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Stability and Substructure of Cardiac Myosin Subfragment 1 and Isolation and Properties of Its Heavy-Chain Subunit†

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Received June 5, 1985

ABSTRACT: The substructure and the thermal stability of the subunit interactions of bovine cardiac myosin subfragment 1 (SF1) have been examined. The results are in agreement with previous reports that the cardiac protein is cleaved in a very similar manner [Flink, I. L., & Morkin, E. (1982) *Biophys. J. 37*, 34; Korner, M., Thiem, N. V., Cardinaud, R., & Lacombe, G. (1983) *Biochemistry 22*, 5843-5847] but at a much faster rate [Applegate, D., Azarcon, A., & Reisler, E. (1984) *Biochemistry 23*, 6626-6630] than the skeletal protein. Additionally, it is found that the long-lived, steady-state intermediates formed by these proteins with MgATP at high ionic strength differ in their susceptibilities to tryptic attack especially at the 27K/50K junction of the associated heavy chains, suggesting a different conformation for these intermediates of the cardiac and skeletal SF1's. The thermal stability of the subunit interactions under conditions approaching the physiological state was examined by thermal ion-exchange chromatography of cardiac SF1 at 39.5 °C in the presence of MgATP. This results in the separation of part of the protein as the isolated heavy chain which is found to exhibit high levels of ATPase activity in the absence and presence of actin. Tryptic digestion of cardiac SF1 prior to thermal ion-exchange chromatography produces greater dissociation, with the heavy chain in this case being isolated as a complex of 27K, 50K, and 18-20K fragments. This tryptic heavy chain exhibits similar levels of ATPase activities as its parent tryptic SF1, showing the same V_{max} for the actin-activated MgATPase as the undigested cardiac SF1 and heavy chain but a larger K_m for actin.

It is now well established that the contractile properties of cardiac and skeletal muscles differ in accordance with the physiological requirements of these two types of muscles. The slower speed of contraction of cardiac muscle is apparently related to the lower rate of actin-activated MgATPase of its constituent myosin compared to that for the skeletal tissue (Taylor & Weeds, 1976). At the molecular level, it has been shown that despite the similarities in size and subunit structure of these two types of myosin, the chemical structures (primary structure) of the heavy and light polypeptide chains of cardiac myosin differ from their counterparts in skeletal myosin (Hoh et al., 1979; Flink et al., 1979; Chizzonite et al., 1982; Weeds, 1975; Leger & Elzinga, 1977). Since the higher orders of structure necessary for the expression of function are directly related to the primary structure, it is not unreasonable to suggest that the kinetic differences are directly attributable to differences in the structures of these two myosins. The ways in which the conformation of the cardiac protein differs from that of the skeletal protein have not as yet been fully characterized, but it is likely that if these differences do exist, they would be found in the myosin subfragment 1 (SF1)[1] regions where the sites for ATPase and actin binding are located.

The SF1 isolated by chymotryptic digestion of skeletal myosin is comprised of a heavy chain of 95K and a light chain

†Supported by U.S. Public Health Service Grants AM 3327 and NS15319 and a Summer Student Fellowship (B.E.M.) provided by the Northeast Ohio Chapter of the American Heart Association.

[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; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

0006-2960/86/0425-0884$01.50/0

(known as an alkali light chain) (Yagi & Otani, 1974; Weeds & Taylor, 1975). The alkali light chain exists in two distinct but chemically related forms known as A1 and A2, of 21K and 16.5K, respectively (Frank & Weeds, 1974). Cardiac SF1 prepared in the same fashion has a similar subunit composition except that the associated light chain is slightly larger than the corresponding skeletal counterparts, being about 28K.

Recently the substructure of cardiac SF1 has been investigated by examining the course and extent of tryptic cleavage. These studies showed that the cardiac SF1 is attacked at essentially the same two restricted regions of its associated heavy chain as was found previously for skeletal SF1 (Flink & Morkin, 1982; Korner et al., 1983) and results in a tryptic SF1 species comprised of the light chain and the three protease-resistant fragments of about 27K, 50K, and 21K arranged in this order in the linear sequence. These findings suggest that the conformation of the cardiac protein bears a high degree of similarity to that of skeletal SF1. More recently, it was shown that the rates of attack by trypsin at these two sites are appreciably faster for the cardiac protein and, furthermore, the protective effect at the 75K junction due to actin binding is appreciably lower for this SF1 (Applegate et al., 1984).

