Molecular and kinetic alterations of muscle AMP deaminase during chronic creatine depletion

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Rush, James W. E., Peter C. Tullson, and Ronald L. Terjung. Molecular and kinetic alterations of muscle AMP deaminase during chronic creatine depletion. Am. J. Physiol. 274 (Cell Physiol. 43): C465–C471, 1998.—We examined a possible mechanism to account for the maintenance of peak AMP deamination rate in fast-twitch muscle of rats fed the creatine analog β-guanidinopropionic acid (β-GPA), in spite of reduced abundance of the enzyme AMP deaminase (AMPD). AMPD enzymatic capacity (determined at saturating AMP concentration) and AMPD protein abundance (Western blot) were coordinately reduced ~80% in fast-twitch white gastrocnemius muscle by β-GPA feeding over 7 wk. Kinetic analysis of AMPD in the soluble cell fraction demonstrated a single Michaelis-Menten constant ($K_{m}$; ~1.5 mM) in control muscle extracts. An additional high-affinity $K_{m}$ (~0.03 mM) was revealed at low AMP concentrations in extracts of β-GPA-treated muscle. The kinetic alteration in AMPD reflects increased molecular activity at low AMP concentrations; this could account for high rates of deamination in β-GPA-treated muscle in situ, despite the loss of AMPD enzyme protein. The elimination of this kinetic effect by treatment of β-GPA-treated muscle extracts with acid phosphatase in vitro suggests that phosphorylation is involved in the kinetic control of skeletal muscle AMPD in vivo.

AMP activity and protein abundance (13, 23). In spite of the apparent reduced AMPD enzymatic capacity, however, IMP accumulation occurred earlier, and at a similar peak rate compared with normal fast-twitch muscle, under identical conditions of hindlimb muscle stimulation (23). At least two possibilities could compensate for the lower content of AMPD to account for sustained normal high rates of AMP deamination in β-GPA-treated muscle during contractions: a substrate effect and/or altered enzyme kinetic behavior. Elevated substrate (AMP) concentration could increase the reaction velocity ($V$) of a fixed number of enzyme molecules by increasing the concentration of the enzyme-substrate complex. It is possible that larger excursions of ADP and AMP occur during contractions in β-GPA-treated muscle compared with controls due to loss of the CK buffer system. In addition, β-GPA treatment could cause an alteration of the AMPD molecule itself, producing a kinetic effect that increases the sensitivity of the enzyme to AMP (e.g., by lowering the $K_{m}$). This mechanism could compensate for the decreased AMPD abundance by increasing the effective molecular activity of AMPD during physiological contraction conditions. This possibility is supported by a report suggesting that phosphorylation of purified AMPD can lower its $K_{m}$ (22), albeit the effect is small.

To investigate the hypothesis that a kinetic alteration of AMPD may contribute to the observed AMP deamination behavior in β-GPA-treated fast-twitch muscle, we evaluated the enzyme kinetics of AMP deamination and followed the time course of changes in muscle AMPD capacity and 80-kDa AMPD protein abundance in fast-twitch muscle from rats fed β-GPA over 7 wk.

METHODS

Animal care. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 250–300 g were housed three per cage in a temperature-controlled room (20–21°C) with a 12:12-h light-dark cycle. Control animals were fed Purina lab chow, and experimental animals were fed the same diet containing 1% β-GPA (wt/wt), which was synthesized as previously described (15). Both groups were provided with food and water ad libitum. All procedures involving the use of animals were approved by the State University of New York Health Science Center Committee for the Humane Use of Animals and were in accordance with the guidelines for the care and use of animals of the American Physiological Society.

Tissue sampling. Muscle tissues were obtained from control rats and from rats fed β-GPA for 1, 2, 3, 5, and 7 wk, under pentobarbital sodium anesthesia (5 mg/100 g body wt, ip). Muscles taken included the superficial portion of the medial gastrocnemius (the white gastrocnemius; WG), which is composed predominantly of fast-twitch white fibers (3).

