Molecular Cloning of AMP Deaminase Isoform L

SEQUENCE AND BACTERIAL EXPRESSION OF HUMAN AMPD2 cDNA*

(Received for publication, March 27, 1992, and in revised form, July 16, 1992)

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Human AMPD2 cDNA clones have been isolated from T-lymphoblast and placental λgt11 libraries utilizing a previously cloned rat partial AMPD2 cDNA as the probe. Alignment analysis of all cDNA clones indicates the presence of intervening sequences in several placental isolates. This has been confirmed by sequencing human AMPD2 genomic clones. Intervening sequences can be removed from the cDNA clones by restriction with endonucleases at unique sites within the proposed open reading frame. This results in a 3292-base pair cDNA proposed to contain the entire AMPD2 open reading frame, which would encode a 760-amino acid polypeptide with a predicted subunit molecular mass of 88.1 kDa. Nucleotide and predicted amino acid comparisons with the 264 base pairs of proposed coding sequences in the rat AMPD2 cDNA demonstrate 91% similarity and identity, respectively. A comparison of the predicted human AMPD1 and AMPD2 polypeptides demonstrates homology in their C-terminal domains. Included in this region is the conserved motif, SLSTDDP, proposed to be part of the catalytic site of all AMP deaminases. In contrast, the predicted N-terminal domains of the human AMPD1 and AMPD2 polypeptides are unique. When placed in a prokaryotic expression vector, the human AMPD2 cDNA expresses AMP deaminase activity which can be precipitated with polyclonal antisera specific for isoform L.

AMP deaminase (AMPD; EC 3.5.4.6) is an integral enzyme of purine nucleotide interconversion. Higher eukaryotes express multiple isoforms of this allosterically regulated catalytic activity. In humans, four variants have been described (1). These include isoforms M (muscle), L (liver), and E1/E2 (erythrocyte), named after the tissue/cell type from which they were initially characterized. Each isoform has been purified as a tetramer (1, 2), and many tissues and cell types express more than one AMPD isoform (1, 3).

In recent years, the molecular biology of AMPD has begun to emerge. In mammals, AMPD is manifest through the regulated expression of a multigene family. The AMPD1 gene, which has been cloned, partially sequenced, and characterized in rats and humans (4), is expressed primarily in adult skeletal muscle (5–7). A MEF-2-like enhancer element (8), located immediately upstream of the AMPD1 transcription start site (4), is necessary and sufficient to promote muscle-specific expression (9). Recently, a single nonsense mutation in the human AMPD1 gene has been identified in 11 patients presenting with a myopathy and an inherited skeletal muscle deficiency of isoform M (myoadenylate deaminase) (10).

A second AMPD gene, AMPD2, has been identified in rats and humans based on information derived from a partial cDNA clone isolated from a rat brain library (6). RNase protection analyses, however, indicate that this cDNA contains intervening sequences. Subsequent Northern blot and additional RNase protection analyses demonstrate that AMPD2 is expressed primarily in non-muscle tissues in the rat. Genomic Southern blot analyses, furthermore, suggest an AMPD2 gene in humans as well. Although limited to comparisons based on partial sequence, a higher degree of nucleotide similarity and predicted amino acid homology is observed between the rat and human AMPD1 genes relative to that between the rat AMPD1 and AMPD2 genes (11).

Most recently, a third AMPD gene, AMPD3, has been identified in rats and humans (12). The human gene, which has been partially characterized, generates at least three transcripts by alternative splicing of 5′-terminal exons. Bacterial expression of a truncated human AMPD3 cDNA, moreover, produces polypeptide that is immunoreactive with polyclonal antisera raised against purified isoform E1.

This report describes the isolation, sequence, and bacterial expression of a human AMPD2 cDNA which contains an open reading frame encoding a polypeptide with a predicted subunit molecular mass of 88.1 kDa. When placed in a prokaryotic expression vector, this cDNA produces AMPD activity which can be precipitated only with antisera specific in humans for isoform L. A complete alignment with the human AMPD1 cDNA is now possible, and this comparison is discussed in relation to AMPD isoform diversity.

