Specific Interactions of the Alkali Light Chain 1 in Skeletal Myosin Heads Probed by Chemical Cross-Linking†

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ABSTRACT: We have investigated the enzymatic properties of the 120K cross-linked heavy-chain–light-chain derivative formed upon reaction of chymotryptic myosin subfragment 1 (S-1) isoenzymes with the bis(imido esters) dimethyl 3,3′-dithiobis(propionimidate) and dimethyl suberimidate. The formation of the 120K product was accompanied for S-1(A1) but not for S-1(A2) by a loss of the actin-activated ATPase without alteration of the Ca^{2+}-ATPase whereas the Mg^{2+}-ATPase was increased 2-fold. Up to 70%, the inhibition of the acto–S-1(A1) ATPase activity was closely correlated with the extent of cross-linking of the A1 light chain; this activity could be largely restored upon cleavage of the cross-link using the reversible cross-linker dimethyl 3,3′-dithiobis(propionimidate). The covalent link affected the acto–S-1(A1) Mg^{2+}-ATPase activity by reducing 3-fold the V_{max} and increasing 2-fold the K_{app}. On reacting for the first time the hydrophobic, carboxyl group directed cross-linker *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) with the acto–S-1(A1 + A2) complex, we found that the N-terminal tail of the A1 light chain was cross-linked to actin to an extent much larger than observed earlier with the water-soluble 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; like the latter agent, EEDQ elicited the covalent union of the A1 subunit to the COOH-terminal part of actin. This cross-linker appears to be a valuable chemical probe of the F-actin–A1 light-chain interaction. Finally, no cross-linking of actin to the isolated A1 light chain was observed in spite of the reported binding of F-actin to this light chain in the isolated state; in contrast, the cross-linking occurred when the A1 subunit was complexed to the isolated COOH-terminal 20K fragment of the S-1 heavy chain. These results suggest that the heavy chain changes the conformation of the light subunit and thereby determines its cross-linking ability to actin.

The alkali light chain A1[1] or A2 present in the skeletal muscle myosin heads constitutes an obligatory component of the myosin molecule. While the light subunit seems to have no apparent direct influence on the myosin ATPase activities (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982), its actual biological role during energy transduction by the actomyosin complex remains to be elucidated. For molluscan muscles, the regulation of contraction appears to involve a critical movement of this type of light chain relative to the Ca^{2+} binding regulatory subunit (Hardwicke & Szent-Gyorgyi, 1985). In the case of vertebrate striated muscles, the basic N-terminal tail of the A1 light chain in S-1 was shown by ^1H NMR and chemical cross-linking techniques to interact with a COOH-terminal segment of actin (Prince et al., 1981; Sutoh, 1982; Henry et al., 1985). The interaction is observable both in the absence and in the presence of ATP (Chalovich et al., 1984), and it still occurs with the proteolytically isolated N-terminal fragments of the A1 light chain (Henry et al., 1985). The binding is also maintained at physiological ionic strength on using regulated actin and calcium, suggesting a regulatory role of the N-terminal region of the A1 light chain in the contractile process (Trayer & Trayer, 1985). Moreover, a recent ^1H NMR study on the interaction of F-actin with the isolated complex between the A2 light chain and the COOH-terminal 20K heavy-chain fragment of skeletal S-1 showed the conformation of the homologous 140-residue COOH-terminal tail of the light chain to be modified by its interaction with the heavy-chain segment and also by the direct binding of actin to the 20K component (Chaussepied et al., 1986a). These observations are probably relevant of recent findings indicating that the integrity of the carboxyl-terminal 14-residue portion of the alkali light chains is required for their association with the heavy chain (Ueno et al., 1985).

Previously we applied chemical cross-linking approaches to S-1 and acto–S-1 to gain insight into the topography of the heavy-chain–alkali light-chain interface; the results suggested that the NH_2-terminal 27K and COOH-terminal 20K fragments of the S-1 heavy chain contain points in close proximity of the COOH-terminal part of the alkali light chains (Labbé et al., 1981), in agreement with the association of these subunits with the isolated 20K peptide (Chaussepied et al., 1986a) and the orientation of their C-terminal region within the S-1 molecule (Waller & Lowey, 1985).

In the present work, we have further extended our earlier chemical cross-linking studies on S-1 and acto–S-1 to better understand the structural rearrangements occurring between the heavy and the light chains during the activity of the myosin heads.

