases were done as described by Reisler (1980) with the following modification. The incubations and subsequent color determination of the released inorganic phosphate were done by the procedure of Seimankowski as described by White (1982).

Results

Thermal Ion-Exchange Chromatography of Tryptic SF1. The elution profile for tryptic SF1(A2) run on DEAE-cellulose at 39.5 °C in the presence of MgATP is presented in Figure 1. It is clear that it can be resolved into four distinct protein fractions as is also found for the undigested control. It is also apparent that the relative abundances of these fractions differ for these two species, suggesting that they may represent digested and undigested forms of the same components. To examine this possibility, the peak tubes of each fraction obtained from the control and digested SF1(A2) shown in Figure 1 were subjected to further analyses.

The first protein fraction eluted in the case of the undigested SF1 has been previously identified as the free heavy-chain subunit on the basis of its electrophoretic behavior and its

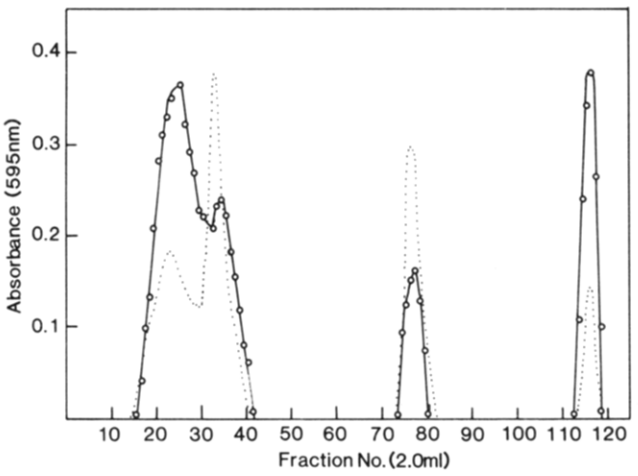

FIGURE 1: Thermal ion-exchange chromatography of tryptic SF1(A2). Tryptic SF1(A2) (15 mg) was loaded on a 1.6 × 33 cm jacketed column of DEAE-cellulose equilibrated at 39.5 °C in 0.093 M imidazole, 10 mM MgATP, and 0.1 mM dithiothreitol, pH 7.0, as described under Materials and Methods. At fractions 30 and 67, steps of 0.15 and 0.5 M NaCl containing the other buffer components, respectively, were applied to the column. A flow rate of 60 mL/h was used. 75-μL aliquots were removed for determination of the protein content by the Bradford method (1976). The dotted profile represents the elution pattern for native SF1(A2) plotted at half the indicated absorbance scale.

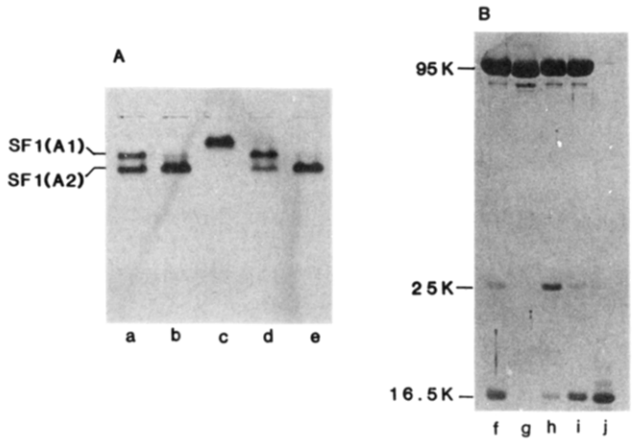

FIGURE 2: (A) Native gel electrophoretograms of SF1 isozymes and peak tubes of fractions resolved in Figure 1 for native SF1(A2): (a) SF1 isozymes; (b) SF1(A2); (c) peak 1; (d) peak 2; (e) peak 3. (B) Sodium dodecyl sulfate gel electrophoretograms of the native SF1(A2) and the peak tubes of the various fractions resolved from it by thermal ion-exchange chromatography shown in Figure 1: (f) native SF1(A2); (g) peak 1; (h) peak 2; (i) peak 3; (j) peak 4.

