regulatory factor. In this model, AppppA would be a positive effector.

APS is a product of the first step of inorganic sulfate reduction, which is catalyzed by ATP-sulfurylase encoded by the *MET3* gene in yeast (Jones & Fink, 1982). Being the first step in a pathway, APS synthesis is tightly regulated both by feedback inhibition of the ATP-sulfurylase (by APS itself, PAPS, $SO_3^{2-}$, or $S^{2-}$) and by repression of the enzyme synthesis by *S*-adenosylmethionine. Since APS can also be a substrate for AppppA synthesis, it would not be surprising if AppppA acted as a feedback inhibitor of ATP-sulfurylase. The hypotheses concerning a role of AppppA in phosphate and APS metabolism in yeast are testable experimentally and point toward new directions in studies of functions of AppppA.

**Registry No.** AppppA, 5542-28-9; APS, 485-84-7; ATP, 56-65-5; ppppA, 1062-98-2; GTP, 86-01-1; AppppA, 41708-91-2; AppppG, 10527-46-5; App(CH$_2$)pA, 101536-13-4; App(CH$_2$)ppA, 88109-92-6; Mn, 7439-96-5; Mg, 7439-95-4; Ca, 7440-70-2; AppppA $\alpha,\beta$-phosphorylase, 96697-71-1; adensoine 5′-($\alpha,\beta$-methylenetriphosphate), 7292-42-4; adenosine 5′-($\beta,\gamma$-methylenetriphosphate), 3469-78-1; adenine, 73-24-5.

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# Spatial Proximity of the Glycine-Rich Loop and the $SH_2$ Thiol in Myosin Subfragment 1†


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ABSTRACT: Subfragment 1 (S1) prepared from rabbit skeletal muscle myosin was digested with trypsin to cleave the 95K heavy chain into three pieces, i.e., the 23K, 50K, and 20K fragments. The trypsin-treated S1 was then cross-linked with *p*-nitrophenyl iodoacetate. The cross-linker bridged one of the reactive thiols ($SH_2$) in the 20K fragment and a lysine residue in the 23K fragment [Hiratsuka, T. (1987) *Biochemistry 26*, 3168–3173]. Location of the lysine residue was mapped along the 23K fragment by "end-label fingerprinting", which employed site-directed antibodies against the N-terminus of the 23K fragment and against the C-terminus of the 24K fragment (the 23K fragment plus nine extra residues at its C-terminus). The mapping revealed that Lys-184 or Lys-189 was the residue cross-linked with $SH_2$. Since the cross-linker used here spans only several angstroms, the result indicates that Lys-184 or Lys-189 is very close to $SH_2$ in the three-dimensional structure of myosin head. Examination of the primary structure of the 23K fragment has revealed that these lysine residues are in and very close to the so-called "glycine-rich loop", whose sequence is highly homologous to those of nucleotide-binding sites of various nucleotide-binding proteins.


Heavy chain of myosin subfragment 1 (S1)[1] contains two reactive cysteine residues called $SH_1$ and $SH_2$ (Sekine & Kieley, 1964). These residues are located in the tryptic 20K fragment of the S1 heavy chain [trypsin cleaves the heavy chain into the 23K, 50K, and 20K fragments which are aligned in this order (Mornet et al., 1979)] and are only 10 residues apart in the primary structure (Elzinga & Collins, 1977; Gallagher & Elzinga, 1980). The structure around the $SH_1$–$SH_2$ region seems flexible; cross-linking experiments showed that these thiols can be as little as 2 Å apart (Burke

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[1] Abbreviations: S1, myosin subfragment 1; LEP, lysyl endopeptidase; NPIA, *p*-nitrophenyl iodoacetate; NaDodSO$_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; IgG, immunoglobulin G.

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& Reisler, 1977; Wells & Yount, 1979) while fluorescence energy transfer experiments revealed a much longer distance (2–3 nm) (Dalbey et al., 1983; Cheung et al., 1985).