EXPERIMENTAL PROCEDURES

Materials

Biochemicals were purchased from Sigma. Restriction endonucleases, nucleotides, modifying enzymes, and molecular biology grade reagents were obtained from Boehringer Mannheim. Bacterial growth media (LB and NCZYM) were purchased from Gibco-BRL Life Science Technologies, Inc. Agar was obtained from Difco Laboratories, and electrophoresis grade agarose was supplied by Bio-Rad Laboratories. Seaplaque low melting agarose was purchased from Life Technologies, Inc.
FMCo Corp. Millipore-NC nitrocellulose filters (0.45 μm) were obtained from Millipore Corp. The prokaryotic expression vector, pKK233-2, was obtained from Pharmacia LKB Biotechnology Inc. Suspended Staphylococcus aureus cells (Pansorbin) were supplied by Calbiochem. All radiotopes used for labeling probes ([α-32P]dCTP) and sequencing (α-35S-dATP) and a random-primer labeling kit were purchased from Amersham. Kits for sequencing and the polymerase chain reaction were obtained from United States Biochemical Corp. and Perkin-Elmer Cetus Instruments, respectively. A multiple tissue Northern (MTN) blot was purchased from Clontech Laboratories.

Library Screening

cDNA—Two different λgt11 cDNA libraries were employed. The first, prepared by random-purging of mRNA isolated from a human acute T-cell leukemic cell line, HuT 102 (HuT), was kindly provided by Dr. Warner Greene, Duke University Medical Center, Durham, NC. The second, prepared by oligo(dT)-priming of mRNA isolated from human placenta (HUT) was kindly provided by Dr. J. Evan Sadler, Washington University, St. Louis, MO, and has been described (13). In both cases, approximately 1.8 × 10^9 plaque-forming units were used to infect Escherichia coli strain Y1090, plated in 150-mm dishes on Luria broth (LB) agar, and duplicate filters were prepared. The initial screen employed the HuT library, and the filters were hybridized as previously described (5) utilizing a 583-base pair rat AMPD2 cDNA (6) as the probe at relatively low stringency (0.6 M NaCl at 60 °C). An overnight exposure of the washed filters identified several strongly reactive duplicate positives. Two of these were plaque purified with additional rounds of screening. The recombinant plasmid and subcloned into plasmid DNA (pBS, Stratagene Cloning Systems), and the resulting recombinant plasmids were labeled HuTA and HuTB (see Fig. 1). The HuTA insert was utilized to screen the second λgt11 cDNA library (HuPL), and four additional positive plaques were purified and their inserts subcloned into pBS. These additional recombinant plasmids were labeled HuPL1A-1D, and HuPL1E. Finally, the 5'-end of the HuPL16 insert was utilized as the probe to screen the HuT cDNA library, and a single positive plaque was purified, its insert was subcloned into pBS, and the recombinant plasmid was labeled HuT6A.

Genomic—A human genomic library, RPMI-8402, prepared from partially Sau3AI-digested DNA isolated from a human T-cell leukemic cell line and cloned into the X2001 phage vector, was obtained from Dr. Richard Baer (Southwestern Medical Center, Dallas, TX). Approximately 1 × 10^9 plaque-forming units were used to infect E. coli host strain, LE-392, and plated on NZCYM medium in 150-mm dishes. Duplicate filters were prepared and screened as previously described (4) utilizing 32P-radiolabeled human AMPD2 cDNA inserts. Two positive plaques were purified and labeled RPMI-1s and RPMI-9. Phage DNA was prepared and restricted with a battery of endonucleases, and the resulting fragments were isolated and subcloned into pBS.

Both strands of all recombinant plasmid cDNA and genomic inserts were sequenced by the dideoxy chain termination method (14) utilizing universal, reverse, and designed oligonucleotide primers. The designed oligonucleotides were derived from cDNA sequence obtained commercially (Operon Technologies, Inc., Alameda, CA) or by the Protein and DNA Synthesis facilities of the Medical College of Wisconsin (funded in part by United States Public Health Service Grant RR03326).

Multiple Tissue Northern Blot Analysis

A charge-modified nylon membrane was obtained from a commercially available source. This membrane contained 2 μg of highly pure poly(A)^+ RNA from eight different human tissues that had been transferred from a denaturing formamide agarose gel. Hybridization was performed by a previously described method (5) utilizing an α-32P-labeled 2.3-kb human AMPD1 cDNA (11) and 32D5 1.3-kb pair human AMPD2 cDNA (HuT6A). Washes were performed at high stringency as previously reported (5).