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[1] Abbreviations: S-1, myosin subfragment 1; acto–S-1, actomyosin subfragment 1; A1, alkali light chain 1; A2, alkali light chain 2; DMS, dimethyl suberimidate; DTP, dimethyl 3,3′-dithiobis(propionimidate); EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; 1,5-IAEDANS, *N*-(iodoacetyl)-*N*′-(5-sulfo-1-naphthyl)ethylenediamine; MES, 2-(*N*-morpholino)ethanesulfonic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate.

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A part of this work has been recently presented (Labbé et al., 1985).

Materials and Methods

Chemicals. Thrombin from bovine plasma, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, and the hydrochlorides of dimethyl suberimidate and dimethyl 3,3′-dithiobis(propionimidate) were purchased from Serva (Heidelberg, West Germany). 1-Ethyl-3-[3-(dimethylamino)propyl]-carbodiimide was from Sigma. All other chemicals were of analytical grades.

Protein Preparations. Rabbit skeletal myosin and actin were prepared according to Offer et al. (1973) and to Eisenberg and Kielly (1974), respectively.

The chymotryptic subfragment 1 was obtained as usual (Weeds & Taylor, 1975) and purified by gel filtration over Sephacryl S-200 (Labbé et al., 1984). The isoenzymes S-1(A1) and S-1(A2) were isolated after DEAE-cellulose chromatography (Weeds & Taylor, 1975).

F-Actin was labeled with the fluorescent dye *N*-(iodoacetyl)-*N*′-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS) as reported by Mornet et al. (1981a).

The preparation of the split S-1 (27K–48K–22K), formed by digestion with *Staphylococcus aureus* V8 protease, was carried out as described by Chaussepied et al. (1983).

The complex of the 20K fragment with the alkali light chains was obtained as reported by Chaussepied et al. (1986a), using the isolated S-1(A1) and S-1(A2) which were converted by trypsin cleavage into (27K–50K–20K)-S-1 according to Mornet et al. (1980).

Pure A1 and A2 light chains were isolated according to Perrie and Perry (1970). Protein concentrations were determined by absorbance using $A^{1\%}_{280nm}$ = 7.5 cm^{-1} for S-1 (Weeds & Pope, 1977) and 11.0 cm^{-1} for actin (West et al., 1967).

Cross-Linking Reactions. The cross-linking of S-1 with dimethyl suberimidate (DMS) was performed as previously described (Labbé et al., 1981, 1982). Cross-linking experiments with the bifunctional disulfide-containing reagent dimethyl 3,3′-dithiobis(propionimidate) (DTP) were carried out as follows: S-1(A1 + A2) at 2 mg/mL in 100 mM triethanolamine hydrochloride buffer (pH 8.5) was supplemented with 10 μL of a cross-linker solution, freshly prepared in the same buffer (10 mg/mL). The modification was allowed to proceed at 20 °C for 0–30 min; at the desired time intervals, the reaction was quenched by the addition of glycine to 100 mM.

The cross-linking of acto–S-1 with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was conducted as follows: F-actin (2 mg/mL) and S-1(A1) or S-1(A2) (2 mg/mL) in 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.5, were reacted with 1 mM EEDQ, freshly dissolved in acetone (20 mg/mL), at 20 °C for 0–45 min. The reaction was terminated by the addition of β-mercaptoethanol to 3 mM. The covalent acto–S-1 formed after 30-min cross-linking was isolated by centrifugation at 140000*g* after addition of 10 mM magnesium pyrophosphate, pH 7.5. The protein pellet was then submitted to a depolymerization process employing 0.6 M KI as recently described by Rouayrenc et al. (1985). After dialysis against 2 mM Tris-HCl buffer (pH 8.0) and centrifugation, the clear supernatant was brought to 5 mM EDTA and digested with thrombin at a protease to actin weight ratio of 1:10 at 25 °C for 180 min.

EDC cross-linking between F-actin and the 20K peptide–light-chain complex and between F-actin and the isolated A1 and A2 light chains was performed essentially as described by Mornet et al. (1981b).