It is well documented that covalent modifications of the reactive cysteine residues drastically affect the ATPase activity. It has been also shown that modifications of the cysteine residues affect the actin–myosin interaction. However, these communications among the $SH_1$–$SH_2$ region, the ATPase site, and the actin-binding site do not necessarily mean steric overlap of these sites on the myosin head. In fact, three-dimensional electron microscopy has revealed that the $SH_1$ thiol, the actin-binding site, and the ATPase site are located at distinct regions on the myosin head (Toyoshima & Wakabayashi, 1985a,b; Sutoh et al., 1984, 1986; Tokunaga et al., 1987). The actin-binding site is close to the tip of the head while the $SH_1$ thiol is at the middle part of the head and on the same side as the actin-binding site. The ATPase site is also at the middle part of the head. However, it is located on the opposite side to the actin-binding site and to the $SH_1$ thiol. Thus, communications among the three sites seem to be transmitted through some distance.

In order to obtain detailed information about the structure around the $SH_1$–$SH_2$ region, many chemical cross-linking experiments have been carried out by using cysteine-specific cross-linking reagents. Two segments in the tryptic 23K and 50K fragments are cross-linked to $SH_1$ by a cross-linker with a several-angstrom span (Lu et al., 1986; Sutoh & Lu, 1987). A cysteine residue (Cys-522) in the tryptic 50K fragment is cross-linked to $SH_1$ by dibromobimane (Mornet et al., 1985; Ue, 1987). Another cysteine-specific cross-linker cross-links $SH_2$ with a cysteine residue, possibly Cys-540, in the 50K fragment (Chaussepied et al., 1986). *p*-Nitrophenyl iodoacetate (NPIA) cross-links $SH_2$ with a lysine residue in the tryptic 23K fragment (Hiratsuka, 1987). These cross-linking studies have shown that various segments in the 23K and 50K tryptic fragments of the heavy chain are in close proximity to the reactive cysteine residues in the 20K fragment.

In this paper, a lysine residue cross-linked with $SH_2$ by NPIA was identified as Lys-184 or Lys-189 in the 23K fragment by employing "end-label fingerprinting" (Sutoh, 1982–1984, 1987; Sutoh & Mabuchi, 1986; Sutoh & Lu, 1987). In the heavy chain sequence (Tong & Elzinga, 1983), Lys-184 and Lys-189 are in and very close to the so-called "glycine-rich loop" (Walker et al., 1982; Fry et al., 1986).

## Materials and Methods

*Cross-Linking of Subfragment 1 with NPIA.* Subfragment 1 (S1) of rabbit skeletal myosin was prepared according to the method of Weeds and Taylor (1975). S1 in 50 mM NaCl, 0.5 mM $MgCl_2$, and 40 mM triethanolamine hydrochloride (pH 8.4) was digested with trypsin at a molar ratio of 1:100 (trypsin/S1) at 25 °C. The trypsin-treated S1 was then cross-linked with NPIA as described (Hiratsuka, 1987). In some cases, S1 was cross-linked with NPIA and then digested with trypsin.

*Isolation of Tryptic Fragments.* The cross-linked product of trypsin-treated S1 was labeled with *N*-[7-(dimethylamino)-4-methylcoumarin-3-yl]maleimide (DACM) (molar ratio of S1 to DACM 1:1) in 1% $NaDodSO_4$ and 20 mM Tris-HCl (pH 7.8) for 30 min at room temperature. The reaction was quenched by addition of 2-mercaptoethanol (final concentration 0.1%). The resulting digest was electrophoresed in the presence of $NaDodSO_4$, and flourescent fragments were visualized by illumination with UV light. Two fluorescent bands corresponding to the tryptic 23K fragment of the heavy chain and the 43K cross-linked product were cut out from the gel. Those fluorescent peptides were then eluted from gels by using an electroelution apparatus. Some of these gel pieces trapping the 23K fragment or the 43K cross-linked product were washed with 50% ethanol and then with ethanol. The washed gel pieces were dried in vacuo.

*Mapping of Cross-Linked Site.* The fluorescent peptides eluted from gel pieces were further digested with lysyl endopeptidase (LEP) (Wako Chemicals Co., Osaka, Japan) at a weight ratio of 1:100 (LEP/peptide) in 0.1% $NaDodSO_4$ and 20 mM Tris-HCl (pH 7.8) containing 0.1% 2-mercaptoethanol. After 20 min, the digestion was quenched by incubating the digest at 95 °C for 5 min. The digested product was electrophoresed in an acrylamide slab gel [15% acrylamide–0.43% bis(acrylamide)] in the presence of $NaDodSO_4$ (Laemmli, 1970). LEP fragments thus separated were blotted onto a Durapore membrane (Millipore, Bedford, MA) (Towbin et al., 1979) and then stained with an antibody directed to the N-terminus of the heavy chain, i.e., the N-terminus of the 23K fragment (Sutoh et al., 1987; Sutoh & Lu, 1987), or to the 23K–50K junction peptide (Sutoh, 1987).