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1 A large fraction of the total ADP and AMP pools is bound to protein in vivo. The subscript “f” refers to free nucleotide that is available for the indicated reactions.
sections were clamp-frozen in aluminum tongs that had been cooled in liquid nitrogen and were stored at \(-80°C\) until analysis. Portions of the mixed gastrocnemius muscles from each animal were weighed wet and dried at \(85°C\) to a constant dry weight to determine muscle water content.

AMPD capacity and kinetics. Portions of muscle sections were homogenized (1.9 or 1.19, wt/vol) in 100 mM KCl, 50 mM imidazole-HCl, and 10 mM reduced glutathione, pH 7.0, and the homogenates were centrifuged for 1 min at 13,000 \(g\) to separate soluble and particulate cell material. The supernatant (soluble cell fraction) was removed, spun again, and retained; there was no visible pellet from the second spin. The pellet of the initial spin (particulate cell fraction) was washed and resuspended in homogenization buffer.

A near-\(V_{\text{max}}\) response was elicited for AMPD in both cell fractions using 15 mM AMP (pH 7.0 and 30°C); this is taken as the enzymatic capacity of AMPD (Table 1). Total muscle AMPD capacity was determined either by direct assay of the initial muscle homogenate or by the sum of the soluble and particulate AMPD capacities, determined separately. The two methods for obtaining total muscle AMPD capacity yielded results that were not systematically or significantly different (\(n = 20, P > 0.05\)).

Reactions for kinetic analysis (15 AMP concentrations in the range of 0.04–15 mM) were initiated by adding aliquots of the cell fraction to reaction buffer (50 mM imidazole, 150 mM KCl, and 0.04–15 mM AMP, pH 7.0) that had been prewarmed to 30°C. The reaction components were mixed by vortex, and the reactions proceeded for 1 min at 30°C and were terminated by dilution in 1–5 volumes of 4.5% (wt/vol) perchloric acid. Acid extracts were neutralized (KOH/ triethanolamine), and IMP produced was quantified by reverse-phase high-performance liquid chromatography. The reduction in AMP concentration that occurred as the reactions proceeded never exceeded 15% of initial AMP concentration. This assay system produced IMP linearly with respect to both time and volume of muscle extract used. The specificity of the assay has been previously confirmed by the complete inhibition of activity by coformycin, an antibiotic inhibitor of AMPD (16).

In experiments examining the effect of phosphatase treatment on AMPD kinetics, acid phosphatase prepared from wheat germ (Sigma) was added to aliquots of the soluble cell fraction of \(\beta\)-GPA-treated WG (7-wk animals) at a dose of 10 U/ml. These mixtures were incubated at room temperature for 30 min, after which they were used for kinetic analysis of AMPD as described above. Preliminary experiments demonstrated that these conditions of dose and time were sufficient to elicit the maximal acid phosphatase-induced response in AMPD kinetics. Preliminary experiments demonstrated that these conditions of dose and time were sufficient to elicit the maximal acid phosphatase-induced response in AMPD kinetics.

AMPD Western blots. Pieces (5–10 mg) of frozen WG muscles were pulverized under liquid nitrogen and homogenized by hand in 19 volumes of extraction buffer containing 10 mM NaH\(_2\)PO\(_4\), 1% sodium dodecyl sulfate (SDS), and 6 M urea, pH 7.4. This extract was incubated for 3 h at 37°C, and an aliquot was used to determine the protein content (bicinchoninic acid protein assay kit; Pierce). \(\beta\)-Mercaptoethanol was added to the remaining extract to a final concentration of 1% SDS-polyacrylamide gel electrophoresis (PAGE) was performed using 10% polyacrylamide gels. A total of 6 \(\mu\)g total muscle protein was loaded per sample lane. Prestained molecular mass standards (low range, Bio-Rad) were included in each gel to determine the apparent molecular masses of AMPD species. Proteins were transferred to nitrocellulose membranes, and Western blot procedures and densitometric analyses were performed as previously described (23). The primary antibody was a previously characterized (10) polyclonal antiserum raised in rabbit against purified rat skeletal muscle AMPD. Aliquots of two particular muscle samples were included in each of the gels, to serve as internal standards for comparison of the stain density measurements across gels.