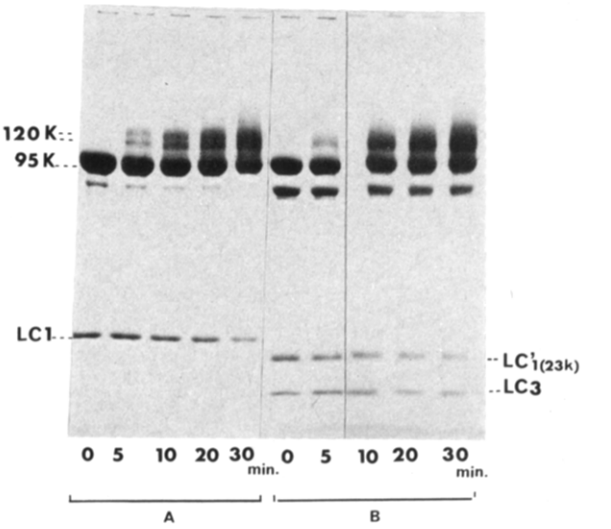

FIGURE 1: Electrophoretic patterns of DMS-cross-linked S-1(A1) (A) and S-1(A2) (B). The enzymes (20 μM) were reacted with DMS (1 mM) at pH 8.5, 20 °C. At the times indicated, protein samples were analyzed on $NaDodSO_4$ 5–18% gradient acrylamide gels.

Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out in a 5–18% polyacrylamide slab gel in the presence of 0.1% sodium dodecyl sulfate (Laemmli, 1970). For the analysis of the protein samples treated by DTP, β-mercaptoethanol was omitted in the electrophoresis buffer. Gels were stained with Coomassie blue and destained according to Weber and Osborn (1969). Protein band intensities were determined by scanning the gels at 600 nm in a Joyce-Loebel densitometer as described earlier (Labbé et al., 1984).

ATPase Measurements. The K^+-, Ca^{2+}-, Mg^{2+}-, and actin-dependent ATPase activities were estimated by using an automated phosphate analyzer as described by Mornet et al. (1979). The recovery of the actin-activated ATPase activity of DTP-treated S-1 was measured after incubation of the samples (2 mg/mL) in the presence of 20 mM DTT in 100 mM triethanolamine hydrochloride buffer (pH 8.5) at 37 °C for 5 min.

Results

Change in the Actin-Activated ATPase of S-1(A1) upon Heavy-Chain–Light-Chain Cross-Linking. Earlier, we reported that the treatment of chymotryptic S-1(A1 + A2) with DMS and cleavable diimidates such as DTP results in the covalent union of the 95K heavy chain and either of the alkali light chains with the concomitant production of a new 120K cross-linked S-1 derivative (Labbé et al., 1981, 1982). As shown in Figure 1, the 120K species generated by DMS from S-1(A1) migrated on the electrophoretic gel as a double protein band whereas that produced from S-1(A2) behaved as a single major band. A qualitative comparison of the extent of this cross-linking in the absence and presence of F-actin suggested also that actin hinders cross-link formation between the heavy and light chains. In this study, we have characterized quantitatively this influence of actin on the cross-linkability of S-1(A1 + A2) by performing densitometric evaluations of electrophoretic gels containing the 120K band produced by DMS. The formation of the acto–S-1 complex led to at least a 2-fold decrease in the amounts of 120K entity formed during the entire course of the reaction. Since this result reflects the occurrence of molecular movements between the heavy and light subunits consequent on actin binding to S-1, we have reciprocally analyzed the impact of the bis(imido ester)-mediated reaction on the actin-dependent ATPase activity of

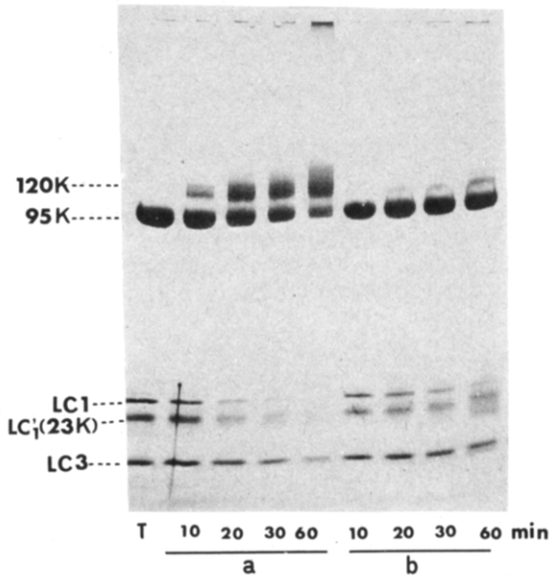

FIGURE 2: Electrophoretic patterns of DTP-cross-linked S-1. At the times indicated, samples of DTP–S-1, formed as reported in Figure 1A, were subjected to electrophoretic analysis on 5–18% gradient acrylamide gels before (a) and after (b) reduction with DTT. T = native S-1(A1 + A2); LC_1' is a 23K degradation product of LC1.