Bacterial Expression of Human AMPD2 cDNA

Bacterial expression was accomplished by employing the commercially available prokaryotic expression vector, pKK233-2, which contains the tri-d-galactosidase-inducible trc promoter. An NcoI restriction endonuclease site (CCATGG) was created around the putative start codon (CCATGC) of the hybrid AMPD2 cDNA (HuPL10B/16; see “Results”) by oligonucleotide-directed mutagenesis employing the polymerase chain reaction. The mutated cDNA was digested with Ncol (5'-end) and HindIII (3'-end) restriction endonucleases that excised a 5358-base pair fragment which was recovered and subcloned into the pKK233-2 vector. The resulting plasmid, labeled pKK-16, was utilized to transform competent E. coli host cells (JM105), as was pKK233-2 alone as a negative control. Five ml of an overnight culture derived from a single transformed bacterial colony were added to 500 ml of LB broth and incubated with shaking at 37 °C to an A550 of 0.3 to 0.4. Induction was initiated by the addition of isopropyl-thio-β-D-galactopyranoside to a final concentration of 2 mM, and the cultures were allowed to grow an additional 3.5 h.

Assay and Immunological Characterization of Bacterially Expressed AMPD2

Isopropyl-thio-β-D-galactopyranoside-induced cultures were pelleted, resuspended in 2 ml of extraction buffer (50 mM imidazole, pH 7.0, containing 100 mM potassium chloride and 1 mM dithiothreitol), and sonicated on ice with bursts of 30 s until the suspension cleared. Sonicated extracts were pelleted to remove debris, and the supernant was dialyzed overnight against 1000 volumes of extraction buffer in order to remove endogenous nucleotides. Dialedelate was aliquoted for AMPD activity at 37 °C in the presence of 50 mM substrate (AMP). Assay conditions were 37.5 mM imidazole, pH 7.0, 100 mM potassium chloride, and 1 mM diithiothreitol. Substrate and product (IMP) were separated by anion exchange high performance liquid chromatography as previously described (15). Immunoreactivity of AMPD activity was evaluated by a previously described solution-hybridization assay (16) employing available isoform-specific antisera (1, 17).

Computer Analysis of Nucleotide and Predicted Amino Acid Sequence

All computer-assisted analyses of nucleotide and predicted amino acid sequence were performed utilizing the sequence analysis software package of the University of Wisconsin Genetics Computer Group (UWGG; Madison, WI).

RESULTS

Cloning and Sequence of Human AMPD2 cDNA and Genomic DNA—Previous immunological studies have demonstrated cross-reactivity of antisera raised against rat AMPD isoform B with human AMPD isoform L (1). We reasoned that this could be due to similarity at the nucleotide level and exploited a previously cloned rat AMPD2 cDNA (6), proposed to be specific for rat isoform B (embryonic) (16). The rat AMPD2 cDNA probe identified two positive clones in an initial screening of a human acute T-cell leukemic cDNA (HuT) library. The cDNA inserts were isolated and subcloned into pBS for DNA sequencing. The resulting recombinant plasmids were labeled HuTA and HuTB. The HuTA insert was then used to screen a human placental cDNA (HuPL) library resulting in the isolation of four additional inserts, and the resulting recombinant plasmids were labeled HuPL1A, HuPL1B, HuPL13, and HuPL16. Finally, the 5'-end of the HuPL16 cDNA insert was used to rescreen the HuT library, and an additional insert was isolated and subcloned, and the resulting recombinant plasmid was labeled HuT6A. The linear relationship between all positive cDNA clones is presented in Fig. 1. A peculiarity of the HuPL clones is that a mutant fragment with each other, as well as with the HuT clones, three of them appear to contain intervening sequences. As illustrated in Fig. 2, this contention has been confirmed by alignments with human AMPD2 genomic clones.

Utilizing unique BstXI and BssHII restriction endonuclease sites in the overlapping clones HuPL10B and HuPL16, the intervening sequence in the open reading frame of the latter was removed to yield a 3359-base pair cDNA (HuPL10B/16; see Fig. 1). This construct contains a 2283-base pair open reading frame.
Cloning, Sequence, and Expression of Human AMPD2 cDNA

Cloning, Sequence, and Expression of Human AMPD2 cDNA. The human AMPD2 cDNA contains a region which is 91% (240/264) similar to the nucleotide level to the proposed coding sequences of the rat AMPD2 cDNA (Fig. 4). Alignment of the human AMPD2 cDNA with the analogous region of the human AMPD1 cDNA (11) reveals only 67% (177/264) nucleotide similarity (alignment not shown). An overall alignment of the entire proposed open reading frames of the human AMPD1 and AMPD2 cDNAs requires numerous gaps, particularly over the 5' one-third of the two sequences. However, an alignment covering most of the 3' two-thirds of these two sequences generates a large block of continuous nucleotide similarity, 63% (960/1515), spanning nucleotides +693 to +2205 in the human AMPD2 cDNA (alignment not shown; refer to Fig. 3 for the AMPD2 sequence involved and Ref. 11 for the analogous AMPD1 sequence).