AMPD immunoprecipitations. Homogenates were prepared as described for AMPD enzyme assays. Twenty microliters of the soluble cell fraction were added to 5 \(\mu\)l of antibody solution [1 part of undiluted antiserum to 1 part of tris(hydroxymethyl)aminomethane (Tris)-buffered saline (TBS; 50 mM Tris, 27 mM NaCl, and 0.1% bovine serum albumin, pH 7.0)], and the mixture was incubated at room temperature for 1 h. Twenty-five microliters of Staphylococcus aureus PANSORBIN cells (Calbiochem-Behring, La Jolla, CA) pre-washed in TBS buffer were added. The mixture was incubated at room temperature for 1 h and centrifuged (5 min at 13,000 \(g\) to pellet the sorbent cells and attached antibody-AMPD conjugates. The supernatant was then assayed for AMPD capacity as described above. The conditions were optimized for maximal specific immunoprecipitation. Anti-skeletal muscle AMPD antibody was the same as that employed in Western blots. Immunoprecipitated AMPD capacity was calculated as the difference between the AMPD capacity remaining in the supernatant of contemporaneous samples with and without antibody.

Calculations and statistics. To determine \(K_m\) kinetic data were plotted as double-reciprocal plots (1/V vs. 1/S, where S is substrate concentration) and as normalized double-reciprocal plots (1/\(V_{\text{max}}\) vs. 1/S) in which V values were expressed as a percent of the \(V_{\text{max}}\) elicited at 15 mM AMP (\(V_{\text{max}}\); see Fig. 2). The \(K_m\) estimations were verified by plotting the data in an Eadie-Hofstee (V/S vs. V) format.

Depending on the nature of the comparison, either one-way analysis of variance followed by Tukey’s procedure or the unpaired t-test was used to determine significant differences (\(P < 0.05\)). Where significant differences exist, \(P\) values are indicated. The AMPD activity data are expressed per gram of wet muscle mass, since neither the total water content nor the total protein content of gastrocnemius muscle was altered by \(\beta\)-GPA feeding.

### RESULTS

AMPD capacity. As anticipated from previous studies (13, 23), the total WG AMPD capacity measured in vitro

| Table 1. White gastrocnemius muscle AMPD capacity during \(\beta\)-GPA feeding |
|-----------------|----------------|--------|--------|--------|--------|--------|--------|
|                 | Control        | 1      | 2      | 3      | 5      | 7      |
| Total           | 664 ± 35       | 568 ± 52| 526 ± 12*| 349 ± 14†| 191 ± 12†| 144 ± 11†|
| Soluble         | 415 ± 30       | 338 ± 32| 303 ± 26| 180 ± 7| 97 ± 6| 50 ± 13†|
| Particulate     | 248 ± 15       | 230 ± 25| 222 ± 26| 170 ± 17*| 94 ± 6| 93 ± 5|

Values are means ± SE in \(\mu\)mol·min\(^{-1}\)·g wet weight\(^{-1}\); \(n = 3\) per group. AMPD, AMP deaminase; \(\beta\)-GPA, \(\beta\)-guanidinopropionic acid. *\(P < 0.05\), †\(P < 0.01\) vs. control.

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was decreased by β-GPA feeding to ~20% of control values at 7 wk (Table 1). The rate and extent of decline in AMPD capacity during β-GPA treatment were both greater in the soluble than in the particulate cell fraction. Solely as a result of the continuing decline in soluble AMPD capacity, the total AMPD capacity continued to decline between 5 and 7 wk (Table 1).

AMPD Western blots. The predominant species of AMPD protein in control WG muscle migrates in SDS-PAGE at ~80 kDa (Fig. 1, Table 2). An additional, much less abundant species occurs at ~60 kDa, and a further species at ~56 kDa is detectable at very low levels in some control muscles. The abundance of 80-kDa AMPD protein in WG decreased progressively with time during β-GPA treatment to ~15–20% of control levels at 7 wk (Fig. 1, Table 2). Increases in the abundance of the 60- and 56-kDa forms also occurred with β-GPA feeding, but these did not appear to be stoichiometric to the decreases in the 80-kDa AMPD.