polypeptide composition (Sivaramakrishnan & Burke, 1982). The remaining three protein fractions were examined by native gel electrophoresis in the present work, and the reults are shown in Figure 2A. On the basis of a comparison of their mobilities with those of the SF1 isoenzymes, the following assignments can be made. The second protein fraction contains two components, the predominant one being the SF1(A1) isoenzyme with a smaller amount of SF1(A2) (Figure 2, lane d). The third protein fraction eluted with the 0.15 M NaCl is comprised predominantly of SF1(A2) with a much smaller amount of SF1(A1) (Figure 2, lane e). The protein fraction eluted with the 0.5 M NaCl step runs as a single band with a mobility similar to that of the free A2 light chain, and although just detectable in the original electrophoretogram, it is not visible in the photoreproductions (data not shown). These assignments are consistent with the peptide compositions of these fractions shown in the sodium dodecyl sulfate electrophoretograms of Figure 2B (lanes g–j).

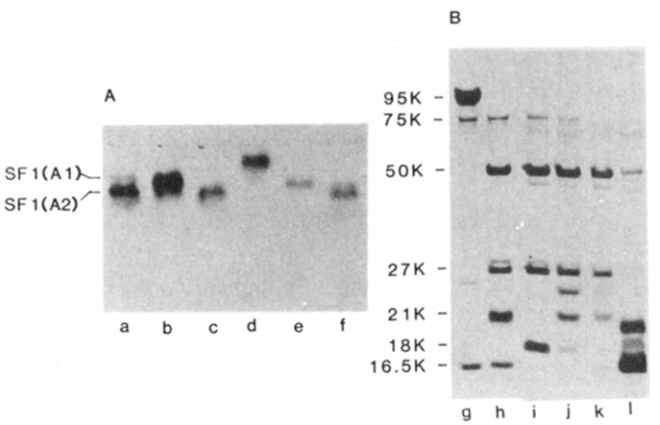

FIGURE 3: (A) Native gel electrophoretograms of tryptic SF1(A2) and the peak tubes of the fractions isolated from it by thermal ion-exchange chromatography shown in Figure 1: (a) SF1(A2); (b) tryptic SF1(A1); (c) tryptic SF1(A2); (d) peak 1; (e) peak 2; (f) peak 3. (B) Sodium dodecyl sulfate gel electrophoretograms of SF1(A2), tryptic SF1(A2), and the peak tubes of the fractions shown in Figure 1: (g) native SF1(A2); (h) tryptic SF1(A2); (i) peak 1; (j) peak 2; (k) peak 3; (l) peak 4.

Table I: Densitometric Analyses of SDS Gel Electrophoretograms of Tryptic SF1(A2) and the Tryptic Heavy Chain[a]

protein	M_r				
	50K	25–27K	21K	18K	16.5K
tryptic SF1(A2)	0.78	0.66	0.84		1.0
(27K, 50K, 18K)-HC	0.74	0.82		1.0	
(27K, 50K, 21K)-HC	0.90	0.86	1.0		

[a] Assumes that all bands show the same dye sensitivity.

The same analysis was also used to identify the corresponding four protein fractions isolated from the tryptic SF1(A2). The first of these fractions is homogeneous with a much lower mobility than any of the native or digested SF1 species (Figure 3, lane d). The second fraction has a mobility close to that of tryptic SF1(A1) (Figure 3, lane e). The fraction eluted by the 0.15 M NaCl step is heterogeneous since it is comprised of two components. Their mobilities are similar to those of the tryptic SF1 isoenzymes (Figure 3, lanes b and c). The fraction obtained with the 0.5 M NaCl step has the same mobility as the free A2 light chain but is not visible in the photoreproduction (data not shown). The peptide compositions of these fractions and the tryptic SF1(A2) prior to chromatography are shown in the sodium dodecyl sulfate electrophoretograms of Figure 3B. The first fraction is comprised of peptides of 50K, 27K, and 18K (Figure 3, lane i) which by densitometric analysis are present in close to equimolar amounts (Table I). In a parallel experiment using tryptic SF1(A2) prelabeled in its 21K segment at its SH1 thiol (Burke et al., 1983) with *N*-[^{3}H]ethylmaleimide, the 18K fragment was found to contain all of the radioactivity, thereby demonstrating that it is a truncated form of the 21K peptide. The lack of any peptide corresponding to the alkali light chains suggests that this component is the severed heavy-chain subunit of tryptic SF1. The peptide compositions of the next two fractions (Figure 3, lanes j and k) are consistent with the assignments based on their electrophoretic behavior presented above. The peptide composition of the high-salt fraction shows that it is predominantly the free A2 light chain (Figure 3, lane l).