Another difference between cardiac and skeletal myosins is the stability of the subunit interactions. In this regard, the cardiac protein appears to be more stable to thermal dissociation. A substantial dissociation of its light chains without loss in ATPase activity has been shown to occur in the presence of ATP at 37 °C in the absence of divalent cations while, on the other hand, the presence of divalent cations was found to prevent subunit dissociation (Higuchi et al., 1978).

In the present study, we have examined a number of properties of the cardiac SF1 to determine to what extent these may differ from those observed for the skeletal protein. We show, in agreement with the data recently reported by Applegate et al. (1984), that the cardiac SF1 is cleaved in its heavy chain at a faster rate by trypsin at sites corresponding closely to those attacked in skeletal SF1. This difference in cleavage rate of the associated heavy chains is also observed at high ionic strengths in the presence of MgATP, but, whereas these conditions are found to protect the 27K/50K junction in the skeletal protein, a much lower degree of protection is observed with the cardiac protein.

The stability of cardiac SF1 has also been examined with regard to its subunit interactions under conditions that more closely approach the physiological state (37–39.5 °C and 10 mM MgATP). The results indicate that ion-exchange chromatography of cardiac SF1 under these conditions leads to the isolation of a portion in the form of its free heavy chain, and suggest that it exists in these conditions in reversible equilibrium with its dissociated subunits in a similar fashion to that found previously for the skeletal protein (Sivaramakrishnan & Burke, 1981).

Additional similarities between these two SF1 species are found with respect to the effect of tryptic digestion on their thermal stabilities estimated by the dissociation induced on thermal ion-exchange chromatography. The fact that the severed heavy chain of tryptic cardiac SF1 can be isolated by thermal ion exchange as a complex of the three protease-resistant fragments shows that the heavy chain is stabilized by interactions between these fragments and that the associated light chain is not essential for maintaining the integrity of the structure of the tryptic heavy chain.

The isolated cardiac native and tryptic heavy chains are shown to exhibit essentially the same levels of ATPase activities in the absence and presence of actin as the corresponding SF1 species, indicating that the 28K light chain is nonessential for this function. The combined data indicate that in terms of its thermal stability and properties of its isolated heavy chain, cardiac SF1 is remarkably similar to that of the skeletal form. The only significant difference which was found in the present study is in the rates of cleavage of the protease-sensitive regions of the heavy chain to tryptic attack which suggests that their accessibility in this SF1 species may differ and influence the interactions between the three protease-insensitive segments of the heavy chain.

Materials and Methods

Distilled water was purified to reagent grade by a Millipore QTM system and used throughout. Trypsin, bovine pancreatic trypsin inhibitor, and ATP were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were analytical grade.

Preparation of Proteins. Cardiac myosin was prepared from freshly dissected bovine hearts according to the method of Taylor & Weeds (1976). Cardiac subfragment 1 was prepared by chymotryptic digestion of myosin and purification on DEAE-cellulose as described by Weeds & Taylor (1975). Skeletal myosin was isolated from male albino New Zealand rabbits by the method of Godfrey & Harrington (1970). Skeletal SF1 was prepared by chymotryptic digestion and separation into its isomeric forms according to the method of Weeds & Taylor (1975). Actin was prepared according to the method of Spudich & Watt (1972). Protein concentrations were determined either by absorption using values for $E^{1\%}_{280nm}$ of 5.6, 6.4, 5.5, 7.5, and 11.0 for cardiac myosin, cardiac subfragment 1, skeletal myosin, skeletal subfragment 1, and actin, respectively, or by the colorimetric method of Lowry et al. (1951).