Fluorescent peptides trapped in gel pieces were digested in situ with 50 mM CNBr in 70% formic acid for 1 h at 37 °C. After digestion, gels were washed thoroughly with 50% ethanol containing 1% 2-mercaptoethanol and then with ethanol. The resulting gel pieces were dried in vacuo. The dried gels were then soaked in 2% $NaDodSO_4$, 20 mM Tris-HCl (pH 7.8), 1% 2-mercaptoethanol, and 10% glycerol for 1 h at 40 °C. The resulting gel pieces were put on the top of a second slab gel [15% acrylamide–0.43% bis(acrylamide)]. CNBr fragments were electrophoresed, blotted, and then stained.

Antibody staining of blots was carried out as previously described (Sutoh et al., 1987; Sutoh & Lu, 1987; Sutoh, 1987) with slight modifications. Antiserum to the N-terminus of the 23K fragment was diluted 2000-fold by 0.5 M NaCl and 20 mM Tris-HCl (pH 8.0) containing 0.05% Tween 20. Antiserum to the 23K–50K junction was diluted 1000-fold by the above solvent. Secondary antibody was anti-rabbit IgG antibody coupled with alkaline phosphatase from Kirkegaard & Perry Laboratories, Inc. (KPL) (Gaithersburg, MD). Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (KPL) were used as chromogen. To visualize all peptides on a membrane, they were stained by gold colloid according to the methods of Moeremans et al. (1985).

*Antibodies.* Antibodies against the N-terminus of the 23K fragment and against the 23K–50K junction peptide was described (Sutoh et al., 1987; Sutoh, 1987).

*Gel Electrophoresis.* The $NaDodSO_4$ gel electrophoresis was carried out according to Laemmli (1970). Apparent molecular weights were estimated by standard proteins and peptides as follows: aldolase ($M_r$ 40 000), carbonic anhydrase ($M_r$ 29 000), and myoglobin and its CNBr fragments ($M_r$ 17 000, 14 000, 8200, 6200, and 2500).

## Results

*Cross-Linking of $SH_2$ and a Lysine Residue in the 23K Tryptic Fragment.* Myosin subfragment 1 (S1) was treated with trypsin to cleave the 95K S1 heavy chain into three fragment (23K, 50K, and 20K fragments) (Mornet et al., 1979). The trypsin-treated S1 was then cross-linked with NPIA. As previously described (Hiratsuka, 1987), the reaction generated a cross-linked product with an apparent molecular weight of 43 000 (lane B in Figure 1), a complex of the 23K and 20K fragments bridged at a lysine residue in the 23K fragment and the $SH_2$ thiol in the 20K fragment.

The 23K fragment and the 43K product were electrophoretically purified (lanes C and D in Figure 1). When the

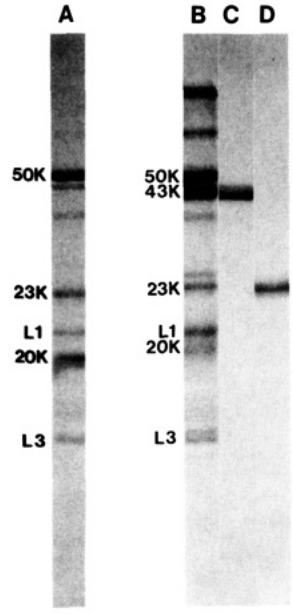


FIGURE 1: Cross-linking of the tryptic 20K and 23K fragments of the heavy chain with NPIA. $NaDodSO_4$ gels [12.5% acrylamide–0.35% bis(acrylamide)] of trypsin-treated S1 before (A) and after (B) the NPIA cross-linking. The cross-linking 43K product and the 23K fragment were electrophoretically purified: (C) isolated 43K product; (D) isolated 23K fragment. Peptides were stained with Coomassie blue.