Predicted Primary Amino Acid Sequence Alignment of Human AMPD1 and AMPD2 Polypeptides—A computer-generated alignment (BESTFIT program of the GCG software package) of the entire predicted primary amino acid sequence of the human AMPD1 and AMPD2 polypeptides is presented in Fig. 5. Considering the nucleotide sequence alignment (see above), it is not surprising that these two human AMPD polypeptides are predicted to exhibit regional homology as well. An alignment of their N-terminal domains requires that several gaps be included. Conversely, their C-terminal domains display significant homology, except at the extreme C-terminal ends. The large block of nucleotide similarity in the 3' two-thirds of the human AMPD1 and AMPD2 cDNAs described above would encode a highly homologous stretch of amino acids, 62% (314/505), spanning residues 232 to 736 in the AMPD1 polypeptide and residues 228 to 732 in the AMPD2 polypeptide (see Fig. 5). Included within this conserved domain of amino acids are the predicted 88 residues from all cloned AMPD cDNA sequences, i.e. yeast, rat, and human (12). The length of this region is defined by the limiting nucleotide sequence available for the rat AMPD2 cDNA (see Fig. 4). Inspection of the predicted primary amino acid sequence encoded by this stretch of human AMPD2 cDNA (residues 567 to 554; see Fig. 5) demonstrates identity with the rat AMPD2 polypeptide (see Fig. 4) and lower homologies (73-74%) with other mammalian cDNAs and the yeast cDNA. This region of amino acid residues also contains the conserved motif, SLSTDDP (residues 645 to 651 in the AMPD1 polypeptide and residues 641 to 647 in the AMPD2 polypeptide; see Fig. 5), proposed to be involved in the catalytic site of AMPD (18).

Multiple Tissue Northern Blot Analysis of Relative AMPD1 and AMPD2 Transcript Abundance—Previous analyses in the rat have indicated relatively high levels of AMPD1 expression in skeletal muscle, whereas relative AMPD2 gene expression is greater in non-muscle tissues and undifferentiated muscle (6). In order to examine whether a similar profile of AMPD
Messenger RNA for human AMPD is expressed in brain, placenta, lung, liver, kidney, and pancreas, but relatively little in heart or skeletal muscle (longer exposures show low level expression in these two tissues). Together, these results are consistent with previous data regarding relativeAMPD1 and AMPD2 gene expression in the rat.

The estimated size of the human AMPD2 transcript is somewhat larger than that reported for the rat AMPD2 transcript (3.4-3.6 kb; Ref. 6), results that also indicate the cloned human AMPD2 cDNA is lacking several hundred base pairs, most likely from its 5'-end. Finally, there appear to be two AMPD2 transcripts expressed in brain, the most abundant of which appears to be smaller than that expressed in other tissues. Conversely, an approximately 4-kb AMPD2 transcript is expressed in brain, placenta, lung, liver, kidney, and pancreas, but relatively little in heart or skeletal muscle (longer exposures show low level expression in these two tissues). Together, these results are consistent with previous data regarding relative AMPD1 and AMPD2 gene expression in the rat.

Bacterial Expression of AMPD2 cDNA and Immunoprecipitation of the Resulting Enzymatic Activity—A lack of endogenous enzymatic activity is an attractive feature of bacterial expression as an approach to functionally characterize a cloned AMPD cDNA. For this purpose, the commercially available prokaryotic expression vector, pKK233-2, was chosen to functionally examine the human AMPD2 cDNA. Extracts prepared from induced host (E. coli strain JM105) containing AMPD2 cDNA exhibit AMPD activity which shows linearity over time with respect to the formation of IMP (Fig. 3). The leaky nature of the trc promoter is evident by detectable enzymatic activity (<2 nmol/min/mg of protein). However, those prepared from vector alone contain no detectable AMPD activity.

The amino acid sequence in this region of the human cDNA (6) is 91% identical in the human and rat isoforms. The putative start codons are identical in the human and rat isoforms.

Fig. 3. Nucleotide sequence of AMPD2 cDNA. Base pair numbering is relative to the putative start codon as +1. Coding sequences in upper case letters and 5' and 3'-untranslated sequences in lower case letters. Locations of designed complimentary oligonucleotides used in sequencing AMPD2 cDNA are denoted by underlining.