S-1(A1 + A2) as well as of the isolated S-1(A1) and S-1(A2) isoenzymes. To discriminate between the influence of cross-link formation and the effects linked to amino blocking, we carried out reversible cross-linking with the disulfide-containing reagent DTP, an analogue of DMS. This reagent caused a progressive and significant decrease of the actin-stimulated ATPase of S-1(A1 + A2). For several enzyme preparations, the residual activity plateaued, after 20–30-min reaction, at 40–50% of the original value. DMS elicited the same inhibitory effect. Incubation of the DTP–S-1 samples with DTT to abolish the cross-links before ATPase measurements led to a large, although not complete, regain of the enzymatic activity (data not shown). The comparison of the gel electrophoretic patterns obtained before and after reduction with DTT (Figure 2) indicated that most of the cross-linked 120K species which grows at the expense of the heavy and light chains was suppressed by DTT. Only a minute amount of 120K band was still observable, accounting, at least in part, for the fraction of nonreactivated S-1. Thus, the recovery of activity seems to occur concomitantly with the cleavage of the cross-links between the heavy and light chains.

In a parallel study, S-1(A1) and S-1(A2) were cross-linked separately with DTP and DMS under the same experimental conditions. Surprisingly, the results presented in Figure 3 show that the two isoenzymes are affected quite differently. The actin-activated ATPase of S1(A2) remained unchanged in spite of an extensive heavy-chain–light-chain cross-linking. In contrast, for S-1(A1) this activity progressively decreased as observed above for the mixture of S-1(A1 + A2). Up to 70% inhibition, the time course of activity loss followed that of the disappearance of free un-cross-linked A1 light chain. On the other hand, for both isoenzymes, the intrinsic Mg^{2+}-ATPase activity was enhanced nearly 2-fold after 40-min reaction, whereas the Ca^{2+}-ATPase remained unaffected (Figure 3). Consequently, the decrease of the actin-dependent ATPase activity in S-1(A1) and S-1(A1 + A2) was not due to a damage of the ATPase site but rather to a specific alteration of this particular function in the cross-linked S-1(A1) isoenzyme. Examination of the actin-activated Mg^{2+}-ATPase activity of DMS-treated S-1(A1) (60% inhibited), obtained as a function of F-actin concentration, showed that the

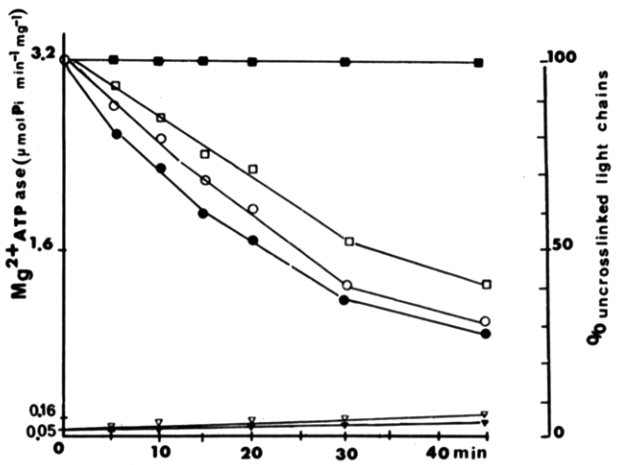

FIGURE 3: Influence of S-1(A1) and S-1(A2) cross-linking on their actin-activated ATPase. S-1(A1) (□) and S-1(A2) (■) were cross-linked with DMS as in Figure 1. At the indicated interval times of the reaction, samples were subjected to acto–S-1 ATPase assays. The Mg^{2+}-ATPase activities were also measured in the absence of actin for S-1(A1) (▽) and S-1(A2) (▼). The percent of un-cross-linked light chain present in DMS–S-1(A1) (○) and DMS–S-1(A2) (●) is also shown.

cross-linking lowers the maximal turnover rate, V_{max}, and increases the apparent affinity of S-1 for actin (K_{app} = 2 × 10^5 M^{-1} for the modified S-1 vs. 5 × 10^5 M^{-1} for the native enzyme).