Tryptic Digestion of Cardiac and Skeletal Subfragment 1. SF1 was digested in a method similar to the one used by Mornet et al. (1979). The low ionic strength digestion was carried out in 0.05 M imidazole and 0.1 mM dithiothreitol, pH 7.0, buffer at 25 °C with a ratio of subfragment to trypsin of 100:1 (w/w). The high ionic strength digestion was carried out in a 0.5 M imidazole, 0.5 M NaCl, 10 mM $MgCl_2$, 10 mM ATP, and 0.1 mM dithiothreitol, pH 7.0, solution using a ratio of subfragment to trypsin of 200:1 and 100:1 for the cardiac and skeletal proteins, respectively. Both digestions were terminated by the addition of an equal weight of bovine pancreatic trypsin inhibitor. The rate of cleavage was assayed by gel electrophoresis in the presence of sodium dodecyl sulfate and scanning the electrophoretograms at 550 nm with a Shimadzu CS930 TLC scanner and a DR-2 data recorder. Normalization of the amount of 50K fragment produced is expressed as the percentage of the theoretical yield expected for complete cleavage of SF1.

Thermal Ion-Exchange Chromatography. This was performed essentially as described earlier by Sivaramakrishnan & Burke (1982). The samples were loaded on the column at a concentration of 2 mg/mL, and 15 mg was applied. The internal temperature of the column was 39.5 °C. The solvent conditions and collection methods remain unchanged from the previous study. The elution profile was determined by removing a 75-μL aliquot from each fraction and using the Bradford procedure (1976) to detect the presence of protein. The tryptic samples applied to the column were digested for 30 min according to the low ionic strength conditions described earlier.

The eluted peaks were concentrated by dialysis vs. saturated ammonium sulfate at 4 °C, and the precipitated protein was

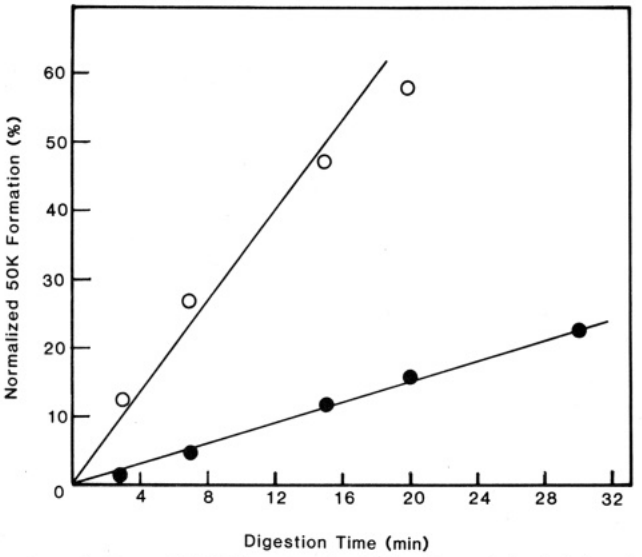

FIGURE 1: Rate of 50K fragment formation from skeletal (●) and cardiac (○) SF1's digested in the presence of MgATP (10 mM) in high ionic strength (0.5 M NaCl) with 0.05 M imidazole, pH 7.0 at 25 °C. The protein concentrations were 2.0 mg/mL, and the SF1 to trypsin ratios were 100:1 and 200:1 for the skeletal and cardiac proteins, respectively. The normalized amount of 50K fragment is expressed as the percentage of the theoretical amount for complete digestion of the heavy chain.

examined by polyacrylamide gel electrophoresis under both nondenaturing and denaturing conditions.

Gel Electrophoresis under Nondenaturing Conditions and in the Presence of Sodium Dodecyl Sulfate. Native gel electrophoresis was performed as described by Burke & Sivaramakrishnan (1981). Sodium dodecyl sulfate gel electrophoresis was done by the procedure of Laemmli (1970) using 12.5% polyacrylamide in the separating portion of the gel.

ATPase Activity Measurements. The Ca^{2+}- and EDTA-activated ATPase activities were performed according to the method of Kielley & Bradley (1956) and Kielley et al. (1956). The actin-activated ATPases were done by using a colorimetric method described by Reisler (1980) with the following modification. The incubations and color determination of the released phosphate were performed according to the procedure of Seimankowski as described by White (1982).