The correlation between total AMPD capacity and 80-kDa AMPD abundance over the experimental time points yielded a strong linear relationship (Fig. 2; r = 0.997, slope significantly > 0, P < 0.001) with a near-zero intercept. The slope of this relationship (0.264), which can be interpreted as an index of enzyme activity per quantity of enzyme, is not different from the same ratio (0.274 ± 0.022; n = 7) determined for control WG muscle that contains essentially no 60- or 56-kDa AMPD. Furthermore, there was no consistent relationship between the abundance of the 60- or 56-kDa species and the AMPD capacity. These results imply that only the 80-kDa protein is enzymatically active.

AMPD kinetics. The kinetics of AMP deamination in the soluble cell fraction of control WG muscle consisted of a single linear phase in the double-reciprocal plot (Fig. 3). This indicates simple Michaelis-Menten kinetics and no cooperativity. The K_m did not depend on the range of AMP concentrations over which it was determined and was ~1.5 mM (Table 3).

β-GPA treatment induced biphasic AMPD kinetics in the soluble cell fraction of WG muscle (Fig. 3). For simplicity, we chose to quantitate this response as two distinct linear kinetic phases: one of lesser slope in the AMP concentration range of 0.04–0.15 mM and the other of greater slope in the 0.2–15 mM range (Fig. 3, Table 3). The K_m over the AMP concentration range ≤0.15 mM was decreased at the 1-wk time point, and the decrease was exaggerated as the time course progressed, to a maximum of ~45-fold lower than control at 7 wk (Fig. 3, Table 3). The result of the kinetic change is that at low AMP concentrations a greater percentage of the available capacity (%V_15mM) is active in β-GPA-treated muscle than in control muscle (Fig. 3, Table 4), i.e., the molecular activity of the AMPD enzyme molecule is increased. The K_m in the higher AMP concentra-

| Western blot density analysis of white gastrocnemius muscle AMPD during β-GPA feeding |
|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                                  | Control       | 1             | 2             | 3             | 5             | 7             |
| 80 kDa                           | 2.416 ± 113   | 2.053 ± 189   | 1.839 ± 168   | 1.088 ± 239†  | 599 ± 70†     | 470 ± 78†     |
| 60 kDa                           | 236 ± 87      | 2.196 ± 691   | 3.138 ± 1.067 | 3.809 ± 1.038 | 4.319 ± 801*  | 3.229 ± 1.036 |
| 56 kDa                           |               | 975 ± 330     | 254 ± 36      | 2.148 ± 262†  | 1.216 ± 340*  | 545 ± 11      |
| %80 kDa                          | 91.1 ± 3.4    | 47.0 ± 4.7*   | 40.6 ± 5.2†   | 27.8 ± 9.2†   | 12.9 ± 2.5†   | 14.1 ± 1.6†   |

Values are means ± SE in standardized density units generated from analysis of digitized images of nitrocellulose membranes used in Western blots; n = 3 per group except for control, where n = 7. No 56-kDa signal was detected in control group. * P < 0.05, † P < 0.01 vs. control for 80-, 60-, and %80-kDa data or vs. zero for 56-kDa data.
tion range (0.2–15 mM) was also decreased during β-GPA feeding, but to a relatively modest extent (to approximately one-half of control), and this was significant only after 5 wk (Table 3). The concave hyperbolic shape of the double-reciprocal plot for β-GPA-treated WG (Fig. 3) implies negative cooperativity in the AMPD reaction.

Acid phosphatase treatment of the soluble cell fraction of 7-wk β-GPA-treated WG eliminated the low-\(K_m\) kinetic phase (Fig. 4). This resulted in kinetics with a half-saturating substrate concentration (\(S_{0.5}\)) of ~3 mM, similar to the \(K_m\) found for control muscle (Table 3). The kinetics of acid phosphatase-treated β-GPA WG samples were distinct from those of the control, however, because they were slightly convex (implying slight positive cooperativity). Furthermore, a much lower \%\(V_{15mM}\) was elicited at low AMP concentrations in the phosphatase-treated β-GPA extracts than in control extracts (compare scale of ordinate in Fig. 4 to that of Fig. 3 and see Table 4).