The above results indicate that thermal ion-exchange chromatography of tryptic SF1(A2) does result in dissociation of a fraction of the protein into its constituent subunits. However, it is evident that during the chromatography the 21K

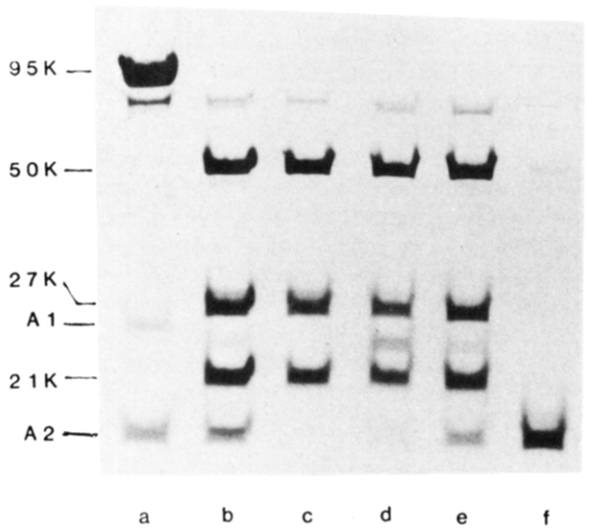

FIGURE 4: Sodium dodecyl sulfate electrophoretograms of SF1(A2), tryptic SF1(A2) terminated with bovine pancreatic trypsin inhibitor, and the peak tubes from the four fractions resolved by thermal ion-exchange chromatography of this tryptic SF1(A2). (a) Native SF1(A2); (b) tryptic SF1(A2); (c) peak 1; (d) peak 2; (e) peak 3; (f) peak 4.

segment of the heavy chain is digested to form the smaller 18K species. This probably arises from some dissociation of the soybean trypsin inhibitor–trypsin complex.

This degradation can be reduced by employing bovine pancreatic trypsin inhibitor, and essentially the same elution profile as shown in Figure 1 was obtained for tryptic SF1(A2) when this inhibitor was used to terminate the tryptic digestion (data not shown). The peptide compositions of the four fractions isolated in this case are shown in Figure 4 (lanes c–f) in order of their elution from the column. Clearly, the first fraction is comprised of peptides of 50K, 27K, and 21K molecular weight as found in the sample before thermal chromatography (Figure 4, lanes c and b, respectively). No peptides corresponding to the alkali light chains are present in the first component eluted. This result and the observation that the fourth component is predominantly the A2 light chain (Figure 4, lane f) strongly support the conclusion that the first fraction is the severed heavy-chain subunit.

Estimation of the Stability of Native and Tryptic SF1(A2). The above data show that the corresponding fractions obtained from either native or tryptic SF1(A2) represent the same species in undigested or digested forms, respectively. Since it was also observed that the elution patterns of each of these species were very reproducible from preparation to preparation, it appeared that these profiles could be analyzed to yield a semiquantitative measure of the dissociation induced by thermal chromatography, provided the chromatography is done under identical conditions. The dissociation is clearly given by the ratio of the area of peak 1 (free heavy chain) to the sum of the areas of peaks, 1, 2, and 3 (all species containing heavy chain), and by assuming the same color yield for the heavy chain and the SF1 species. The results of this analysis are presented in Table II. It is clear that tryptic cleavage significantly increases the degree of dissociation, indicating that the binding interaction between the two subunits is destabilized at least under the conditions of thermal chromatography.

Reassociation Studies with Severed Heavy Chains and A1 and A2 Light Chains. Previous studies with the native heavy chain have shown that it is capable of reassociating with either alkali light chain to form reconstituted SF1 isoenzymes (Sivaramakrishnan & Burke, 1982). It was also observed that

Table II: Degree of Dissociation of Tryptic SF1(A2) and Native SF1(A2) Induced by Thermal Ion-Exchange Chromatography at 39.5 °C in the Presence of 10 mM MgATP

protein	deg of dissociation (%)
native SF1(A2) P1[a]	33
native SF1(A2) P2	36
native SF1(A2) P3	34
tryptic SF1(A2) (27K, 50K, 21K) P1	58
tryptic SF1(A2) (27K, 50K, 21K) P2	61
tryptic SF1(A2) (27K, 50K, 18K) P1	60
tryptic SF1(A2) (27K, 50K, 18K) P2	68
tryptic SF1(A2) (27K, 50K, 18K) P3	64

[a] P represents different preparations of protein.