Fig. 4. Nucleotide sequence alignment between human (upper) and rat (lower) AMPD cDNAs. Bases are numbered according to Fig. 3 (human) and Ref. 6 (rat). Dashes in the rat nucleotide sequence indicate base identity with the human nucleotide sequence, which overall displays 91% (241/264) similarity. Slashes in rat sequence indicate the relative positions of proposed intervening sequences in this partial cDNA (6). Also included is the predicted amino acid sequence in this region of the AMPD2 polypeptide, which is identical in the human and rat isoforms.

**Discussion**

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Fig. 5. Predicted primary amino acid sequence alignment between the human AMPD1 (upper) and AMPD2 (lower) polypeptides. Amino acid identities are numbered from the predicted N terminus. Amino acid identity is denoted by shaded boxes. This alignment was generated by the BESTFIT program of the GCG software package, and numerous gaps (...) are evident in the N-terminal domains.

Fig. 6. Multiple tissue Northern blot analysis of human AMPD1 and AMPD2 expression. A commercially available, charge-modified nylon membrane containing 2 μg of poly(A) RNA isolated from eight different human tissues was sequentially hybridized with α-32P-labeled cDNA inserts specific for AMPD1 (left panel) and AMPD2 (right panel). The lane designations are as shown on the figure. RNA size markers are indicated in kilobases.

Fig. 7. AMPD activity in bacterial extracts. Total activity (nmol of IMP formed/mg of protein in extract) is plotted against time (min). AMPD activity is linear over time in extracts prepared from both uninduced (pKK-L uninduced) and induced (pKK-L induced) cultures transformed with a prokaryotic expression vector containing human AMPD2 cDNA. AMPD activity was less than 2 nmol/min/mg of protein (cross-hatched area) in extracts prepared from induced cultures containing prokaryotic expression vector alone (pKK-induced).

Fig. 8. Anion exchange high performance liquid chromatography analysis of a solution-hybridization AMPD immunoassay. Aliquots of an extract prepared from an induced (pKK-L) culture were incubated with preimmune (A), anti-M (B), anti-E1 (C), and anti-L (D) serum, precipitated with a suspension of S. aureus (PANSORB) cells, and the residual activity was assayed by anion exchange high performance liquid chromatography as previously described (16). The abscissa indicates elution time in minutes. Peaks of substrate (AMP) and product (IMP), detected by absorbance at 254 nm (the scale shown is 400 mV), are labeled in panel A.
only 67% nucleotide similarity. These nucleotide sequence comparisons suggest that the present cDNA represents transcript produced from the human AMPD2 gene. This interpretation would also be consistent with a previous report which demonstrated higher nucleotide similarities for related AMPD coding sequences across mammalian species relative to different isoform-encoding sequences within a species (11).

By subcloning the entire predicted coding region of the human AMPD2 cDNA into a prokaryotic expression vector, it is possible to produce AMPD activity which is precipitable only with antiserum specific for the major activity isolable from rat kidney, termed isoform B (19). This antiserum specifically cross-reacts in humans with the predominant AMPD activity isolable from liver, termed isoform L (1). Together, this demonstrates that the human AMPD2 gene produces a transcript that can be translated into AMPD isoform L. By analogy, this also supports the proposal that the rat AMPD2 gene is specific for isoform B (embryonic) (16).

The open reading frame contained within the human AMPD2 cDNA predicts that the isoform L subunit is a 760-amino acid polypeptide with a subunit molecular mass of 88.1 kDa. This size estimate, which is exclusive of potential post-translational modifications, is consistent with a previous report of isoform L purification. AMPD purified from outdated human platelets displayed a subunit molecular mass of 83–85 kDa (20). Although not immunologically identified as isoform L in that study, a subsequent report demonstrated that human platelets express this isoform exclusively (3). A second isoform L purification, which utilized autopsied liver as the source of protein, indicated a subunit molecular mass of only 68 kDa (1). The reason for the size discrepancy between these two purifications is unknown, but may be the result of variable proteolysis, known to be characteristic of AMPD purification from other sources (21–23).