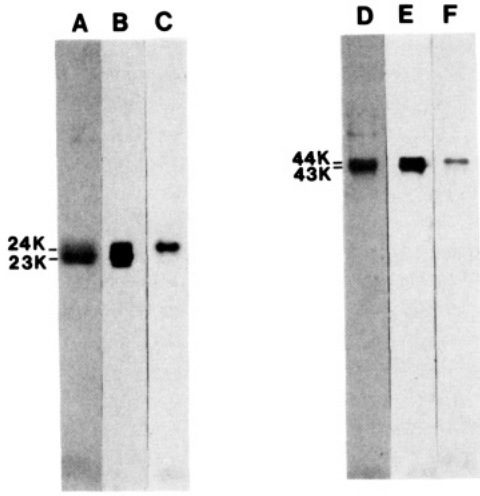


FIGURE 2: Antibody staining of the purified 23K and 43K peptides. The purified 23K fragment and the 43K cross-linked product were electrophoresed [12.5% acrylamide–0.35% bis(acrylamide)] in triplicate and blotted onto a Durapore membrane. Lanes A and D: Stained with gold colloid. Lanes B and E: Stained with the antibody against the N-terminus of the 23K fragment. Lanes C and F: Stained with the antibody against the 23K–50K junction. Lanes A–C: 23K fragment. Lanes D–F: 43K cross-linked product. Broad 23K and 43K bands observed in lanes B and E actually consist of closely spaced doublets. Note that narrower bands are observed in lanes C and F and that those bands correspond to the upper halves of the broad bands in lanes B and E, respectively.

isolated 23K fragment was stained with the antibody against its N-terminus (Sutoh et al., 1987; Sutoh & Lu, 1987; Sutoh, 1987), a broad 23K band was observed (lane B in Figure 2). The broad band actually consisted of two closely spaced bands, which were identified individually only at an early stage of the staining. The same fragment was also stained with the antibody against the 23K–50K junction. It has been previously shown that the antibody does not react with the 23K fragment, but with a longer fragment consisting of the 23K fragment and an extra nine residues at its C-terminus (24K fragment)

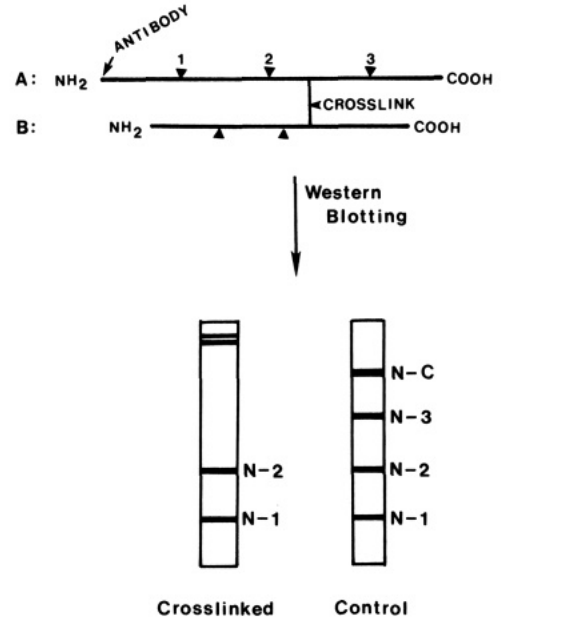


FIGURE 3: Schematic drawing of strategy of the end-label fingerprinting to map the cross-linking site. Two lines at the top represent peptides A and B which participate in cross-linking. Cleavage sites along these peptides are shown by triangles. The cross-linking site in peptide A is in the third segment from its N-terminus. After cross-linking, the product is subjected to partial digestion. As a control, peptide A is also subjected to the same partial digestion. These digests are electrophoresed, blotted, and then stained with the antibody against the N-terminus of peptide A. On the blot of non-cross-linked peptide A ("control"), three fragments from the N-terminus to cleavage site 1 (N-1), to cleavage site 2 (N-2), and to cleavage site 3 (N-3) as well as the parent peptide (N-C) are visible. In contrast to the control blot, only two fragments (N-1 and N-2) are visible on the blot of the cross-linked product ("cross-linked").