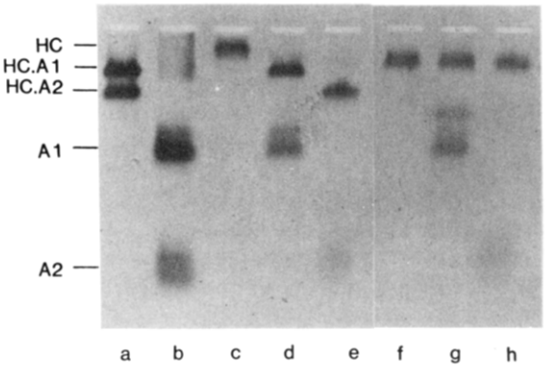

FIGURE 5: Native gel electrophoretograms of SF1 isozymes, A1 and A2 light chains, and 21K- and 18K-severed heavy chains before and after incubations with the free A1 and A2 light chains. Lanes a and b are the SF1 isoenzymes and the free light-chain mixtures, respectively. Lanes c, d, and e correspond to the 21K heavy chain alone and after incubation with free A1 and A2 light chains, respectively. Lanes f, g, and h represent the 18K heavy chain alone and together with the free A1 and A2 light chains, respectively.

cleavage of the free heavy chain at a site about 3 kilodaltons from its carboxyl terminus abolished this property and resulted subsequently in the formation of a truncated 21K carboxyl fragment of only 18K (Burke et al., 1983). It was of interest, therefore, to examine the reassociation potential of the two forms of severed heavy chain. The gel electrophoretograms obtained under native conditions for the two forms of the severed heavy chains alone and after incubation with the A1 and A2 light chains are presented in Figure 5. Each of these heavy chains runs as a single band (Figure 5, lanes c and f) with slightly different mobilities but slower than the corresponding SF1 isoenzymes (Figure 5, lane a). On incubation with the A1 and A2 light chains, it is found that the band corresponding to the 21K heavy chain (Figure 5, lane c) has disappeared and in its place bands with mobilities similar to those of SF1(A1) and SF1(A2), respectively, are now present (Figure 5, lanes d and e). In contrast, there is no change at all in the mobility of the 18K heavy chain (Figure 5, lane f) for the corresponding situations (Figure 5, lanes g and h), suggesting that no reassociation with the light chain has occurred. These observations indicate that cleavage at the T1 and T2 sites of the heavy chain does not abolish the capacity of the heavy chain to recombine with the free alkali light chains, but cleavage of the 21K segment to the 18K form does abolish this property. In this regard, the present results are consistent with the previous work with the native heavy chain (Burke et al., 1983) and suggest that the missing 3K peptide that gives rise to the 18K severed heavy chain is lost from the carboxyl-terminal end of the 21K segment. The reconstituted tryptic SF1 isoenzymes can be subsequently isolated by DEAE-cellulose chromatography under the conditions of Weeds & Taylor (1975).

Table III: ATPase Activities of Various Subfragment 1 and Heavy-Chain Species

protein	Ca-ATPase (s^{-1})	EDTA-ATPase (s^{-1})
SF1(A2)	8.8	15.4
tryptic SF1(A2)	8.4	12.5
native heavy chain	7.4	15.0
(27K, 50K, 18K)-HC	7.4	14.3
(27K, 50K, 21K)-HC	6.0	13.9
tryptic SH1-NEM[a] SF1(A2)	15.8	0.77
SH1-NEM (27K, 50K, 18K)-HC	14.3	0.60

[a] NEM is *N*-ethylmaleimide.

ATPase Activities of the Severed Heavy Chains of Tryptic SF1. Although it has been previously reported that the isolated heavy chain of SF1 is active, the levels of ATPase reported have been in poor agreement between laboratories (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982; Wagner & Stone, 1983). We have reinvestigated this for the native heavy chain, and we find that the ATPase levels are in reasonable agreement with those of the corresponding parent SF1. We typically find that the actin-activated ATPase of the heavy chain is between 76% and 100% of that of the SF1. The Ca^{2+}- and EDTA-activated ATPases of the heavy chain are always at least 80% of the corresponding activities of the SF1 (Table III). The ATPases of the two forms of the severed heavy chain are presented in Table III together with those of the tryptic SF1(A2). Depending on the preparation, small differences between the activities of the 21K and 18K forms of the severed heavy chains are sometimes observed, but the levels are generally quite close to those of the parent molecule. Also shown in Table III are the activities of the 18K form of the severed heavy chain isolated from tryptic SF1(A2) that was prelabeled with *N*-ethylmaleimide at the SH1 thiol. It is clear that the altered ATPase activities associated with this modification (Sekine & Kielley, 1964) are also expressed in this severed heavy chain.

