Identification of a Noncatalytic Domain in AMP Deaminase That Influences Binding to Myosin†

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ABSTRACT: AMP deaminase (AMP-D) plays a critical role in energy metabolism in skeletal muscle. Prior studies have demonstrated AMP-D binds to myosin heavy chain in vitro, and it decorates the end of the A band in the myofibril. The present study presents evidence that proteolytic removal of 14 kilodaltons, presumably from the carboxy terminus, of the native 80K peptide does not eliminate catalytic activity but this deletion has a pronounced influence on binding of AMP-D to myosin in the presence of ATP. Comparison of the sequence of the rat skeletal muscle form of AMP-D to that of yeast AMP-D demonstrates conservation of an ATP binding site in the carboxy-terminal domain of the rat protein. These results provide a mechanism for regulating binding of AMP-D to myosin heavy chain in response to changes in ATP concentration and suggest a potential function for AMP-D/myosin complex formation in myocytes.

AMP deaminase (EC 3.5.4.6) (AMP-D) catalyzes a reaction that is important in nucleotide interconversion and preservation of energy charge in many cell types, it generates NH_3 that may be used to buffer H^+ in some specialized cell types, and it is an integral part of the purine nucleotide cycle, a series of reactions that links amino acid metabolism with production of citric acid cycle intermediates in some tissues (Sabina et al., 1989). AMP-D is an allosteric enzyme regulated by nucleoside triphosphates that inhibit enzyme activity at low concentrations and by nucleoside diphosphates and monophosphates that stimulate enzyme activity (Coffee & Solano, 1977; Ashby & Frieden, 1978; Wheller & Lowenstein, 1979). AMP-D and the purine nucleotide cycle play a particularly important role in energy metabolism in skeletal muscle as evidenced by the high activity of this enzyme in skeletal muscle, the unique isoform(s) of this protein produced in this tissue (Ogasawara et al., 1975), and the myopathy that develops in patients with inherited deficiency of AMP-D (Sabina et al., 1989).

In skeletal muscle a variable fraction of AMP-D has been reported to be bound to the myofibril, ranging from 20 to 90% depending upon the extraction conditions and extent of muscle activity prior to extraction (Byrnes & Suelter, 1965; Ashby & Frieden, 1977; Ashby et al., 1979; Wheeler & Lowenstein, 1979; Shiraki et al., 1981; Barshop & Frieden, 1984; Cooper & Trinick, 1984). Histochemical studies have demonstrated AMP-D is bound predominantly, if not exclusively, at the end of the A band in the myofibril (Ashby et al., 1979; Cooper & Trinick, 1984). The abundance of AMP-D relative to myosin heavy chain, i.e., 2–6 mol of native AMP-D tetramer/myosin filament, is sufficient for 1–3 native AMP-D tetramers to decorate the end of each myosin filament (Ashby et al., 1979; Cooper & Trinick, 1984).

While it is not possible to exclude that AMP-D binds to more than one protein in the myofibril (Cooper & Trinick, 1984), numerous studies have documented specific binding of AMP-D to myosin heavy chain in vitro (Byrnes & Suelter, 1965; Ashby & Frieden, 1977, 1978; Barshop & Frieden, 1984). The binding site on myosin has been localized to the α-helical hinge region of the myosin heavy chain (Ashby & Frieden, 1977; Barshop & Frieden, 1984). However, little is known about the domains in AMP-D that determine its binding to myosin heavy chain. In this paper we present evidence that a truncated form of rat skeletal muscle AMP-D resulting from in vitro proteolysis retains catalytic activity and ability to bind to myosin heavy chain, but the fragment deleted as a consequence of proteolysis has a pronounced influence on binding of AMP-D to myosin in the presence of physiological concentrations of ATP. Identification of a potential ATP binding site in the carboxy-terminal domain of AMP-D provides a mechanism for regulating binding of this enzyme to myosin in response to changes in ATP concentration and suggests a potential function for AMP-D/myosin complex formation in myocytes.

Materials and Methods

Male Sprague-Dawley LD strain rats were purchased from Charles River Breeding Laboratories, Wilmington, MA. Protease inhibitors benzamidine, phenylmethanesulfonyl fluoride, leupeptine, soybean trypsin inhibitor, pepstatin, α-2-macroglobulin, chymostatin, aprotinin, and antipain were obtained from Sigma Chemical Co., St. Louis, MO. Reagents for polyacrylamide gel electrophoresis were all purchased from Bio-Rad Laboratories. Nitrocellulose sheets were purchased from Schleicher and Schuell. Goat serum and peroxidase-conjugated goat anti-rabbit IgG were obtained from Tago, Inc., Burlingame, CA. Phosphocellulose (P 11) was purchased from Whatman. Activated CH-Sepharose 4B was obtained from Pharmacia Fine Chemicals. PEI-TLC plates were obtained from Merck, Darmstadt. [^{14}C]AMP was purchased from New England Nuclear Corp., Boston, MA. Polyclonal anti-myosin heavy chain antibody was purchased from Miles Scientific Laboratories.