(Sutoh, 1987). Thus the antibody recognizes the C-terminus of the N-terminal 24K heavy chain fragment. As observed on the stained blot (lane C in Figure 2), a 24K band, which corresponded to the upper half of the broad 23K band in lane B, was visualized, indicating that the isolated 23K fragment actually contained the longer 24K fragment as a minor component. Hereafter, we will refer to the isolated 23K fragment as the 23K + 24K fragments.

When the isolated 43K product was stained with the N-terminus antibody, again a broad 43K band was observed (lane E in Figure 2). The broad 43K band actually consisted of two closely spaced bands, which were identified individually only at an early stage of staining. When the same 43K product was stained with the antibody against the 23K–50K junction, however, the visualized band in lane F corresponded to the upper half of the broad 43K band in lane E (Figure 2). The isolated 43K product actually contained the cross-linked species of the 24K fragment and the 20K fragment (44K product) as a minor component together with the major 23K–20K cross-linked product. Hereafter, we will refer to the isolated 43K product as the 43K + 44K products.

The 23K + 24K fragments and the 43K + 44K products were used to map the cross-linking site in the 23K fragment by the end-label fingerprinting.

*General Strategy for End-Label Fingerprinting To Identify a Residue Cross-Linked to $SH_2$.* It has been established that segments participating in covalent cross-linking can be easily identified by the end-label fingerprinting, which employs either chemical labels or site-directed antibodies to the N- and/or C-termini of polypeptide chains (Sutoh, 1982–1984, 1987; Sutoh & Mabuchi, 1986; Sutoh & Lu, 1987; Jay, 1984; Jue & Doolittle, 1985; Matsudaira et al., 1985). General strategy

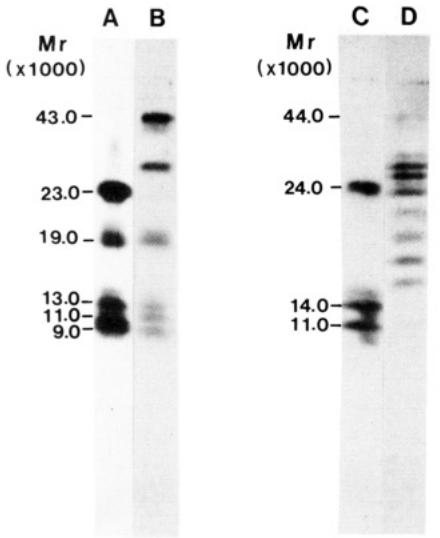


FIGURE 4: Mappings of the cross-linking site in the 23K fragment by CNBr digestion. The 23K + 24K fragments and the 43K + 44K cross-linked products were subjected to partial CNBr digestion. Digests were electrophoresed in duplicate in a $NaDodSO_4$ slab gel [15% acrylamide–0.43% bis(acrylamide)], blotted onto a Durapore membrane, and stained with the antibody against the N-terminus of the 23K fragment (lanes A and B) or with the antibody against the 23K–50K junction (lanes C and D). Lanes A and C: 23K + 24K fragments. Lanes B and D: 43K + 44K cross-linked products.

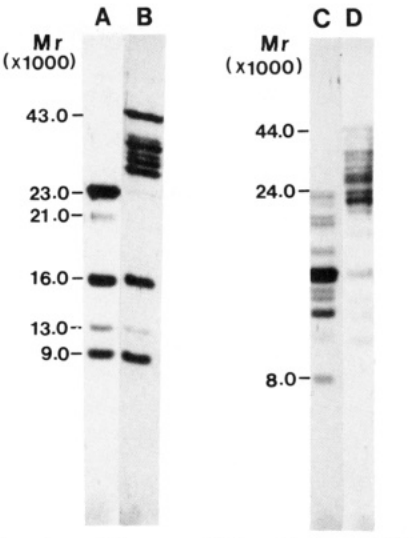


FIGURE 5: Mappings of the cross-linking site in the 23K fragment by LEP digestion. The 23K + 24K fragments and the 43K + 44K cross-linked products were partially digested with LEP. Digests were then electrophoresed in duplicate in a $NaDodSO_4$ slab gel [15% acrylamide–0.43% bis(acrylamide)], blotted onto a Durapore membrane, and stained with the antibody against the N-terminus of the 23K fragment (lanes A and B) or with the antibody against the 23K–50K junction (lanes C and D). Lanes A and C: 23K + 24K fragments. Lanes B and D: 43K + 44K cross-linked products.