The actin-activated ATPases of native and tryptic SF1(A2) and their corresponding heavy chains have also been measured to determine whether the proteolytically induced changes in the K_m for actin (Botts et al., 1982) could be attributed solely to the effects of cleavage on the heavy chain or to changes involving the bound alkali light chain. For this study, the 21K form of the severed heavy chain was chosen since it most closely corresponds to the state of this subunit in tryptic SF1(A2). The results are presented in Figure 6. The linear regression analyses of the data for the native and tryptic SF1(A2) are consistent with the previous findings of Botts et al., (1982), who showed that the V_{max} was unchanged but that the K_m was increased by tryptic cleavage. The results obtained with the native and tryptic heavy chains closely match the regression lines of their SF1 counterparts and indicate that the changes in the K_m of SF1 do not require the presence of the associated alkali light chain.

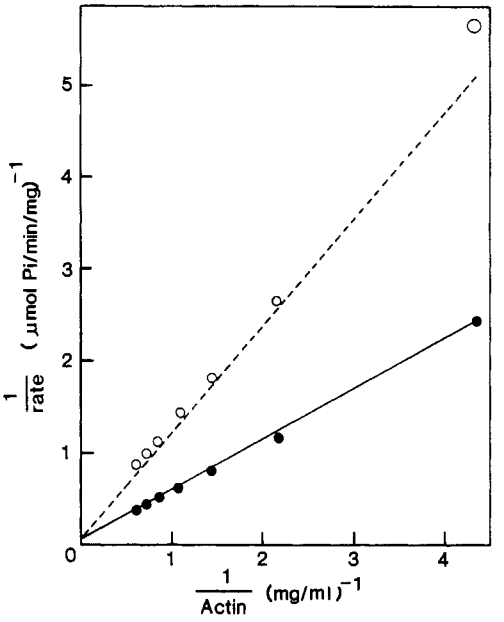

FIGURE 6: Lineweaver–Burk plots of the actin-activated ATPase of native heavy chain (●) and tryptic heavy chain (○). The continuous and discontinuous lines represent linear regression fits to the kinetic data for native and tryptic SF1(A2), respectively. SF1 and heavy chains were at 0.05 mg/mL in 0.05 M imidazole, 5 mM KCl, 3 mM $MgCl_2$, and 3 mM ATP, pH 6.95 at 25 °C. Actin concentrations ranged from 0.02 to 1.6 mg/mL.

Discussion

The present study was undertaken to examine (i) the consequences of tryptic cleavage on the stability of the subunit interactions in SF1, (ii) whether the altered affinity for actin accompanying tryptic cleavage was associated with such a change in this interaction, and (iii) the stability of the interactions between the three protease-resistant segments of the heavy chain. This was stimulated by recent observations that cleavages located at about 75 kilodaltons from the amino terminus markedly lowered the actin affinity of SF1 (Botts et al., 1982) and that the alkali light chain can communicate with actin in the acto–SF1 complex (Wagner & Weeds, 1977; Prince et al., 1981). It seemed possible, therefore, that the tryptic-induced change in the affinity for actin could be attributed to an alteration of the subunit interactions in the tryptic SF1.

To examine this possibility, the procedure of thermal ion-exchange chromatography was chosen for the following reasons. It had been previously shown that when SF1(A2) is subjected to DEAE-cellulose chromatography a portion of it is dissociated (Sivaramakrishnan & Burke, 1982). This observation suggested that the degree of dissociation occurring under standardized conditions could be used as an empirical estimate of the stability of the subunit interactions in SF1. It is assumed that a change in the degree of dissociation reflects an altered subunit interaction although it is realized that the converse does not necessarily apply.