Protein Purification. AMP deaminase was purified by phosphocellulose chromatography according to the method of Smiley et al. (1967). Protein concentrations were determined by assuming an $A^{1\%}_{280nm}$ value of 9.13 (Zielke & Suelter, 1967) or by the Bradford (1976) method. Myosin was prepared by the method of Perry (1955), and myosin concentration was determined spectrophotometrically by using an $A^{1\%}_{280nm}$ value of 5.43 (Kielley & Harrington, 1960).

AMP Deaminase Antibody Column. Polyclonal antisera were raised in rabbits against AMP deaminase purified from adult rat skeletal muscle (Marquetant et al., 1987). AMP

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deaminase antisera were coupled to cyanogen bromide activated Sepharose in 100 mM $NaHCO_3$ and 100 mM NaCl, pH 8.0, according to the method of the manufacturer.

Myosin Affinity Column. Purified myosin was coupled to cyanogen bromide activated Sepharose according to the method of the manufacturer as described above. The column was washed exhaustively with 500 mM KCl to remove any aggregated, noncovalently coupled myosin prior to use.

AMP Deaminase Assays. AMP deaminase activity was quantitated by determining the conversion of [^{14}C]AMP to [^{14}C]IMP (Marquetant et al., 1987). All assays were linear with respect to time and enzyme amount, and AMP disappearance equaled IMP formation.

SDS Gel Electrophoresis and Immunoblotting. Samples were electrophoresed by using 5 or 8% SDS–polyacrylamide gels according to the method of Laemmli (1970). Proteins were electrophoretically transferred to nitrocellulose membranes as described by Towbin et al. (1975). Conditions for performing immunoblots have been described previously (Marquetant et al., 1987). Proteins were visualized in duplicate gels by Amido-Schwarz or Coomassie blue stain.

Electroelution of AMP-D from SDS–PAGE Gels. The 80K and 66K forms of AMP deaminase were eluted from SDS gel slices in an electrophoretic chamber concentrator (Isco) according to the method of Hunkapiller (Hunkapiller et al., 1983). Amino acid composition (Tommasselli et al., 1986a) and N-terminal (Tommasselli et al., 1986b) analyses were performed by Dr. R. Frank at the EMBL Laboratory, Heidelberg.

Analysis of AMP-D Peptide Sequence. Hydropathy analysis of rat AMP-D was performed by the Kyte and Doolittle method (1982), and secondary structure predictions were made by the method of Garnier and Robson (1978). Dot-matrix comparison of rat and yeast AMP-D primary structure was performed by using a program written by Lawrence and Goldman (unpublished data) with a requisite minimum length of 10 residues for homology domains.

Results

In Vitro Proteolysis. We established previously that the predominant isoform of AMP-D in adult rat skeletal muscle has an apparent subunit molecular weight of 80K (Marquetant et al., 1987). However, purification of AMP-D in the absence of protease inhibitors yields a single peptide with an apparent subunit molecular weight of 66K (Figure 1). Wheeler and Lowenstein (1979) reported a similar molecular weight for AMP-D purified from rat skeletal muscle by the same procedure, while Coffee and Kofke (1975) reported a value of 60K using a procedure that employed several additional chromatography steps. Comparison of immunoblots from fresh muscle extract and the purified enzyme indicates that the 66K peptide is not present in vivo (Figure 2). These results suggest rat muscle contains a protease that cleaves the 80K native peptide to a 66K peptide. A precursor–product relationship between the 80K and 66K AMP-D peptides was documented by incubating muscle extract at 37 °C for several hours (Figure 3). The faint 80K and 66K bands observed at the 1-h time point presumably reflect accumulation of proteolytic intermediates at this time point since the 80K band predominates at 0 time and the 66K band is the predominant AMP-D species at the 2- and 3-h time points.