for the fingerprinting is shown in Figure 3. First, cross-linked product of two polypeptides (A and B) is purified and subjected to partial cleavage by an enzyme or a chemical. For simplicity of our argument, we assume that there are three cleavage sites along polypeptide A and that the cross-linking site is on the third segment from the N-terminus. After the partial cleavage, the digest is electrophoresed and blotted. Then fragments containing the N-terminus of polypeptide A are visualized by using an antibody directed to the N-terminus. At the same time, as a control, polypeptide A is subjected to the partial cleavage, blotted, and then stained by the same antibody ("control" in Figure 3). From polypeptide A, three fragments containing its N-terminus are generated by the partial cleavage. They are fragments from the N-terminus to cleavage site 1 (N-1), to cleavage site 2 (N-2), and to cleavage site 3 (N-3). Thus four peptides are visible on the control blot stained with the N-terminus antibody; N-1, N-2, N-3, and the parent peptide (N-C). When the blot of the cross-linked product ("cross-linked" in Figure 3) is compared with the control blot ("control"), it is observed that two peptides N-3 and N-C are missing on the former blot because these two peptides contain the cross-linking site. These peptides appear at a higher molecular weight region on the blot as cross-linked species. Thus just by comparing band patterns of the control blot and the blot of the cross-linked sample, location of the cross-linking site can be identified. Similar experiments are carried out by using an antibody against the C-terminus of polypeptide A in place of the antibody against its N-terminus. Thus by using two antibodies directed to the N- and C-termini of the polypeptide chain (the antibodies against the N-terminus of the 23K fragment and against the 23K–50K junction peptide in this study), we can locate the cross-linking site from both ends.

*Mapping the Cross-Linking Site by CNBr Digestion.* The 23K + 24K fragments and the 43K + 44K cross-linked products were electrophoretically isolated and then partially digested with CNBr. The digests were electrophoresed in the presence of $NaDodSO_4$, blotted onto a Durapore membrane, and then stained with the antibody against the N-terminus of the 23K fragment (lanes A and B in Figure 4). The CNBr digestion of the 23K + 24K peptides generated a fragment with an apparent molecular weight of 19 000 together with three smaller fragments with apparent molecular weights ranging from 9000 to 13 000 (lane A). All fragments visible in the blot contained the N-terminus of the parent 23K fragment since they are stained with the antibody against this region. Judging from the primary structure of the 23K fragment (Tong & Elzinga, 1983), it is most likely that the 19K fragment was generated by cleavage at Met-165 (Sutoh, 1987). The CNBr digestion of the 43K + 44K cross-linked products also generated the 19K fragment together with three smaller fragments (lane B). The band pattern generated from the 23K + 24K fragments and that from the 43K + 44K cross-linked products showed complete alignment up to the 19K fragment. Thus the cross-linking site in the 23K tryptic fragment is located outside the N-terminal 19K segment, i.e., within a segment of residues 166–204 (Figure 6).

The CNBr digests of the 23K + 24K fragments and the 43K + 44K cross-linked products were blotted and then stained by the antibody against the 23K–50K junction peptide (lanes C and D in Figure 4). Since the 24K fragment, but not the 23K fragment, was stained with the antibody (Sutoh, 1987), the 24K fragment was the parent peptide visible on the blot. Besides the parent 24K peptide, two major CNBr fragments with apparent molecular weights of 11 000 and 14 000 were visible on the control blot (lane C). However, both of these CNBr fragments were missing in the digest of the 43K + 44K cross-linked products (lane D). The result indicates that the cross-linking site resides within the C-terminal 11K segment of the parent 24K fragment (Figure 6), consistent with the above result by the N-terminus antibody.