The kinetics of AMP deamination in the particulate cell fraction of control WG muscles indicated slight positive cooperativity in the reaction, with an \(S_{0.5}\) of ~1.1 mM AMP (data not shown). This resulted in the same whether the kinetics were assessed in the crude particulate material or after partial purification from the particulate material (extraction in 500 mM KCl to solubilize myofibrils and AMPD, removal of the particulate material, and then dilution back to 100 mM KCl). β-GPA treatment had no effect on AMPD kinetics in the particulate fraction of WG muscles using either method of preparation.

AMPD immunoprecipitation. In addition to the skeletal muscle isoform of AMPD, WG muscle homogenates contain small amounts of cardiac and nerve isoforms (21). This raises the possibility that changes in the relative expression of nonskeletal muscle AMPD isoforms could account for the kinetic differences we observed. To address this issue, immunoprecipitation was used to assess the fractional contribution of the skeletal muscle AMPD isoform to the homogenate AMPD capacity in control and β-GPA-treated WG muscle. Immunoprecipitation using anti-skeletal muscle AMPD isoform antibody removed similar fractions of the AMPD capacity from control and 7-wk β-GPA-treated WG samples (91.3 ± 0.4% and 88.5 ± 0.5%, respectively; \(n = 3\) per group). The isoform specificity of the antibody was confirmed by the observation that none of the AMPD capacity from heart samples (which contain only the cardiac AMPD isoform; Ref. 21) was immunoprecipitated by the anti-skeletal muscle AMPD isoform antibody (\(n = 3\)).

DISCUSSION

β-GPA feeding results in the displacement of Cr and PCr from skeletal muscle by β-GPA and β-GPA-P. In spite of the impaired CK reaction, steady-state muscle force production is normal, endurance performance is excellent, and steady-state energy balance is well maintained during contractions. Similarly, the normal high rates of AMP deamination that occur during intense muscle contractions are found in β-GPA-treated muscle, in spite of an ~80% reduction in the content of AMPD protein (23). The findings of this study indicate that the induction of unique, high-affinity AMPD reaction kinetics is available to compensate for the reduced enzyme protein and allow the normal high rates of AMP deamination to occur in contracting β-GPA-treated muscle. The observation that in vitro phosphatase treatment eliminates this kinetic effect implicates phosphorylation in the regulation of AMPD in vivo.
AMPD enzymatic capacity is directly proportional to 80-kDa abundance. The direct relationship between enzymatic capacity of AMPD and 80-kDa abundance during β-GPA treatment illustrated in Fig. 2 suggests that the loss of enzymatic capacity is due to a loss of 80-kDa enzyme protein in muscle. The predicted AMPD capacity in the absence of 80-kDa AMPD (the y-intercept) is near zero (Fig. 2), even though 60- and 56-kDa AMPD species are increased 20- to 40-fold compared with control (Table 2). Furthermore, the slope of the AMPD capacity-abundance relationship over time (Fig. 2) was not different from that for control muscle in which 60- and 56-kDa AMPD species are essentially absent (see RESULTS, AMPD Western blots). These results suggest that the AMPD capacity is dependent on the abundance of the 80-kDa AMPD protein and independent of the 60- and 56-kDa forms, although we cannot completely eliminate the possibility that the lower-molecular-mass species may be active in a complex with some other intracellular factor.

Decreases in AMPD capacity and abundance could be due to interfiber and/or intrafiber alterations. Slow-twitch muscle fibers have inherently lower AMPD capacity than do fast-twitch fibers (16), and therefore an increased fraction of slow fibers would be expected to result in decreased AMPD capacity. The decrement in AMPD capacity is not confounded by a simple increase in abundance of slow-twitch fibers, however, since there is no significant increase in slow-twitch fiber fraction in the limb muscles of adult rats treated with β-GPA (1). It is instead likely that the decreased amount of AMPD protein occurs within the existing muscle fibers. This may be part of a coordinated series of changes that also affects the content of mRNA, protein, and/or enzymatic activity of other components involved in fast-twitch skeletal muscle energy transduction. These other components include α-actin (9), parvalbumin (12), the GLUT-4 glucose transporter (14), and many glycolytic and mitochondrial oxidative enzymes (8, 9, 18).