Proteolysis was demonstrated to be responsible for conversion of the 80K peptide to the 66K peptide by repeating the purification procedure with the following protease inhibitors in the extraction buffer: benzamidine (1 mM); phenylmethanesulfonyl fluoride (1 mM); leupeptin (1 μg/mL); soybean trypsin inhibitor (0.2 mg/mL); pepstatin (5 μg/mL); α-2-macroglobulin (5 μg/mL); chymostatin (2 μg/mL); aprotinin (2 μg/mL); antipain (2 μg/mL); and EDTA (1 mM). Under these conditions the purified AMP-D prepara-

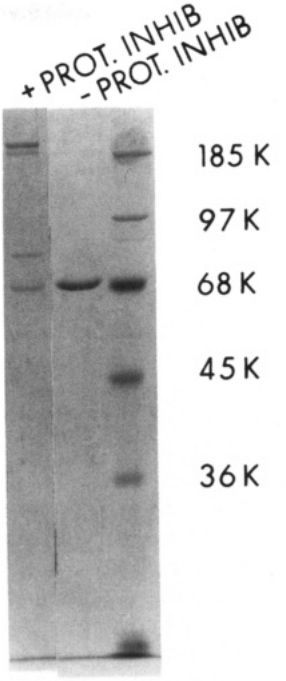


FIGURE 1: Purification of AMP-D in the presence and absence of protease inhibitors. Skeletal muscle from adult rat hindlimb was extracted in 89 mM KP_i and 100 mM KCl, pH 6.5, containing no protease inhibitors, or the following inhibitors were included in the extraction buffer: benzamidine, phenylmethanesulfonyl fluoride, leupeptine, soybean trypsin inhibitors, pepstatin, α-2-macroglobulin, chymostatin, aprotinin, and antipain. Extracts were applied to a phosphocellulose columm equilibrated in extraction buffer, and the column was developed with a linear gradient of KCl (Smiley et al., 1967). AMP-D activity eluted in a single peak and at the same position whether or not protease inhibitors were included in the extraction buffer. (Lane 1) Coomassie staining pattern of the SDS–PAGE for 5 μg of the AMP-D preparation extracted with protease inhibitors. (Lane 2) 5 μg of the AMP-D preparation extracted without protease inhibitors. (Lane 3) Molecular weight standards.

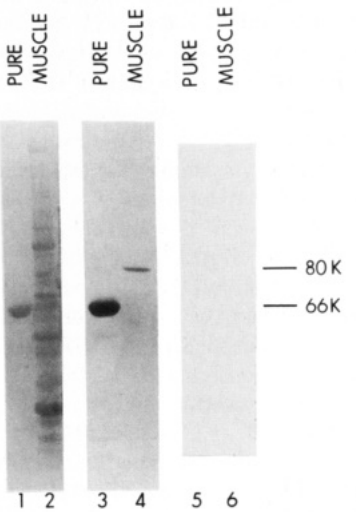


FIGURE 2: Immunoblot of purified AMP-D and crude extract of rat hindlimb. (Lanes 1, 3, and 5) 4 μg of AMP-D purified without protease inhibitors, as described in the legend to Figure 1. (Lanes 2, 4, and 6) 150 μg of rat hindlimb extracted directly into SDS sample buffer. Lanes 1 and 2 are stained with Amido-Schwarz; lanes 3 and 4 are probed with AMP-D antisera; and lanes 5 and 6 are probed with control antisera. The second antibody was a goat, anti-rabbit peroxidase-conjugated immunoglobin.

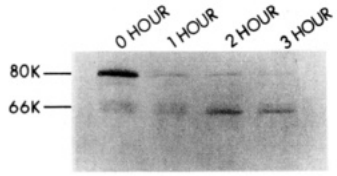


FIGURE 3: In vitro conversion of 80K to 66K form of AMP-D. Rat hindlimb was extracted in 89 mM KP_i and 100 mM KCl, pH 6.5, without protease inhibitors. The extract was incubated at 37 °C; 150-μg aliquots were removed at the indicated times for SDS–PAGE. Immunoblots were prepared as described in the legend to Figure 2 by using AMP-D antisera.

tion contains a mixture of 80K and 66K subunits (Figure 1). We have not been able to obtain a purified preparation of AMP-D that contains only the native 80K subunit irrespective of the precautions employed to block proteolysis during extraction and column chromatography.

The protease(s) responsible for cleavage of the 80K to 66K peptide could remove residues from the amino and/or carboxy termini. Analyses of the amino and carboxy termini of the truncated AMP-D peptide have the potential of providing insight into the location of in vitro proteolysis. The amino terminus of the 66K peptide purified by phosphocellulose chromatography as illustrated in Figure 1 is blocked at the amino terminus (Sabina et al., 1987). Coffee and Kofke (1975) previously reported that the amino terminus of the 60K rat skeletal muscle AMP-D they purified by a combination of phosphocellulose, DEAE-cellulose, and Bio-Gel chromatography is also blocked. These investigators found the carboxy terminus of the truncated AMP-D peptide is heterogeneous–50% valine and 50% leucine residues. The carboxy-terminal residue predicted from the cDNA sequence is glutamate (Sabina et al., 1987). As stated above, we have not been able to purify the native 80K peptide by column chromatography, but we have isolated the 80K and 66K peptides from SDS gels by electroelution (Hunkapillar et al., 1983); both peptides are blocked at their amino termini. While we cannot exclude the possibility that the amino-terminal residue becomes modified following in vitro proteolysis, especially in the peptides isolated by electroelution from SDS gels, the finding of a blocked amino terminus after isolation under the mild conditions of column chromatography in combination with an altered carboxy terminus suggests proteolysis may be restricted to the carboxy terminus.