*Mapping the Cross-Linking Site by Lysyl Endopeptidase Digestion.* The 23K + 24K fragments and the 43K + 44K cross-linked products were subjected to partial cleavage by lysyl endopeptidase (LEP). These digests were electrophoresed, blotted, and then stained by the antibody directed to the N-terminus of the 23K fragment (lanes A and B in Figure 5). From the control 23K + 24K peptides, fragments with ap-

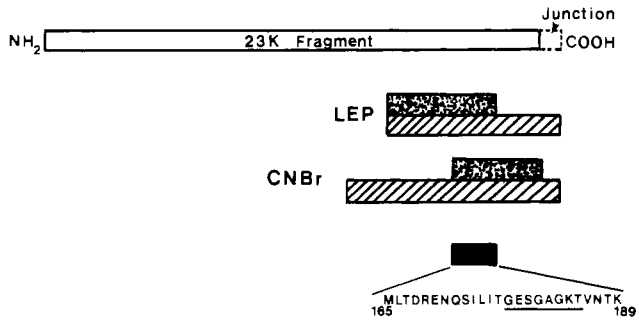


FIGURE 6: Maps of the cross-linking site in the 23K fragment. Top bar represents the 23K polypeptide chain. The 23K–50K junction peptide is shown by the open bar outlined with dots. The two bars marked LEP are segments containing the cross-linking site as determined by LEP digestion. The two bars marked CNBr are segments containing the cross-linking site as determined by CNBr digestion. Segments shaded with dots are located by the antibody against the N-terminus of the 23K fragment. Cross-hatched segments are located by the antibody against the 23K–50K junction. Closed box represents the segment containing the cross-linking site. Its sequence is shown in the one-letter code. The sequence of the glycine-rich loop is underlined.

parent molecular weights of 9000, 13 000, 16 000, and 21 000 were generated (lane A). Since the blot was stained with the antibody against the N-terminus of the parent 23K peptide, all of these fragments necessarily contained its N-terminus. When the LEP digest of the 43K + 44K cross-linked products was blotted and stained with the same antibody, the 9K, 13K, and 16K fragments were visible on the blot while the 21K fragment and the parent 23K peptide were missing (lane B). The result indicates that the cross-linking site in the 23K fragment of the heavy chain resides within a segment spanning two cleavage sites which generate the N-terminal 16K and 21K fragments (Figure 6).

The same band pattern as in lane A in Figure 5 was generated by trypsin digestion of the 23K fragment (Sutoh, 1987). The 16K fragment was generated by tryptic cleavages at a cluster of basic residues locating at residues 143–147 (Sutoh, 1987), whose sequence is Arg-Gly-Lys-Lys-Arg (Tong & Elzinga, 1983). For the case of the LEP digestion, which is specific at lysine residues, it is very likely that proteolytic cleavage at Lys-145 and/or Lys-146 generated the 16K fragment. Molecular weight of the segment of residues 1–145 was calculated as 16 500. Examination of the amino acid sequence of the 23K fragment (Tong & Elzinga, 1983) has revealed that there are only three lysine residues between residue 147 and residue 204, i.e., Lys-184, Lys-189, and Lys-204. Therefore, Lys-184 and Lys-189 are candidates for cleavage sites to generate the 21K LEP fragment. Molecular weights of segments of residues 1–184 and 1–189 were calculated as 20 800 and 21 300, respectively. Thus, the segment spanning the 16K cleavage site and the 21K cleavage site actually corresponds, at most, to residues 147–189.

The LEP digests of the 23K + 24K fragments and the 43K + 44K cross-linked products were blotted as above and stained with the antibody against the 23K–50K junction (lanes C and D in Figure 5). By comparison of these two blots (lanes C and D), it was evident that no band in the blot of the 23K + 24K fragments had its counterpart on the blot of the 43K + 44K products. The smallest fragment visible on the former blot had an apparent molecular weight of 8000. Even this 8K fragment was missing on the blot of the cross-linked products. Since all fragments visible on these blots contained the C-terminal segment of the 24K peptide, it is concluded that the cross-linking site is located within the C-terminal 8K segment of the 24K tryptic fragment (Figure 6), consistent with the above result by the N-terminus antibody.

*Indentification of a Lysine Residue Cross-Linked to $SH_2$.* The CNBr mappings showed that the cross-linking site in the 23K segment was in its C-terminal CNBr fragment, i.e., residues 166–204. As mentioned above, there are only three lysine residues in residues 166–204, Lys 184, Lys-189, and Lys-204. Since NPIA bridges the $SH_2$ thiol with a lysine side chain (Hiratsuka, 1987), these lysine residues are candidates for the cross-linking site. Further mappings by LEP revealed that the cross-linking site was in residues 147–189. Thus it is concluded that Lys-184 or Lys-189 is the residue cross-linked to the $SH_2$ thiol by NPIA. Since NPIA bridges only a short distance, Lys-184 and/or Lys-189 must be in close proximity to the thiol.