Extensive Cross-Linking between Actin and A1 Light Chain in S-1(A1) with N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. To gain further insight into the nature of the A1 light-chain–actin interface, chemical cross-linking experiments on acto–S-1(A1) and acto–S-1(A2) complexes were carried out with the hydrophobic, zero-length cross-linker *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). This reagent induces covalent union of proteins by peptide bond formation through a specific catalytic esterification reaction of carboxyl groups (Belleau & Malek, 1968). The results were compared to those previously observed with the water-soluble EDC (Mornet et al., 1981b; Sutoh, 1982; Yamamoto & Sekine, 1983).

Figure 4 shows the gel electrophoretic patterns of S-1 isoenzymes cross-linked by EEDQ in the presence of fluorescent actin. Under the optimal conditions employed, the reaction led to an extensive cross-linking of the A1 subunit to actin with the production of an intense fluorescent band of M_r 67K, similar to the cross-linked entity which forms, but in a much lower amount, in the presence of EDC. The same entity was obtained with labeled and unlabeled actin. This species is absent in cross-linked acto–S-1(A2), indicating that cross-linking involves the N-terminal tail of A1. On the other hand, a substantial amount of actin trimers can be distinguished together with covalent actin–heavy-chain products with molecular weights in the range 175K–210K, the identification of which will be detailed elsewhere.[2] To assess the actin site which has been cross-linked by EEDQ to the A1 light chain, the covalent acto–S-1(A1) complex was submitted to a rapid and direct enzyme probe based on the thrombic digestion of actin within the depolymerized cross-linked preparation (Figure 4, lanes C and c). Thrombin does not act at all on the heavy or alkali light chains in S-1 (Chaussepied et al., 1986b; Henry et al., 1985), but it cuts selectively G-actin into well-known fragments (Muszbeck et al., 1975). One of these is the COOH-terminal 27K peptide issued from a 37K pre-

[2] R. Bertrand, P. Chaussepied, and R. Kassab, unpublished results.

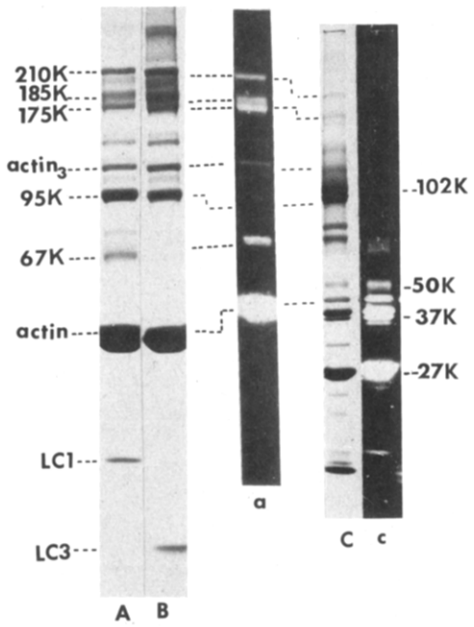

FIGURE 4: EEDQ-catalyzed covalent cross-linking between F-actin and S-1 isoenyzmes. Fluorescent actin (40 μM) was mixed with S-1(A1) and S-1(A2) (20 μM) in the presence of 1 mM EEDQ in 100 mM MES buffer, pH 6.5; after 20-min reaction at 20 °C, the protein samples were analyzed by gel electrophoresis. (A and B) Protein banding patterns for acto–S-1(A1) and acto–S-1(A2), respectively. (a) Fluorescence profile of the gel corresponding to acto–S-1(A1). (C) Covalent S-1(A1)–actin complex formed after 30-min cross-linking was isolated and digested with thrombin as described under Materials and Methods. (c) Same gel viewed under UV light.

cursor, both of which contain Cys-374 and incorporate the initial actin fluorescence. After proteolysis, most of the fluorescence of the 67K band was transferred to a new product of M_r 50K which could be only the adduct of the A1 light chain with the 27K actin fragment. This suggests that EEDQ also promotes, like EDC, the cross-linking of the actin COOH-terminal tail to the A1 subunit. Furthermore, the digest contained a nonfluorescent and new species of M_r 102K which corresponds to the actin N-terminal thrombic peptide cross-linked to the intact S-1 heavy chain.[2]