Properties of Native and Truncated AMP-D. The enzyme purified in the absence of protease inhibitors consists of only 66K subunits (Figure 1) and has a specific activity of approximately 1000 units/mg, a value comparable to that reported by others (Coffee & Solano, 1977; Ashby & Frieden, 1978; Wheeler & Lowenstein, 1979). Thus, the fragment of the protein that is removed by proteolysis in vitro is not essential for catalytic activity. Wheeler and Lowenstein (1979) demonstrated previously that the 66K truncated form of rat AMP-D responds to the inhibitory effect of low concentrations of purine nucleoside triphosphates, and it is stimulated by low concentrations of AMP and ADP. We have obtained similar results with the 66K truncated form of rat skeletal muscle AMP-D (data not shown). Thus, the truncated 66K form of rat AMP-D retains nucleotide inhibitory sites and nucleotide stimulatory sites in addition to its catalytic site. It has not been possible to purify AMP-D consisting of only native 80K subunits from rat skeletal muscle, and consequently, we have not been able to compare the kinetic properties of the native and truncated rat enzymes.

Since the segment of rat skeletal muscle AMP-D removed by in vitro proteolysis is not required for catalytic activity or

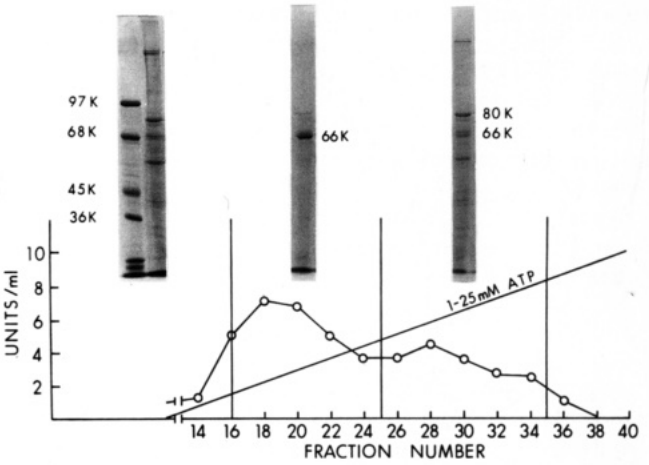


FIGURE 4: Binding of partially purified AMP-D to myosin affinity column. Myosin heavy chain was purified and coupled to Sepharose 4-B as described under Materials and Methods. An AMP-D preparation partially purified in the presence of protease inhibitors was applied to the myosin affinity column that had been equilibrated in 89 mM KP_i and 180 mM KCl, pH 6.5, with 1 mM β-mercaptoethanol and 2 mM $MgCl_2$. After washing with 2 column volumes of this buffer, a linear gradient of 1–25 mM ATP and 2–26 mM $MgCl_2$ was used to elute AMP-D. AMP-D activity of each fraction is shown on the ordinate (O). The two peak fractions, as well as the starting preparation, were analyzed by SDS–PAGE (gel insets above the activity profile). Molecular weight standards are included in the lane on the left.

regulation of this enzyme by purine nucleotides, it is possible that this region of the protein has some other function. A role for this segment in binding of AMP-D to myosin was first suggested by the results of the purification procedures performed in the presence and absence of protease inhibitors (Figure 1). AMP-D elutes in the same position from the phosphocellulose column in the presence and absence of protease inhibitors. However, when protease inhibitors are included, a peptide of ≈200K copurifies with AMP-D (Figure 1). This peptide does not react with AMP-D antisera but does bind anti-myosin antibodies (see immunoblot in Figure 5). Thus, the native peptide of AMP-D binds to myosin heavy chain under conditions that lead to dissociation of the truncated peptide.

Binding of Native and Truncated AMP-D to Myosin Heavy Chain. Myosin heavy chain purified from rat skeletal muscle was coupled to Sepharose 4B, and an AMP-D preparation purified in the presence of protease inhibitors was passed over this column (Figure 4). After the column was washed with KP_i buffer, pH 6.5, AMP-D was eluted with a linear gradient of MgATP in the same buffer. AMP-D activity elutes from the column in two peaks; the first peak elutes at 4–5 mM ATP and contains a large preponderance of 66K AMP-D subunits; the second peak elutes at 10–12 mM ATP and contains a mixture of 80K and 66K AMP-D subunits (Figure 4). Since most of the AMP-D tetramers in the first peak will be homopolymers of 66K subunits, we conclude that the 66K subunit is capable of binding to myosin at pH 6.5. Tetramers that are homopolymers of 80K subunits or heteropolymers in which 80K subunits predominate (second peak) also bind to myosin, but this myosin/AMP-D complex is less sensitive to ATP-induced dissociation. We conclude from these observations that the 14-kDa fragment removed from the native AMP-D peptide by in vitro proteolysis has a pronounced influence on the binding of AMP-D to myosin in the presence of ATP.