## Discussion

Comparison of the primary structure of nucleotide-binding proteins has revealed that many of them contain highly homologous glycine-rich sequences ("glycine-rich loop") (Walker et al., 1982; Fry et al., 1986). The glycine-rich sequence is believed to be a diagnostic sequence of the nucleotide-binding site (Walker et al., 1982), though some exceptions have been reported (MacLaughlin et al., 1984). The myosin heavy chain also contains the glycine-rich loop at residues 178–185, whose sequence is Gly-Glu-Ser-Gly-Ala-Gly-Lys-Thr (Tong & Elzinga, 1983; Walker et al., 1982). Lys-184 and Lys-189, which are candidates for a residue cross-linked to the $SH_2$ thiol, are in and very close to the glycine-rich loop. Does the result mean that the reactive thiol is in proximity to the ATPase site?

By use of electron microscopy, it has been shown that on the myosin head the $SH_1$ thiol occupies a location distinct from the ATPase site (Sutoh et al., 1984, 1986; Tokunaga et al., 1987). Although both sites are at the middle part of the head, they are located on the opposite side of the head. Therefore, some distance (several nanometers) is expected between the ATPase site and the $SH_1$ thiol, consistent with fluorescence energy transfer experiment (3–4 nm) (Tao & Lamkin 1981; Cheung et al., 1985).

Although $SH_1$ and $SH_2$ are only 10 residues apart in the primary structure (Elzinga & Collins, 1977), the three-dimensional structure around these residues seems very flexible; spatial distance between the thiols changes significantly depending on conditions as revealed by chemical cross-linking and fluorescence energy transfer experiments (Burke & Reisler, 1977; Wells & Yount, 1979; Dalbey et al., 1983; Cheung et al., 1985). Maximum distance between the two thiols can be 3 nm (Dalbey et al., 1983; Cheung et al., 1985) while minimum distance can be as little as 2 Å (Burke & Reisler, 1977; Wells & Yount, 1979). Therefore, one way to interpret our result is that the glycine-rich loop in the myosin heavy chain is really a part of the ATPase site and that the $SH_2$ thiol is away from the $SH_1$ thiol and close to the ATPase site (nine intervening residues between the two thiols can span as much as 3 nm), at least when the cross-linking reaction occurs. Another possibility is that the glycine-rich loop is not directly involved in the ATPase site and that both the glycine-rich loop and the $SH_2$ thiol are in proximity to the $SH_1$ thiol. Further studies are required to decide which is the case.

The glycine-rich loop of myosin contains one of the most conserved sequences along the heavy chain. From residue 175 to 185, the sequence is Leu-Ile-Thr-Gly-Glu-Ser-Gly-Ala-Gly-Lys-Thr for *Dictyostelium* myosin (Warrick et al., 1986), nematode myosin (Karn et al., 1983), rabbit skeletal myosin (Tong & Elzinga, 1983), rat embryonic skeletal myosin (Strehler et al., 1986), chicken skeletal myosin (Maita et al., 1987), and chicken embryonic skeletal myosin (Molina et al.,

1987). The $SH_1$–$SH_2$ region is another segment whose sequence is strikingly conserved (Gallagher & Elzinga, 1980; Karn et al., 1983; Strehler et al., 1986; Warrick et al., 1986; Maita et al., 1987; Molina et al., 1987). Furthermore, Cys-522 and Cys-540, which are in proximity to the $SH_1$–$SH_2$ region (Mornet et al., 1985; Chaussepied et al., 1986; Ue, 1987), are located next to the longest stretch of the conserved sequences of the heavy chain (residues 460–520) (Karn et al., 1983; Strehler et al., 1986; Warrick et al., 1986; Maita et al., 1987; Molina et al., 1987). Therefore, highly conserved segments in the 20K, 50K, and 23K fragments of the heavy chain participate in the structure around $SH_1$–$SH_2$. This finding implies that the area around $SH_1$–$SH_2$ may play an important role in myosin function, though it has been established that these residues are not directly involved in ATP hydrolysis.

**Registry No.** Lys, 56-87-1; ATPase, 9000-83-3.

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