As discussed above, sequence alignments have detailed greater degrees of nucleotide similarity between related AMPD cDNAs across mammalian species relative to different AMPD cDNAs within a species. This generalization can be extended to the predicted amino acid level as well. For example, over a span of 88 amino acids located in the C-terminal domain of all predicted AMPD polypeptides, homologies of 73% (rat) and 74% (human) are observed between AMPD1 and AMPD2, whereas this stretch exhibits identity (AMPD2) or near identity (99%; AMPD1) when related cross-species variants are compared. Clearly, this region of the predicted AMPD polypeptide is highly conserved. Even alignments between individual mammalian isoforms and the yeast protein generate 60% (AMPD1) and 74% (AMPD2) homologies over this stretch of amino acids. Interestingly, within this span of 88 amino acid residues, all cloned forms of AMPD contain the conserved motif, SLSSTDPP. Recently, x-ray crystallographic analysis of murine adenosine deaminase has confirmed the presence of the 2 aspartate residues contained within the highly homologous sequence, SLNTDDP, in the catalytic site of this enzyme (24). The first aspartate residue sequesters a zinc atom, which in turn interacts with the substrate (adenosine), and the second binds adenosine directly. Because AMPD is also a zinc-containing enzyme which catalyzes a similar reaction, the aspartate residues in its SLSTDPP motif are proposed to play a similar role in the deamination of AMP (24).

Nucleotide similarities and predicted amino acid homologies between AMPD variants within a species appear to be regional. Unlike the 3’-ends of the cloned human AMPD1 and AMPD2 cDNAs, there is little conservation of sequence at their 5’-ends. An alignment of the amino acid sequences predicted from these two cDNAs indicates a divergent domain encompassing approximately the first 250 N-terminal residues within each polypeptide (see Fig. 5). N-terminal domain divergence was also observed in comparisons between either of the predicted mammalian AMPD1 polypeptides and that encoded by the yeast amy1 gene (25). In contrast, a high degree of nucleotide similarity and predicted amino acid homology is maintained throughout the mammalian AMPD1 cDNAs (11). This demonstrates that the patterns of 5’-end and corresponding N-terminal divergence between different intraspecies AMPD genes have persisted for the millions of years of rat to human evolution, suggesting that they do have a function.

Although the catalytic site of the enzyme is postulated to be contained within the conserved C-terminal domain (discussed above), it is possible that divergent N-terminal domains play roles in determining kinetic and regulatory parameters. In fact, kinetic analyses of purified AMPD isoforms indicate isoform-specific differences (1, 2, 20, 26). For example, in the presence of 0.15 M potassium chloride, a substrate versus velocity curve for isoform M (AMPD1) is hyperbolic (1, 26), whereas a similar plot for isoform L (AMPD2) is sigmoidal which can be converted to a hyperbolic shape upon addition of ATP (1, 20). The addition of ATP also increases the affinity of isoform L for substrate, as the apparent Km changes from 6.6 mM to 0.6 mM. Conversely, the presence of ATP does not alter substrate affinity for isoform M. With regard to the regional diversity between the predicted human AMPD1 and AMPD2 polypeptides (discussed above), it is possible that the allosteric binding site for ATP in isoform L may reside within its N-terminal domain. This hypothesis could be tested by kinetic analyses of normal and chimeric AMPD polypeptides, the latter created in domain exchange experiments utilizing the cloned cDNAs.

It is also possible to speculate that the unique N-terminal domains play structural roles, such as directing different AMPD isoforms to specific intracellular locations. In fact, it is well established that skeletal muscle AMPD (AMPD1) is localized at the A-band of the myofibril (27–30), where it specifically interacts with sarcomeric myosin heavy chain (22, 31–38). Although little is known regarding the intracellular distribution of the AMPD2 polypeptide, recent immunohistochemical analyses of rat skeletal muscle sections have demonstrated anti-B (AMPD2) staining associated with the muscle spindle and the endomysium (39). Combined, the available molecular and cellular data warrant an electron microscopic examination of AMPD isoform localization.

Whatever role the N-terminal domain might play, it clearly does not need to be intact for catalytic activity of at least two AMPD variants. The yeast AMPD polypeptide is known to be susceptible to N-terminal proteolysis without apparent effect on catalytic function (23). Furthermore, at least 215 base pairs can be removed from the 5’-end of the bacterially expressed rat AMPD1 cDNA without losing catalytic activity (7). To what extent the N-terminal domain is involved in AMPD isoform-specific properties has yet to be determined. However, the further development of expression systems utilizing available cDNAs should allow for an examination of this and other questions concerning AMPD isoform diversity.

Acknowledgments—We acknowledge Carolyn K. Snyder for her computer graphic work and Sue Tjepkema-Burrows for her photographic work in the preparation of this manuscript. We are also indebted to Drs. Joe Barbieri, and Ed Krug for their helpful comments during the organization of this manuscript.
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J. Biol. Chem. 1992, 267:22407-22413.

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