To examine binding of native AMP-D to myosin in the presence of other myofibrillar proteins, rat skeletal muscle extracted in the presence of protease inhibitors was applied

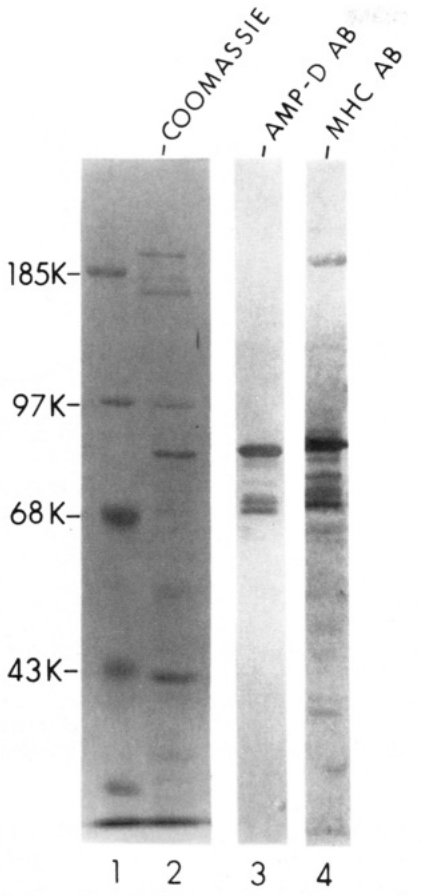


FIGURE 5: Binding of AMP-D in muscle extract to antibody affinity column. AMP-D antisera was coupled to Sepharose 4-B as described under Materials and Methods. Rat hindlimb extracted in 89 mM KP_i and 200 mM KCl, pH 6.5, containing protease inhibitors was applied to the antibody affinity column equilibrated in extraction buffer. The column was washed sequentially with 2 volumes of extraction buffer containing 3 M KCl, 1 mM GTP, 100 mM PP_i, and 500 mM $MgCl_2$. A final wash was performed with 100 mM glycine hydrochloride, pH 2.5, and the eluate immediately neutralized. Each fraction was assayed for AMP-D activity and analyzed by SDS–PAGE, including immunoblotting. No AMP-D activity was detectable in any of the eluted fractions, and AMP-D antigen was only found in the acid wash. (Lane 2) Pattern of proteins eluted in the final acid wash (Coomassie stain). (Lane 1) Molecular weight standards. (Lane 3) Immunmoblot of the acid elutate probed with AMP-D antisera. (Lane 4) The same filter strip shown in lane 3 reprobed with an anti-myosin heavy chain antisera.