RESULTS

Course and Extent of Tryptic Cleavage of Cardiac and Skeletal SF1. Recent work by Applegate et al. (1984) has shown that the associated heavy chain of cardiac SF1 is cleaved at a higher rate at sites which are similar to those that occur in the skeletal SF1. In the present work, we have examined the tryptic susceptibility of cardiac SF1 under two different sets of conditions: (i) in the absence of NaCl and (ii) in the presence of both NaCl (0.5 M) and MgATP (10 mM). By quantitative gel densitometry, it is found that the presence of high salt and MgATP together results in a similar degree of protection to the heavy chains of both the cardiac and skeletal SF1's. Thus, in the absence of salt and nucleotide, the rates of digestion of the cardiac and skeletal heavy chains were found to be 0.648 and 0.246 min^{-1}, respectively, whereas in their combined presence the rates were found to be 0.286 and 0.108 min^{-1}, respectively. For both sets of conditions, we find in agreement with the findings of Applegate et al. (1984) that the cardiac heavy chain is more susceptible than its skeletal counterpart. An additional difference between the two forms of SF1's is the degree of protection afforded the 27K junction by the steady-state intermediate SF1**MgADP·P_i conformations favored by the high ionic strength conditions (Harrington et al., 1975). This can be readily seen from the percentage of the amount of 50K formed from the cardiac and skeletal forms plotted as a function of digestion time as shown in Figure 1. It is clear that the 27K junction is more exposed to attack in the case of the cardiac protein.

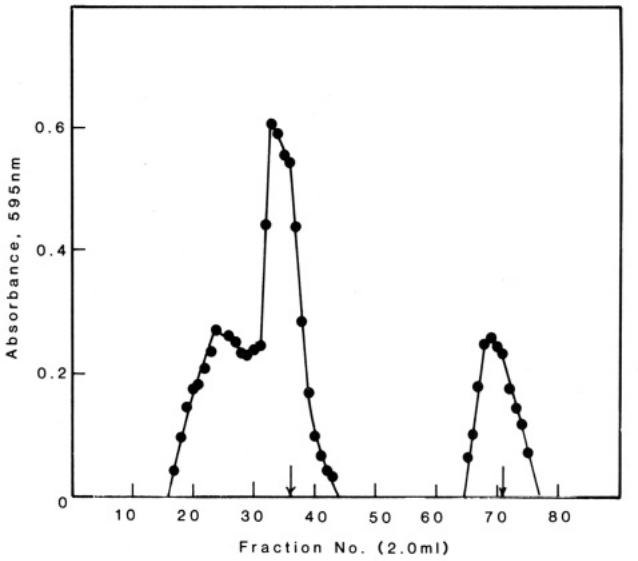

FIGURE 2: Elution pattern of cardiac SF1 from thermal ion-exchange chromatography. The ordinate represents the absorption obtained by the addition of 75-μL aliquots from each fraction to 1.0 mL of the Bradford (1976) reagent. The first arrow indicates the start of the 0.5 M NaCl step, and the second, a 2 M NaCl step.

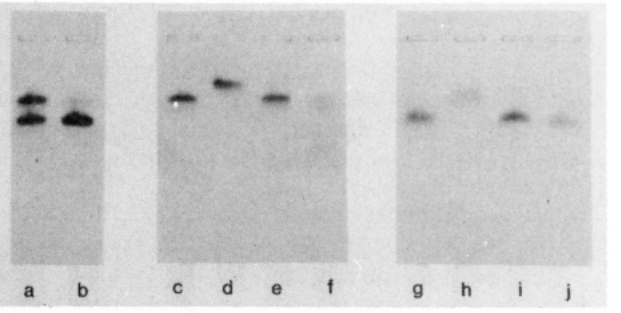

FIGURE 3: Gel electrophoretograms obtained under nondenaturing conditions of cardiac SF1 and fractions isolated by thermal ion-exchange chromatography. (a) Skeletal SF1(A1) and SF1(A2) mixture; (b) skeletal SF1(A2); (c) cardiac SF1; (d, e, and f) first, second, and third protein fractions respectively eluted from the thermal column as shown in Figure 2; (g) tryptic cardiac SF1; (h, i, and j) first, second, and third protein fractions respectively eluted from thermal ion-exchange chromatography of tryptic cardiac SF1.