The nature of the dissociation that is induced by subjecting native and tryptic SF1(A2) to thermal ion-exchange chromatography is apparent from their respective elution patterns shown in Figure 1. The electrophoretic analyses presented in Figures 2–4 established that the corresponding fractions represent undigested and digested forms of the same components and, in the order of their elution, these fractions are (i) the dissociated heavy chain, (ii) the undissociated SF1(A1), (iii) the undissociated SF1(A2), and (iv) the dissociated alkali light chain. The presence of SF1(A1) in these patterns is due to its contamination of the SF1(A2) preparations as has been reported by Weeds & Taylor (1975). It could be argued that peak 1 is a peculiar form of SF1 containing tightly bound, degraded A2 chains but this view is difficult to reconcile with the observations that (i) peak 1 readily reassociates at 4 °C with A1 and A2 chains to reconstitute the appropriate SF1 species and (ii) peak 4 is predominantly the free A2 light chain whose presence dictates that a portion of the SF1 must also be present as the free heavy chain.

The difference in the relative amounts of these fractions from the two forms of SF1 is a clear indication of an alteration in the subunit interactions, since it clearly reflects a difference

in the extent of dissociation induced in them by thermal chromatography. The results presented in Table II indicate that tryptic cleavage does significantly destabilize the subunit interactions. The fact that the severed heavy chain can be isolated as a complex comprised of either the 50K, 27K, and 21K peptides or the 50K, 27K, and 18K peptides demonstrates that the interactions between the heavy chain segments are considerably more stable than those between the two subunits. It is likely that this can be attributed in part to the presence of bound substrate, since both the heavy chain and SF1 are rapidly denatured at 37 °C or higher in the absence of the Mg nucleotide. Since there is accumulating evidence that these protease-resistant fragments may represent functional domains capable of extensive intercommunication (Mornet et al., 1981a,b, 1984; Muhlrad & Morales, 1984), a very stable interaction among them would not be unexpected.

The very similar ATPase properties of the two forms of the severed heavy chain to those of tryptic SF1(A2) in the absence of actin (Table III) signify that the cleavage near the carboxyl-terminal end of this subunit does not have a significant effect on the ATPase site. This would be in accord with the findings that the ATPase site apparently involves portions of the 27K segment (Szilagyi et al., 1979; Okamoto & Yount, 1983) and perhaps the 50K segment (Mahmood et al., 1984). On the other hand, since the 18K severed heavy chain isolated from the tryptic digest of SH1-modified SF1(A2) exhibits the same altered ATPase properties as the modified SF1, it may be concluded that the communication between the SH1 thiol in the 21K segment and the ATPase site of SF1 is independent of the associated alkali chain and can be expressed even with the cleavages at the T1 and T2 sites in the isolated severed heavy chain.

The high levels of ATPase activities found for these severed heavy chains can be attributed to the lack of aggregation of these preparations as seen from the gel electrophoretograms under native conditions (Figures 2A, 3A, and 5). Wagner & Stone (1983) have reported lower levels of ATPase activities for the heavy chain isolated from native SF1(A2) by thermal chromatography, but they state that their preparations appear to be highly aggregated on the basis of their electrophoretic data. We have no explanation for these differences.

The actin activation data presented in Figure 6 show that the native and digested heavy chains closely mimic their respective SF1 counterparts, and on this basis, we may conclude that the altered affinity for actin induced by the tryptic digestion of SF1, noted earlier by Botts et al., (1982), is primarily a property of changes solely involving the heavy chain. It is also clear from this work that the communication between the actin binding region and the ATPase site in tryptic SF1 occurs independently of the associated light chain.

The reassociation studies shown in Figure 5 demonstrate that cleavages at the T1 and T2 sites of the heavy chain do not prevent it from reassociating with the free alkali light chain, even though this interaction is weakened near physiological conditions. The inability of the 18K form of the severed heavy chain to reassociate with the free light chains suggests that it is missing the carboxyl-terminal 3K segment of the heavy chain. This conclusion is supported by previous studies with the native heavy chain where it was shown (i) that the 18K fragment was produced by cleavage at this site and (ii) that this cleavage abolished light-chain rebinding (Burke et al., 1983). It is of interest to note that with the A1 chain, the reconstituted tryptic SF1(A1) represents a species not previously seen since it contains an undegraded form of the A1 chain and, thus, differs from the standard tryptic SF1(A1) preparations which possess a partially degraded form of this chain.