Dependence of A1 Light-Chain Cross-Linking to Actin on Its Association with the 20K Heavy-Chain Segment. Figure 5 illustrates the relationship between the association of the A1 light chain with the COOH-terminal 20K heavy-chain region and its cross-linkability to actin. When a mixture of actin and the isolated intact A1 and A2 light chains was treated with EDC under standard conditions, no cross-linked actin–A1 light-chain product could be observed. In contrast, the reaction of EDC with the isolated, renatured binary complex of A1 and the tryptic 20K fragment resulted in the formation of two covalent species corresponding to actin–20K peptide and actin–LC1′ (23K) light-chain, respectively (Figure 5A). Only the former entity was present in the reaction mixture containing actin and the 20K peptide–A2 light-chain complex (Figure 5B). As previously observed for the intact A1 in the native S-1, we have noted that its 23K derivative is cross-linked by EDC to F-actin to a relatively small extent. Our results are also in agreement with the observations of Ueno et al. (1985) that cosedimentation between F-actin and isolated A1 occurs only in the presence of 20K fragment. The data suggest that a close contact between actin and the cross-linking sites on the N-terminal tail of the A1 light chain requires the attachment of the light subunit to the COOH-terminal 20K portion of the S-1 heavy chain.

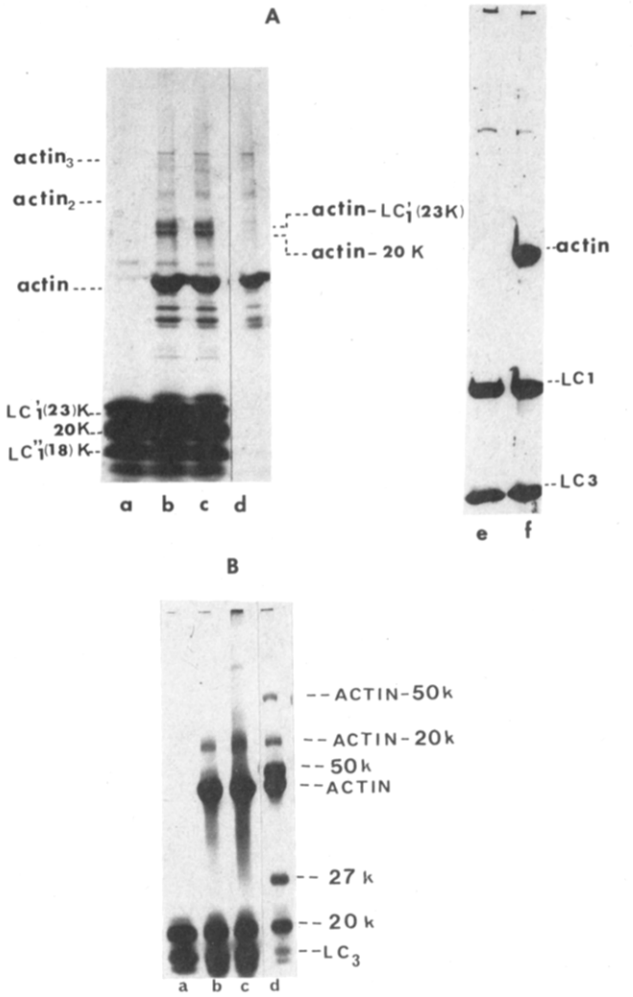

FIGURE 5: Cross-linking between actin and the A1 light chain complexed to the isolated 20K heavy-chain fragment. (A) F-actin (2 mg/mL) was incubated with 20K peptide–A1 light-chain complex (2 mg of protein/mL) in the presence of 15 mM EDC in 100 mM MES buffer, pH 6.5, 20 °C. Protein samples were analyzed by gel electrophoresis after 5 min (b) and 10 min (c) of cross-linking reaction. a = starting 20K peptide–A1 complex; A1 is present as the two tryptic products of M_r 23K and 18K. d = control 10-min EDC–actin. e = 10-min reaction mixture of EDC and isolated A1 + A2 light chains. f = 10-min reaction mixture of EDC, F-actin, and isolated A1 + A2 light chains. (B) F-Actin and the 20K peptide–A2 complex were reacted with EDC under the conditions specified in (A). Protein samples were subjected to gel electrophoresis after 5 (b) and 10 min (c) of cross-linking reaction. a = starting 20K peptide–A2 complex. d = control trypsin split (27K–50K–20K)-S-1(A2) cross-linked to F-actin for 10 min.