The observation that β-GPA treatment did not affect the AMPD mRNA level (23) suggests that transcription of the AMPD gene is not impaired. If translation of the AMPD mRNA is likewise not impaired, AMPD protein synthesis rate is expected to be normal. This implies that the degradation rate of AMPD protein may be increased by β-GPA treatment to account for the lower 80-kDa AMPD abundance. In support of this argument is the increased abundance of lower-molecular-mass species (60 and 56 kDa; Fig. 1, Table 2) that are probably degradation products of the 80-kDa AMPD.

β-GPA treatment alters the kinetics of AMPD from fast-twitch muscle. The induction of low- $K_m$ kinetics in the soluble fraction of WG by β-GPA treatment was progressive with treatment time to an apparent steady state after 3 wk (~45-fold reduction in $K_m$ assessed at low AMP concentrations, i.e., ~0.15 mM; Table 3). The inflection point of the double-reciprocal plot (at ~0.15 mM) did not change as the $K_m$ was progressively lowered, suggesting that the kinetic alteration did not affect all AMPD molecules gradually over time. Rather, it is likely that the individual alterations enveloped a greater number of AMPD molecules over time, until all molecules were altered to yield the high-affinity $K_m$ of ~0.03–0.04 mM (Table 3). The high-affinity kinetics observed at AMP, ~0.15 mM is a reflection of increased available AMPD molecular activity. As a result, the predicted soluble AMPD activities of β-GPA and control muscle (extrapolated using the kinetic equations described in RESULTS) were similar in low, physiologically relevant concentrations of AMP (i.e., 0.1–10 µM). Furthermore, these data suggest that similar AMP deamination rates observed in control and β-GPA-treated WG muscle during intense in situ contractions (23) are elicited at similar AMP, concentra-

**Table 3.** $K_m$ values of soluble white gastrocnemius muscle AMPD during β-GPA feeding

<table>
<thead>
<tr>
<th>Weeks of β-GPA Feeding</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>0.04–0.15 mM</td>
<td>1.439 ± 0.039</td>
<td>0.603 ± 0.307$^*$</td>
<td>0.174 ± 0.045†</td>
<td>0.041 ± 0.007†</td>
<td>0.065 ± 0.021†</td>
</tr>
<tr>
<td>Beta-GPA</td>
<td>0.2–0.15 mM</td>
<td>1.577 ± 0.099</td>
<td>1.486 ± 0.090</td>
<td>1.232 ± 0.084$^*$</td>
<td>1.494 ± 0.115$^*$</td>
<td>1.101 ± 0.15§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 per group. *P < 0.05, †P < 0.01 vs. control; ‡P < 0.1, §P < 0.05 vs. the 0.04–0.15 mM AMP value at same time point.