Thermal Ion-Exchange Chromatography of Cardiac SF1. The elution pattern obtained when cardiac SF1 is subjected to thermal ion-exchange chromatography is presented in Figure 2. Under isocratic conditions, it is found that the protein is partially resolved into two distinct fractions. On application of a salt step (0.5 M NaCl), a further fraction is eluted from the column. These fractions have been examined by gel electrophoresis under nondenaturing conditions, and the resulting electrophoretograms are shown in Figure 3. The preparation of cardiac SF1 prior to thermal ion-exchange chromatography is quite homogeneous as evidenced by the presence of one major protein band [Figure 3(c)]. The first fraction obtained from the thermal column exhibits a mobility which is significantly slower than that of the cardiac SF1 (Figure 3, lanes d and c, respectively). Its polypeptide composition and that of the unchromatographed cardiac SF1 are presented in the sodium dodecyl sulfate electrophoretograms

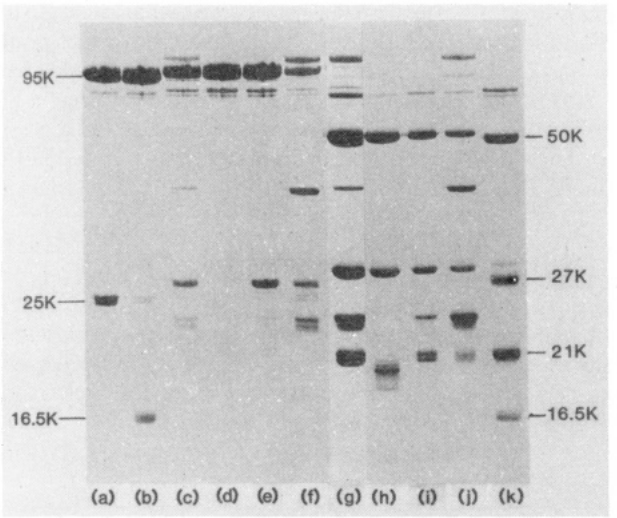

FIGURE 4: SDS gel electrophoretograms of (a) skeletal SF1(A1), (b) skeletal SF1(A2), (c) cardiac SF1, (d, e, and f) the first, second, and third protein fractions shown in Figure 2, (g) tryptic cardiac SF1, (h, i, and j) the first, second, and third protein fractions respectively isolated by thermal ion-exchange chromatography of tryptic cardiac SF1, and (k) tryptic skeletal SF1(A2).

of Figure 4 (lanes d and c, respectively). It is clear that, whereas the cardiac SF1 is comprised primarily of peptides of 95K and 27K corresponding to the heavy and light chains, respectively, the first fraction from the thermal chromatography is comprised solely of the 95K heavy chain, and on this basis, we believe that this first fraction is the free heavy-chain subunit of cardiac SF1. The compositions of the remaining fractions can be readily identified from the mobilities of their constituents under native conditions (Figure 3) and their polypeptide compositions shown in Figure 4. On this basis, we attribute the second fraction to undissociated cardiac SF1 while the third fraction appears to contain a small amount of SF1, some free light chain, and a band corresponding to a weight of 40K.

Prior trypsin digestion of cardiac SF1 was found to have little effect on its elution behavior, and the resulting chromatogram was found to consist of three major components similar to those shown in Figure 2, although the relative amounts of the first and second fractions were reversed from that found for the native SF1. The electrophoretic behavior of these fractions and that of the tryptic cardiac SF1 prior to thermal chromatography under native and sodium dodecyl sulfate conditions are also shown in Figures 3 and 4. It is clear that the tryptic SF1 moves with a faster mobility than the undigested protein (Figure 3, lanes g and c, respectively). The first fraction isolated from tryptic cardiac SF1 [Figure 3(h)] has a mobility distinctly slower than that of either undigested or tryptic SF1 (Figure 3, lanes c and g, respectively). The second and third fractions isolated from the tryptic SF1 (Figure 3, lanes i and j, respectively) have similar mobilities to that of the tryptic SF1. The polypeptide compositions of these fractions are shown in the sodium dodecyl sulfate electrophoretograms of Figure 4. The tryptic cardiac SF1 prior to thermal ion-exchange chromatography is comprised predominantly of the heavy-chain fragments of 50K, 28K, and 21–22K and the degraded light chain of about 23K [Figure 4(g)]. The first fraction isolated by thermal chromatography of the tryptic cardiac SF1 has the polypeptide composition shown in Figure 4(h). It is clearly comprised of 50K and 28K fragments and peptides ranging from about 18K to 20K. These latter peptides are presumed to represent degraded forms of the 21–22K fragment on the basis of earlier observations