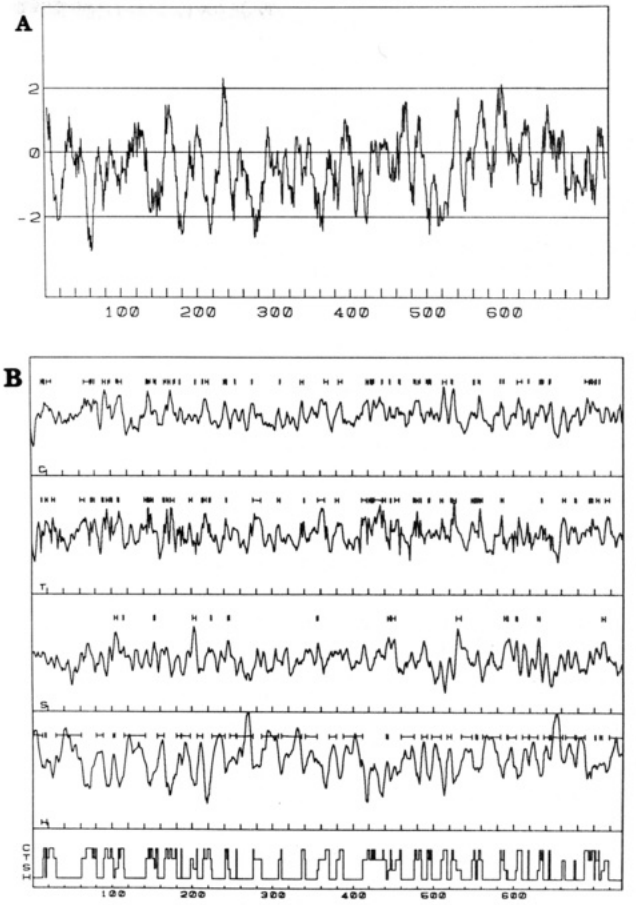


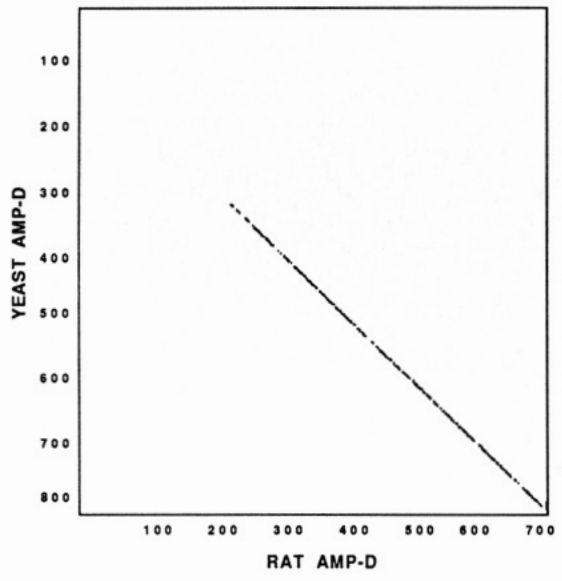
FIGURE 6: AMP-D structural analysis. (A) Hydropathy analysis of rat skeletal muscle AMP-D. (B) Predicted secondary structure of AMP-D; C = coil, T = turn, S = β-sheet, and H = α-helix.

to an affinity column constructed with AMP-D antisera linked to Sepharose 4B. As shown in Figure 2, this antisera does not recognize any other proteins in rat muscle extract. After application of muscle extract, the antibody affinity column was washed sequentially with 89 mM KP_i buffer, pH 6.5, containing 3 M KCl, 1 mM GTP, 100 mM PP_i, and 500 mM $MgCl_2$. Neither AMP deaminase activity nor AMP-D antigen was detectable in any of these column washes. The column was then developed with 100 mM glycine hydrochloride, pH 2.5, to dissociate antigen/antibody complexes. The elute was immediately neutralized, and a small group of peptides was identified in the acid wash (Figure 5). While no AMP-D activity was detectable, the prominent peptide with apparent molecular weight of 80K was shown to be AMP-D on the basis of its reactivity with AMP-D antisera (Figure 5, lane 3). The prominent 200K peptide reacted with anti-myosin antisera (Figure 5, lane 4). The prominent 43K peptide is probably actin, another major component of the myofibril. These results demonstrate that the native form of AMP-D remains bound to myosin even in the presence of other myofibrillar proteins, and it cannot be displaced by high salt concentrations (3 M KCl) or high concentrations of GTP, PP_i, or Mg^{2+}.

Structural Analysis of AMP-D. We have previously reported the amino acid sequence for adult rat skeletal muscle AMP-D deduced from the cDNA sequence (Sabina et al., 1987). Hydropathy analysis of this peptide, shown in Figure 6A, does not indicate clustering of hydrophobic or charged residues in any region of the peptide. Predicted secondary structure of this peptide is not unusual for a globular enzyme (Figure 6B).

Dot-matrix comparison of the primary sequence of rat skeletal muscle AMP-D to that of yeast AMP-D (Meyer et al., 1989), the only other form of AMP-D sequenced thus far, indicates that some regions of AMP-D have been highly conserved during evolution while others have not (Figure 7). [Sequence alignment for individual amino acid residues is presented in the accompanying paper by Meyer et al. (1989).] The primary sequence of the amino-terminal 240 residues of the rat protein is quite divergent from that of the amino terminus of the yeast enzyme. This comparison suggests the amino-terminal region of the rat peptide may not be essential for catalysis, a conclusion supported by studies in which up to 175 amino acid residues have been deleted from the amino terminus of the rat peptide in a bacterial expression vector with retention of catalytic activity, albeit at a reduced level (unpublished observations). From residue 241 to the carboxy terminus, the rat peptide is 52% identical with the yeast protein. Within this conserved presumably catalytic region a potential ATP binding site has been identified by comparison to the yeast protein. Residues 717–732 of the yeast peptide have been demonstrated to bind ATP (Merkler & Schramm,