**Table 4.** Soluble white gastrocnemius muscle AMPD activities at low AMP concentration

<table>
<thead>
<tr>
<th>AMPD Activity at Indicated AMP Concentration</th>
<th>Measured at 40 µM</th>
<th>Predicted at 1 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>%V$_{15	ext{mm}}$</td>
<td>V</td>
</tr>
<tr>
<td>Control</td>
<td>12.40 ± 0.90</td>
<td>3.39 ± 0.22</td>
</tr>
<tr>
<td>β-GPA</td>
<td>6.36 ± 0.30</td>
<td>13.99 ± 2.63$^*$</td>
</tr>
<tr>
<td>+ Acid phosphatase</td>
<td>0.31 ± 0.02</td>
<td>0.93 ± 0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 per group. V, reaction velocity in µmol·min$^{-1}$·g tissue$^{-1}$; %V$_{15	ext{mm}}$, reaction velocity expressed as a percentage of velocity elicited at 15 mM AMP within the same muscle soluble fraction. V$_{15	ext{mm}}$ = 415 ± 30, 50 ± 13, and 33 ± 5 µmol·min$^{-1}$·g tissue$^{-1}$ in control, β-GPA, and acid phosphatase-treated β-GPA groups, respectively. AMPD rates at 40 µM AMP are measured values. AMPD rates at 1 µM AMP are values calculated using kinetic parameters determined at low AMP concentration (i.e., <0.15 mM) for each muscle in each treatment; $K_m$ or S$_{0.5}$ = 1.439 ± 0.039, 0.032 ± 0.006, and 2.970 ± 0.099 mM, and V$_{max}$ = 415 ± 30, 31 ± 1, and 33 ± 5 µmol·min$^{-1}$·g tissue$^{-1}$ for control, β-GPA, and acid phosphatase-treated β-GPA groups, respectively. Note that for β-GPA group V$_{max}$ extrapolated from AMP ≤ 0.15 mM data is less than V$_{15	ext{mm}}$ because of biphasic nature of AMPD kinetics in this group. Calculated values for AMP concentrations in the range 0.1–10 µM show similar relative results as those illustrated for 1 µM. †P < 0.01 vs. control.
illustrate that an exaggerated increase in AMPf is not to the absence of a PCr buffer pool. However, our results ability of be the most important factor preserving the observed tent to offset the decrease in AMPD abundance. It may an increased molecular activity, appears fully compe-
tions. Thus the kinetic alteration in AMPD, manifest as an increased molecular activity, appears fully competent to offset the decrease in AMPD abundance. It may be the most important factor preserving the observed ability of β-GPA-transformed fast-twitch muscle to deaminate AMP to IMP (13, 23), which has been shown to be the predominant entry reaction to adenosine nucleotide degradation in skeletal muscle, occurring at rates several thousandfold higher than that of the alternate AMP disposal pathway: dephosphorylation to adeno-
sine by cytosolic AMP-5′-nucleotidase (2). This does not exclude the possibility that AMPf is elevated in β-GPA-treated muscle or that such elevations may affect deamination in situ. For example, the earlier onset of deamination during intense in situ contractions in β-GPA-treated muscle (23) may be the result of a higher AMP, concentration near the onset of contractions due to the absence of a PCr buffer pool. However, our results illustrate that an exaggerated increase in AMPf is not an essential or sole mechanism to account for the normal AMP deamination rate observed in β-GPA-treated muscle during extreme contraction conditions (23).

On the basis of the currently available evidence, it is impossible to identify the modification that is respon-
sible for the change in AMPD kinetics. It could be due to changes in factors that serve to modulate the AMPD molecule or to changes in the 80-kDa AMPD molecule itself. The kinetic alteration is probably independent of changes in the relative abundance of other tissue-specific AMPD isoforms, since 90% of the AMPD capacity in both control and β-GPA-treated WG was immuno-
precipitated by the skeletal muscle isoform-specific antibody. It is also unlikely that the 60- and 56-kDa molecules are responsible for the change in kinetic behavior of AMPD, since they likely are catalytically inactive. The possibility that these molecules could form heterotetramers with active 80-kDa molecules and that the kinetics of such complexes could be biphasic is unlikely, since 60- and 56-kDa AMPD mole-
cules were not found as components of AMPD tetra-
mers isolated in native PAGE experiments (unpub-
lished observations). It seems more likely that the 80-kDa molecule itself is influenced by β-GPA treatment in a manner that produces the observed kinetic changes. No naturally occurring tight binding effectors of AMPD are known, and neither β-GPA nor β-GPA affects AMPD activity (23). Other soluble effectors would not be expected to account for the observations in vitro because they would dissociate during extraction of tissues and assay of the extracts (∼2,000-fold dilution).

Protein-protein interactions and covalent modification of the 80-kDa enzyme are possibilities because of the observed stability of the response to dilution and over time in vitro. It has been reported that myosin binding as a result of muscle stimulation affects AMPD kinetics in a manner similar to that observed in the present study in response to β-GPA treatment (17). In unstimu-
lated control muscle, however, we found that putative myosin binding of AMPD in the particulate cell fraction did not induce this kinetic behavior. Thus there is some unique aspect of intense muscle contractions that influ-
ences AMPD kinetics. If simple myosin binding played a role in the β-GPA-induced AMPD kinetic response, it would be expected to affect the kinetics of the enzyme in the particulate cell fraction (myosin is a component of the particulate material) and not in the soluble cell fraction. We observed exactly the opposite response in the current study; the particulate fraction kinetics were not affected, whereas those of the soluble fraction were. Furthermore, the kinetics of soluble cell fraction AMPD from β-GPA-treated muscle were not affected by ultrafiltration (0.22 µm), which has previously been demonstrated to retain myosin-bound AMPD (17). This evidence undermines an obligatory role of myosin binding in the β-GPA-induced kinetic alteration in resting muscle but does not discount the possible significance of myosin binding during intense contrac-
tions as an important determinant of AMPD kinetic behavior. The lack of effect of β-GPA treatment on particulate fraction AMPD kinetics implies either that the association of AMPD with the particulate cell material protects AMPD from the modification seen in the soluble fraction or that the modified AMPD cannot bind to the particulate material. The possible influence of other protein-protein interactions involving AMPD on its kinetics have not been studied.