Table I: ATPase Activities of Native and Tryptic Cardiac SF1's and Their Respective Free Heavy Chains

protein	ATPase act. (s^{-1})	
	Ca-ATPase	EDTA-ATPase
SF1	2.6	4.5
tryptic SF1	2.8	4.3
heavy chain	4.2	4.7
tryptic heavy chain	3.1	5.6

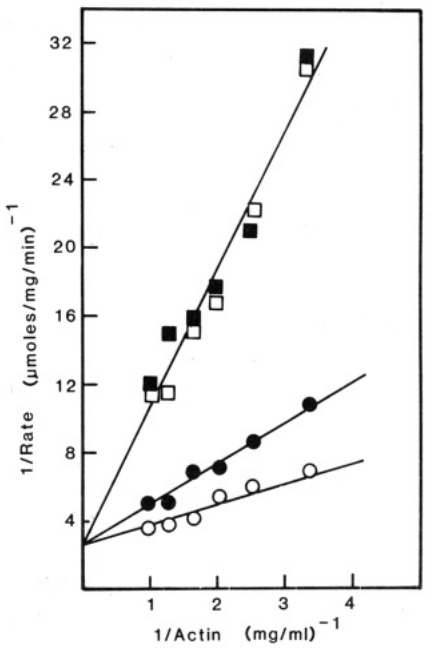

FIGURE 5: Double-reciprocal plots of actin activation of native and tryptic cardiac SF1's and their respective heavy chains isolated by thermal ion-exchange chromatography. Incubations were done in 0.05 M imidazole, 5 mM KCl, 3 mM ATP, and 3 mM $MgCl_2$ at 25 °C. The concentrations of SF1 and heavy chain were 0.05 mg/mL. (○) Native cardiac SF1; (●) native cardiac heavy chain; (□) tryptic cardiac SF1; (■) tryptic cardiac heavy chain.

that the 21K fragment of tryptic skeletal SF1 is degraded during thermal ion-exchange chromatography to an 18K peptide (Burke et al., 1983). Thus, it appears that the severed heavy chain of tryptic cardiac SF1 can be isolated as a stable complex comprised of the three interacting, protease-resistant fragments of 50K, 28K, and 21–22K. The second fraction obtained from tryptic cardiac SF1 is comprised of 50K, 28K, and 21–22K heavy-chain fragments as well as the 23K degraded light chain [Figure 4(i)], and from its electrophoretic mobility under native conditions [Figure 3(i)], it appears to be undissociated tryptic cardiac SF1. The third fraction of thermal ion exchange of tryptic SF1 shows bands of 50K, 28K, and small 21–22K heavy-chain fragments together with a band at about 40K and a large amount of 23K degraded light chain.

ATPase Properties of the Free Heavy Chains Isolated from Undigested and Digested Cardiac SF1. The ATPase properties of the different cardiac SF1 species and their corresponding heavy chains in the absence of actin are presented in Table I. It is clear that these heavy chains exhibit greater Ca-activated ATPase activities than their corresponding SF1 species. The K/EDTA-ATPase activities are also somewhat higher for the free heavy chains. Thus, as demonstrated previously by Wagner & Giniger (1981) and by this laboratory (Sivaramakrishnan & Burke, 1982), the heavy chain of myosin is the catalytic subunit and does not require the associated light chain for activity. The ability of actin to activate the cardiac SF1 and the corresponding heavy chains is shown in Figure 5. The data indicate that the V_{max} for the undigested cardiac

heavy chain is similar to that of its parent SF1 although the K_m for actin appears to be slightly increased by removal of the light chain. It is also evident that the effect of tryptic cleavage is to increase the K_m for actin and since this is observed for both the cardiac SF1 and its heavy chain, this change in K_m is caused by changes in the heavy-chain subunit and not by changes in the subunit interactions. Although this increase in the K_m makes estimates of the V_{max}'s of the tryptic forms less reliable, the data suggest that these values may approach those of the undigested cardiac proteins.