A. DOT-MATRIX COMPARISON



B. CONSERVATION OF ATP BINDING SITE

RAT: EPLMEEYAIAAQVFKLSTCDMCEVARNSVLQ
YEAST: EPLI EEYSVAAQIYKLSNVDMCELARNSVLQ

C. PROPOSED DOMAINS IN AMP-D

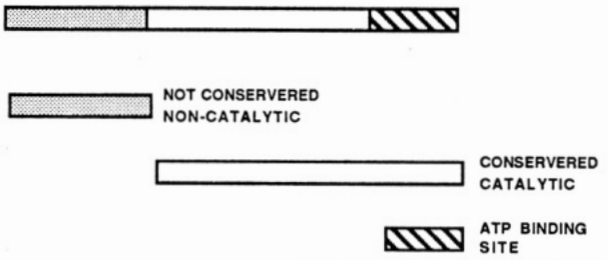


FIGURE 7: Functional domains in AMP-D. (A) Dot-matrix comparison of the primary amino acid sequence of rat skeletal muscle AMP-D and yeast AMP-D. Amino acid residues in each peptide are numbered beginning at the amino terminus and recorded on the abscissa and ordinate. The dotted line indicates regions of similar sequence. (B) Comparison of conserved sequences in the carboxy-terminal domain of yeast and rat AMP-D. The ATP binding site of the yeast peptide is underlined. The yeast sequence begins at residue 717, and the rat sequence begins at residue 660. Identical residues are noted by a line connecting the two sequences. (C) Putative functions assigned to the various domains of rat skeletal muscle AMP-D.

1988). In the analogous portion of the rat carboxy-terminal region, 23 of 31 residues surrounding the ATP binding site are identical with those of the yeast enzyme (Figure 7).

Discussion

Prior studies have demonstrated AMP-D binds to myosin heavy chain; there is one AMP-D binding site per myosin monomer, in the native myosin dimer there is cooperativity with respect to addition of the two molecules of AMP-D, and the AMP-D binding site is localized to the α-helical hinge region of myosin (Ashby & Frieden, 1977, 1978; Barshop & Frieden, 1984). The present study provides insight into the domains of AMP-D that participate in formation of this intermolecular complex.

The native, 80K skeletal muscle isoform of AMP-D can be subdivided into two domains on the basis of comparison to the yeast enzyme (Figure 7): a nonconserved, noncatalytic amino-terminal domain and a conserved, catalytic carboxy-terminal domain. The latter domain contains not only the active site for binding of AMP but also an ATP binding site located within the terminal 70 residues. The 66K proteolytic product of the native 80K AMP-D peptide provides further definition of these putative functional domains. The truncated peptide is catalytically active, exhibits allosteric regulation by purine nucleotides, and binds to myosin heavy chain, indicating an AMP binding catalytic site, a nucleotide binding regulatory site, and a binding site for myosin heavy chain must be located on this truncated peptide. This conclusion does not assume that any of these binding sites are necessarily comprised of contiguous amino acid residues. The region deleted from the native peptide, while not essential for any of the above functions, has a pronounced influence on AMP-D binding to myosin heavy chain in the presence of ATP.

Location of the truncated AMP-D peptide with its various binding sites within the native 80K peptide has not been determined with certainty, but for reasons discussed earlier we favor the interpretation that in vitro proteolysis may be restricted to the carboxy terminus. If this conclusion is correct, it suggests a model of the AMP-D peptide in which the catalytic domain of the protein is located in the conserved mid portion of the peptide; the amino and carboxy termini are not required for catalytic activity. In this model the myosin binding site is located in some portion of the molecule other than the carboxy-terminal 14 kDa since this region is presumably removed from the native peptide by in vitro proteolysis. The ATP binding site in the carboxy-terminal region will be removed from the native 80K peptide following in vitro proteolysis.

This model suggests a mechanism by which the carboxy-terminal domain could influence binding of AMP-D to myosin. Binding of ATP in the carboxy-terminal domain may induce a conformational change in the native enzyme which alters the properties of the myosin binding site of the native peptide. This hypothesis is consistent with the observation that ATP induces dissociation of the native 80K form of AMP-D from myosin. This cannot be the only nucleotide site that affects binding of AMP-D to myosin, however, since the 66K truncated form of AMP-D is also dissociated from myosin by ATP. The 66K truncated form of rat skeletal muscle has a nucleoside triphosphate inhibitory site, as well as an NXP stimulatory site (Wheeler & Lowenstein, 1979), and binding of ATP to one of these sites presumably accounts for its effect on dissociating the truncated form of AMP-D from myosin. The ATP concentration required for dissociation of the native form of AMP-D from myosin is 2–3 times higher than for the truncated enzyme, suggesting that conformational changes resulting from inclusion of the carboxy-terminal domain alter the accessibility or affinity of nucleotide binding sites as well as myosin binding sites on the amino-terminal and/or central domains of AMP-D.