It is still unclear how cleavage of the heavy chain in SF1 at the T1 and T2 sites causes a destabilization of the subunit interactions. From cross-linking studies, it appears that the associated light chain is proximal to both the 27K and the 21K segments of the intact heavy chain (Labbé et al., 1981), but the actual locations of the contact regions have not to our knowledge been identified. It is conceivable that contact regions for the light chains involve both these segments of the heavy chain and that cleavage at one or both of these "connector" sites alters the affinity at the contact regions. Studies are currently under way to determine the contribution to the subunit destabilization of cleavage at each of these proteolytically sensitive regions of the heavy chain.

Registry No. ATPase, 9000-83-3.

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Pressure Effects on Actin Self-Assembly: Interspecific Differences in the Equilibrium and Kinetics of the G to F Transformation†

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ABSTRACT: Purified skeletal muscle actins from species whose ambient pressures range from 1 to >500 atm were examined for the sensitivity to hydrostatic pressure of the globular (G) to filamentous (F) self-assembly reaction. Both the equilibrium position and the kinetics of self-assembly were affected by pressure. Increased pressure shifted the self-assembly equilibrium toward the monomer (G) state and reduced the rate of F-actin assembly. For most of the actins studied, the perturbation by pressure of F-actin formation decreased with increasing measurement of pressure, indicating that F-actin has a higher compressibility than G-actin. The increase in system volume and compressibility concomitant with the assembly of F-actin can be interpreted as reflections of the major role played by hydrophobic effects in stabilizing F-actin and of the existence of "hard" binding sites, in the terminology of Torgerson et al. [Torgerson, P. M., Drickamer, H. G., & Weber, G. (1979) *Biochemistry 18*, 3079-3083], in the actin subunits. For actin from the deepest occurring species studied, the teleost fish *Coryphaenoides armatus*, which occurs to depths of approximately 5000 m (equivalent to 501 atm of pressure), there was no difference in compressibility between G-actin and F-actin; that is, the effect of increasing pressure on self-assembly was linear over the entire pressure range examined, 600 atm. The self-assembly reaction of the actin from *C. armatus* also differed from that of the other actins examined in that the G to F equilibrium was relatively insensitive to increased pressure; i.e., the volume change (ΔV) of assembly was small. The changes in enthalpy (ΔH) and entropy (ΔS) accompanying self-assembly of *C. armatus* actin also were small, relative to the other actins examined [Swezey, R. R., & Somero, G. N. (1982) *Biochemistry 21*, 4496-4503]. Pressure effects on the kinetics of self-assembly were greater for this pressure-adapted actin than for actin from rabbit muscle, however. The actin from a shallower occurring congener of *C. armatus*, *Coryphaenoides acrolepis* (depth of occurrence of 250-2100 m), displayed responses to pressure similar to those of actins from shallow-living fishes, an observation which suggests that pressure effects on actin assembly may not become selectively important until pressures in excess of 200 atm are reached. On the basis of the calculated volume changes and the changes in protein compressibility upon self-assembly, we propose that the interspecific variations in the effects of pressure on the G to F transformation may result either from differences in the amount of hydrophobic effect contributing to the stabilization of F-actin or from varying degrees of "hardness" of the subunit contact sites in F-actin. In addition, for actin of *C. armatus*, we propose that concomitant with the assembly of F-actin there is an increase in exposure to solvent of polar or charged residues. Hydration of these residues during self-assembly would reduce the enthalpy, entropy, and volume changes accompanying self-assembly and would contribute to the negative free-energy change of self-assembly, thereby compensating for a lower contribution by the hydrophobic effect in this actin self-assembly reaction. The low ΔH, ΔS, and ΔV of self-assembly of *C. armatus* actin, and the similar ΔG of self-assembly relative to the other actins studied, support this model.

Despite the fact that more than three-fourths of the biosphere, when viewed in terms of volume (Childress, 1982), consists of environments where hydrostatic pressures lie in the range of several hundred atmospheres, the influences of pressure on biochemical systems, and the needs for adaptation to high pressure by deep-living organisms, remain poorly understood [cf. Jaenicke (1983), Somero et al. (1983), and Hochachka & Somero (1984)]. There are, in fact, two strong reasons for examining the influences of pressure on biochemical systems. First, such studies may lead to a closer understanding of the processes of molecular evolution, by which the bio-

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