Discussion

This study was undertaken to examine the substructure of cardiac SF1 and the stability of its subunit interactions with the purpose of determining the extent that these properties resemble those of the skeletal protein. Although it has been known for some time that the ATPase activities of cardiac myosin and its SF1 were significantly lower than those of their skeletal counterparts, until recently very little about the substructure of cardiac SF1 was known, in contrast to the situation with the skeletal proteins. The work by Flink & Morkin (1982) and Korner et al. (1983) has revealed that the course of tryptic digestion of cardiac SF1 is quite similar to that of skeletal SF1 and results in the cleavage of the associated heavy chain at sites about 27–29K and 73–75K from its amino terminus. This similarity suggests that the SF1's have essentially the same basic architecture of three interacting, protease-resistant domains of 27–28K, 50K, and 21–22K arranged in this order in the linear sequence connected by the two protease-sensitive linker regions.

Despite the similarity, the rates of tryptic cleavage presented in the present work confirm the recent results of Applegate et al. (1984) that the linker regions of the cardiac SF1 are more accessible to trypsin. As noted by these workers, a comparison of the sequences of the linker region for the two proteins at the 75K junction (Kavinsky et al., 1984) indicates that there are substantial differences at the adjacent 12 or so residues at the amino-terminal so that a more rapid cleavage at this junction may reflect conformational dissimilarities related to these sequence differences.

The recent work of Applegate et al. (1984) has also demonstrated differences in the structure of the two SF1's during their binding to actin especially with regard to the lesser degree of protection given to the 75K junction of cardiac acto-SF1 in the presence and absence of MgADP. The present work has also revealed a difference between the two SF1's in a conformational state directly associated with the ATPase reaction. This difference is found in the extent of protection given to the 27K junction from trypsin digestion in the presence of high ionic strength with MgATP where the long-lived SF1**MgADP·P_i conformation is favored (Harrington et al., 1975). From the extent of 50K formation (Figure 1), it is clear that the 27K junction is greatly protected in the case of the skeletal protein but it is quite susceptible in the cardiac SF1. The difference is substantial when it is noted that the amount of trypsin used in the digest of the cardiac SF1 was half that employed for the skeletal SF1.

The results of the thermal ion-exchange chromatography presented in Figure 2 indicate a high degree of similarity between the cardiac and the skeletal proteins in terms of the stability of the subunit interactions (Sivaramakrishnan & Burke, 1982). The fact that a portion of the cardiac protein can be isolated as the free heavy chain under conditions where the ATPase activity is not lost (Higuchi et al., 1978) suggests that it exists in a rapid, reversible equilibrium with its dissociated subunits under conditions which approach the physiological state. A further similarity in the two forms of SF1 is that prior cleavage at the two protease-sensitive sites destabilizes the subunit interactions (Burke & Kamalakannan, 1985). A comparison of the relative amounts of protein isolated in the first fractions from the undigested and digested cardiac SF1 indicates that more protein was released in this fraction for the tryptic-digested protein (data not shown).

The retention of high levels of ATPase activities in the free cardiac heavy chain (Table I) demonstrates that the associated 27K light chain is not required for this function and supports recent findings by Wagner & Giniger (1981) and by this laboratory (Sivaramakrishnan & Burke, 1982) for the skeletal heavy chain. The fact that, in the case of tryptic cardiac SF1, its severed heavy chain can also be isolated in active form (Table I and Figure 5) demonstrates that the interfragment interactions within the heavy chain are considerably more stable than those between the two subunits as has also been recently demonstrated for the skeletal SF1 (Burke & Kamalakannan, 1985).