Discussion

The S-1 heavy chain contains the ATPase and the main actin sites of myosin (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). Its covalent cross-linking to the nonessential alkali light chains is a useful tool in the study of the ligand-induced structural changes at the intersubunit interface and of the importance of motion of the heavy chain during the catalytic activity of S-1. The cross-link formation can be activated by the zero-length reagent EDC, but irreversibly and with a very low yield (Yamamoto & Sekine, 1983); in contrast, the longer bis(imido esters) are capable of covalently linking the two subunits in a reversible manner and to a relatively large extent (Labbé et al., 1981, 1982). The treatment of the isolated S-1(A1) and S-1(A2) with DTP or DMS results in the production of a corresponding new 120K species whose enzymatic properties can be assessed over the entire course of the cross-linking reaction. The data we ob-

tained show that the ATPase site of the two S-1 isoenzymes was not very sensitive to the covalent union of the heavy and alkali light chains. However, the S-1(A1) preparation displayed a striking behavior as its cross-linking induced a significant inhibitory effect on the actin-dependent ATPase activity. The loss of the acto–S-1(A1) ATPase could be due either to a specific intersubunit cross-linking between the heavy and the A1 light chains or to an intra-heavy-chain cross-linking which could restrict the required motion of the heavy chain during the expression of the acto–S1(A1) ATPase. Indeed, the internal cross-linking between the three 27K–50K–20K fragments of the heavy chain induced by the bis(imido esters) has been illustrated earlier (Yamamoto & Sekine, 1979; Labbé et al., 1982). However, the latter alternative is unlikely for two reasons. First, the actin-activated ATPase of DMS–S-1(A2) remained unchanged; second, the (28K–48K–22K)-S-1 produced by cleavage with staphylococcal protease and containing a truncated 17K A1 light chain (Chaussepied et al., 1983; Mornet et al., 1984) was cross-linked with DMS without alteration of its actin-activated ATPase (data not shown). The overall data suggest that the specific covalent union between the intact A1 light chain and the 95K heavy chain, either through its NH_2-terminal tail or through both the NH_2-terminal and COOH-terminal portions, is not compatible with the normal functioning of the acto–S-1(A1) ATPase.

To better understand the direct interaction of actin with the NH_2-terminal region of A1 light chain in S-1, we cross-linked the acto–S1(A1) complex with the hydrophobic, carboxyl group specific reagent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Because the COOH-terminal sequence of actin between residues 355 and 375, in which resides the A1 recognition sites (Sutoh, 1982), contains several hydrophobic side chains, its cross-linking to the A1 light chain occurs much more readily with this reagent than with the hydrophilic cross-linker EDC. On the other hand, the hydrophobic character of the COOH-terminal actin segment explains its poor activation by the carbodiimide reagent as demonstrated by the low extent of cross-linking between this region and the A_1 or 23K tryptic product and also by the low yield of its substitution by a high concentration of nucleophiles (personal observations). Since the interaction of the A1 light chain with actin is still operant at high ionic strength in the presence of the regulatory proteins and calcium (Trayer & Trayer, 1985), the EEDQ-catalyzed cross-linking reaction would be a useful probe of the dynamics of the actin–A1 light-chain interface under these physiological conditions. Moreover, although EEDQ also induced the cross-linking of the NH_2-terminal segment of actin to the S-1 heavy chain, similarly to EDC, we found no cross-linked species corresponding to the double cross-linking of actin both to the heavy chain and to the A1 light chain. This observation implies either that the two kinds of S-1 subunits were cross-linked to different actins or that the cross-linking of either subunit inhibits that of the other on the same actin monomer.

Finally, a further interesting feature of the A1–actin interaction was revealed by the non-cross-linking of actin to the isolated light chain in spite of the reported binding of the isolated light chain and its proteolytic NH_2-terminal fragments to actin (Henry et al., 1985). Our results are in agreement with the observation of Ueno et al. (1985). This suggests that the contact points on the isolated light chain are not sufficiently close to or conveniently oriented relative to the COOH-terminal tail of actin so that their cross-linking with EDC was abolished. In sharp contrast, the association of the A1 light chain to the COOH-terminal 20K heavy-chain segment led to a recovery of the cross-linkability of the light chain. This result is in agreement with the involvement of this heavy-chain domain in the tight association of the light chains to the S-1 heavy chain (Burke et al., 1983) and with the data of a recent NMR study illustrating the conformational change induced in the light-chain structure by its attachment to the 20K region (Chaussepied et al., 1986a). Thus, the observed cross-linking of the A1 subunit to actin only when complexed to the heavy chain provides additional criteria to the specificity of the interaction of actin with this light chain in the S-1 molecule.

Registry No. ATP, 56-65-5; ATPase, 9000-83-3.