This model provides a framework for interpreting prior studies that have analyzed AMP-D binding to myosin. Nakagawa and associates (Shiraki et al., 1981) examined the fraction of AMP-D bound to the myofibril in freshly isolated rat skeletal muscle from resting animals and following muscle stimulation. These investigators found that the percentage of AMP-D bound to the myofibril increases from ≈20% at rest to ≈75% after 30 s of muscle stimulation in association with a 40% decrease in ATP content of the muscle. We find that changes in ATP concentration over the range which occur during the transition from resting to vigorously contracting muscle (≈10 to 5 mM)[1] are associated with changes in binding

of native AMP-D to myosin (Figure 4). The domain of skeletal muscle AMP-D removed by in vitro proteolysis has a pronounced effect on binding of native AMP-D to myosin, and the presumed location of this domain, i.e., the carboxy terminus, contains a highly conserved sequence shown to be an ATP binding site in the yeast enzyme (Merkler & Schramm, 1988). One interpretation of these results is that the carboxy-terminal domain of the native AMP-D peptide regulates the binding of this enzyme to myosin in response to changes in ATP concentrations during muscle contraction. Since AMP-D is refractory to inhibition by nucleoside triphosphates when bound to myosin (Ashby & Frieden, 1977, 1978), regulating the binding of AMP-D to myosin may provide another mechanism for controlling AMP-D activity in addition to the allosteric effects produced by ATP and other nucleotides binding to AMP-D.

Acknowledgments

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Registry No. AMP-D, 9025-10-9; ATP, 56-65-5.

References

Ashby, B., & Frieden, C. (1977) *J. Biol. Chem. 252*, 1869–1872.

Ashby, B., & Frieden, C. (1978) *J. Biol. Chem. 253*, 8728–8735.

Ashby, B., Frieden, C., & Bischoff, R. (1979) *J. Cell Biol. 81*, 361–373.

Barshop, B. A., & Frieden, C. (1984) *J. Biol. Chem. 259*, 60–66.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Byrnes, E. W., & Suelter, C. H. (1965) *Biochem. Biophys. Res. Commun. 20*, 422–426.

Coffee, C. J., & Kofke, W. A. (1975) *J. Biol. Chem. 250*, 6653–6658.

Coffee, C. J., & Solano, C. (1977) *J. Biol. Chem. 252*, 1606–1612.

Cooper, J., & Trinick, J. (1984) *J. Mol. Biol. 177*, 137–152.

Garnier, J., Osquthorpe, D. J., & Robson, R. (1978) *J. Mol. Biol. 120*, 97–120.

Hunkapillar, M. W., Lujan, E., Ostrander, F., & Hood, L. E. (1983) *Methods Enzymol. 92*, 227–236.

Kielley, W. W., & Harrington, W. F. (1960) *Biochim. Biophys. Acta 41*, 401–421.

Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.

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

Marquetant, R., Desai, N. M., Sabina, R. L., & Holmes, E. W. (1987) *Proc. Natl. Acad. Sci. U.S.A 84*, 2345–2349.

Merkler, D. J., & Schramm, V. L. (1988) *FASEB J. 2*, A552.

Meyer, S. L., Kvalnes-Krick, K. L., & Shramm, V. L. (1989) *Biochemistry* (second of three papers in this issue).

Ogasawara, N., Goto, H., & Watanabe, T. (1975) *Biochim. Biophys. Acta 403*, 530–537.

Perry, S. V. (1955) *Methods Enzymol. 2*, 582.

Sabina, R. L., et al. (1983) *J. Appl. Physiol. 55*, 624–627.

Sabina, R. L., Marquetant, R., Desai, N. M., Kaletha, M., & Holmes, E. W. (1987) *J. Biol. Chem. 262*, 12397–12400.

Sabina, R. L., Swain, J. L., & Holmes, E. W. (1989) in *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., & Valle, D., Eds.) 6th ed., pp 1077–1084, McGraw-Hill, New York.

Shiraki, H., Miyamoto, S., Matsuda, Y., Momose, E., & Hakagawa, H. (1981) *Biochem. Biophys. Res. Commun. 100*, 1099–1103.

Smiley, V. L., Berry, A. J., & Suelter, C. H. (1967) *J. Biol. Chem. 242*, 2502–2506.

Tommasselli, A. T., Frank, R., & Schlitz, E. (1986a) *FEBS Lett. 202*, 303–308.

Tommasselli, A. T., Mast, E., Jones, W., & Schlitz, E. (1986b) *Eur. J. Biochem. 15*, 111–119.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Wheeler, T. J., & Lowenstein, J. M. (1979) *J. Biol. Chem. 254*, 8994–8999.

Zielke, C. L., & Suelter, C. H. (1967) *J. Biol. Chem. 242*, 2502–2506.

[1] ATP concentration was estimated by assuming intracellular water is 60% of wet weight (Sabina et al., 1983).