In terms of the interaction with actin, our results show that the free heavy chains of cardiac SF1 exhibit similar V_{max} values of their MgATPase at infinite actin concentrations (Figure 5). The values of V_{max} obtained in the present study are lower than those reported from other laboratories, but, since cardiac SF1 is known to be very labile (Taylor & Weeds, 1976; Flamig & Cusanovich, 1983; Siemankowski & White, 1984), it is likely that this difference is related to the extra time required in the present study for the isolation of the free heavy chains. It is clear that the increase in the K_m for actin of cardiac SF1 on trypsin digestion (Figure 5) is similar to that shown previously for the skeletal protein (Botts et al., 1982) that is also reflected in the corresponding free heavy chains as has been recently reported for skeletal SF1 (Burke & Kamalakannan, 1985).

Acknowledgments

We thank Jacqueline Bliss, James Craig, and Scott Purvis for technical assistance.

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

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Gangliosides as Markers of Cortisone-Sensitive and Cortisone-Resistant Rabbit Thymocytes: Characterization of Thymus-Specific Gangliosides and Preferential Changes of Particular Gangliosides in the Thymus of Cortisone-Treated Rabbits†

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Received July 1, 1985

ABSTRACT: Neutral glycosphingolipids and gangliosides in rabbit thymus, spleen, bone marrow, and erythrocyte ghosts were analyzed by conventional chemical and enzymatic procedures and negative ion fast atom bombardment mass spectrometry (FABMS). Thymus gangliosides showed a characteristic composition. Major gangliosides comprising 75% of the total thymus gangliosides were sialosyl lacto-*N*-*neo*-tetraosyl- and sialosyl lacto-*N*-*nor*-hexaosylceramides containing NeuGc and palmitic acid. These major thymus gangliosides were not detected in spleen, bone marrow, or erythrocytes, whereas GD1a, which was not present in the thymus even in a trace amount, was present in spleen and bone marrow. In addition, the major gangliosides in rabbit thymus were preferentially reduced when an animal was given an intraperitoneal injection of cortisone acetate, as found on analysis 48 h later. The decrease was accompanied by a concomitant increase in NeuAc-containing GM3 with longer chain fatty acids.

By application of the ganglioside-mapping technique (Iwamori & Nagai, 1978c) to studies on the tissue distribution of gangliosides, we found in rabbit that the ganglioside distribution in the thymus is uniquely different from those in other tissues (Iwamori & Nagai, 1981b,c) and that several gangliosides only occur in this tissue. Recently, considerable attention has been paid to the possible involvement of glycosphingolipids in various important immunological functions such as in the case of cell surface markers of immunocyte subpopulations (Kasai et al., 1980; Momoi et al., 1980; Nakano et al., 1980; Schwarting et al., 1980; Young et al., 1980; Taki et al., 1981; Rosenfelder et al., 1982; Nagai et al., 1984; Ugorski et al., 1984), differentiation antigens (Habu et al., 1980; Schwating et al., 1980; Akagawa et al., 1981; Kannagi, et al., 1983; Taki et al., 1983), lymphokine receptors (Riedl et al., 1982), mitogen-induced activation (Rosenfelder et al., 1978; Ryan & Shinitzky, 1979; Whisler & Yates, 1980; Sela, 1981), and immune recognition (Hakomori, 1981). In mouse, for example, globoside was identified as a serological marker of alloantigen-stimulated T lymphocyte percursors of both helper and cytolytic T cells (Muhlradt et al., 1984), and the

† This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan and a grant for specific diseases from the Ministry of Health and Welfare of Japan and by the Special Coordination Funds for promoting science and technology from the Science and Technology Agency of Japan. Support by the Naito Foundation is also gratefully acknowledged. This study is one of a series of investigations on the characterization of gangliosides in the thymus and related organs, the first five of which were reported elsewhere: 1 (Iwamori & Nagai, 1978a), 2 (Nagai & Iwamori, 1980a), 3 (Nagai & Iwamori, 1980b), 4 (Iwamori & Nagai, 1981b), and 5 (Iwamori & Nagai, 1981c).

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0006-2960/86/0425-0889$01.50/0