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Aminoacyl-tRNA–Elongation Factor Tu–Ribosome Interaction Leading to Hydrolysis of Guanosine 5′-Triphosphate†

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ABSTRACT: We investigated the elongation factor Tu (EF-Tu) dependent binding of Phe-tRNA and Phe-tRNAs with the nicks at positions 46, 37, and 17 to the *Escherichia coli* 70S ribosome–poly(U)–$tRNA^{Phe}$ complex. Binding of Phe-$tRNA_{1-45+47-76}$, Phe-$tRNA_{1-36+38-76}$, or Phe-$tRNA_{1-16+17-76}$ to the 70S ribosome has been found to be poly(U)·tRNA dependent and, similar to that of intact Phe-tRNA, is inhibited by the antibiotic thiostrepton. We have further found that, contrary to a previous report [Modolell, J., Cabrer, B., Parmeggiani, A., & Vazquez, D. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1796], the EF-Tu–ribosome GTPase mediated by Phe-tRNA is not inhibited by thiostrepton; rather, the drug stimulates the endogenous GTPase of the EF-Tu·70S ribosome. Phe-tRNA fragments 47–76, 38–76, and 17–76 all promote the EF-Tu·GTPase reaction in the presence of 70S ribosome–poly(U)–$tRNA^{Phe}_{yeast}$. Moreover, since the GTPase-promoting activities of both the short and long fragments are similar, it appears that the most important aminoacyl transfer ribonucleic acid (aa-tRNA) interaction with EF-Tu occurs alongside its 3′ quarter. Thiostrepton slightly stimulates the GTPase activity of these Phe-tRNA fragments. Although the Phe-$tRNA_{1-36+38-76}$ cannot bind to poly(U) during its binding to 70S ribosomes, its binding at high Mg^{2+} concentration occurs at the A site. Thus, most of the bound modified Phe-tRNA functions as the acceptor in the peptidyltransferase reaction. We interpret these results to mean that the GTP hydrolysis is triggered upon the initial contact of the ternary aa-tRNA·EF-Tu·GTP complex with a ribosomal domain which may not be identical with the A site at which thiostrepton and EF-G act. The aa-tRNA molecule can reach the A site only after hydrolysis of GTP and removal of EF-Tu·GDP from ribosomes.

During the elongation phase of protein synthesis, aminoacyl transfer ribonucleic acid (aa-tRNA)[1] enters the programmed ribosome in the form of a ternary aa-tRNA·EF-Tu·GTP complex. Binding of aa-tRNA to the ribosomal acceptor sites ensues, and in the course of this process, one molecule of GTP is hydrolyzed, and EF-Tu·GDP leaves the ribosome (Miller & Weissbach, 1977). While it is clear that the catalytic activity for GTP hydrolysis is effected by EF-Tu (Chinali et al., 1977) and binding of an aa-tRNA molecule, especially its 3′ terminus, to EF-Tu is a prerequisite for activation of EF-Tu GTPase, it is worth mentioning that this reaction can only occur (under physiological conditions) upon the contact of the ternary complex with a certain, but as yet undefined, ribosomal domain (Liljas, 1982). The other elongation factor, EF-G, binds to the ribosome in the step following peptide bond formation and effects the translocation of the newly formed peptidyl-tRNA from the A to the P site (Brot, 1977). This reaction is also accompanied by hydrolysis of one molecule of GTP, though catalysis by EF-G (de Veridittis et al., 1984) also requires interaction of this factor with the ribosome. While it has been clearly shown that the ternary aa-tRNA·EF-Tu·GTP complex and EF-G compete with each other for binding to the ribosome (Miller, 1972), it does not necessarily follow that the ribosomal domains responsible for the activation of the GTPases of both factors are identical. Thus, protein L11 seems to be involved in an interaction of the ribosome with

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[1] Abbreviations: aa-tRNA, aminoacyl transfer ribonucleic acid; EF-Tu, elongation factor Tu; DTT, dithiothreitol; TEMED, tetramethylethylenediamine; TCA, trichloroacetic acid; A-Phe, 2′(3′)-*O*-L-phenylalanyladenosine (similar abbreviations are used for other oligonucleotide derivatives); BD, benzoyldiethylaminoethyl; A_{260} unit, quantity of material contained in 1 mL of solution which has an absorbance of 1.00 at 260 nm when measured in a 1-cm path-length cell; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Me_2SO, dimethyl sulfoxide. The fragments of $tRNA^{Phe}_{yeast}$ are designated by their terminal nucleosides, e.g., $tRNA^{Phe}_{yeast(38-76)}$; see also Figure 1.

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