It is possible that the kinetic alteration of AMPD involves covalent modification of the native 80-kDa molecule. The observation that treatment of the soluble fractions of β-GPA-treated muscle with a nonspecific acid phosphatase eliminates the high-affinity, low-Km kinetic phase (Fig. 4) suggests that phosphorylation-
mediated events control the kinetic characteristics of skeletal muscle AMPD in vivo. Phosphorylation may therefore be responsible for the appearance of the low-Km phase in β-GPA-treated muscle. This is sup-
ported by the results of Tovmasian and co-workers (22), who demonstrated that phosphorylation of purified

![Figure 4](http://ajpcell.physiology.org/)

Fig. 4. Normalized double-reciprocal plot of AMPD kinetics in soluble fraction of WG muscle from 7-wk β-GPA animals after acid phosphatase treatment (10 U/ml, 30 min) of extracts. Values are means ± SE; n = 3.

C470 HIGH-AFFINITY AMP DEAMINASE KINETICS
80-kDa skeletal muscle AMPD by protein kinase C in vitro resulted in a relatively modest (~65%) reduction in $K_m$ at higher (0.1–1 mM) AMP concentrations. Our observation of an ~55% reduction in $K_m$ at high (0.2–15 mM) AMP concentrations with β-GPA feeding (Table 3) agrees with the data of Tovmasian et al. (22). Unfortunately, Tovmasian et al. did not evaluate enzyme kinetics over a low AMP concentration range (i.e., <0.1 mM) in their purified, phosphorylated AMPD preparation, so direct comparison to our kinetic data cannot be made. On the basis of our data, a substantial reduction (i.e., 45-fold) in $K_m$ of purified skeletal muscle AMPD evaluated at more physiologically relevant AMP concentrations is expected to result from this putative AMPD phosphorylation.

From the current results it is not possible to determine whether the influence of β-GPA treatment on AMPD involves direct effects of β-GPA on pathways that control AMPD abundance, activity, and kinetics, or whether the effects are indirect, as a function of intracellular energetics. Direct effects of β-GPA could be ruled out if similar changes to AMPD occur in CK knockout muscle in which CK flux is eliminated in the absence of Cr analogs. As has been previously proposed (22), intracellular energetics could regulate muscle phenotype through alterations of cellular ATP, ADP, P_i, H^+, or Ca^{2+} levels. Disruptions in these due to β-GPA treatment may therefore be directly or indirectly involved in the signaling pathway that causes the observed alterations in AMPD.

In summary, phosphorylation of AMPD could be important in the in vivo control of AMP deamination, by increasing the molecular activity of the enzyme at physiological AMP concentrations. Such a kinetic alteration occurred when CK-dependent buffering of the ATP pool was impaired by β-GPA treatment. This alteration appears competent to compensate for the decreased AMPD abundance that also results from β-GPA treatment, to allow normal in situ AMP deamination rates. The involvement of this kinetic alteration in the response of AMPD to other physiological states in which adenine nucleotide management is challenged and the mechanism of involvement of phosphorylation in the control of AMPD kinetics remain to be determined.

We gratefully acknowledge the excellent technical assistance of Judy Freshour. We thank R. L. Sabina (Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI) for generously supplying the anti-skeletal muscle AMPD antibody.

This study was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-21617. J. W. E. Rush was the recipient of a Natural Sciences and Engineering Research Council of Canada postgraduate scholarship B predoctoral fellowship.


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Received 2 September 1997; accepted in final form 3 November 1997.

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