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MOLECULAR FORMS OF PIGEON SKELETAL MUSCLE AMP DEAMINASE*

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Phosphocellulose chromatography of pigeon leg muscle extract revealed the existence of two well-separated forms of AMP deaminase. This was in contrast to the pigeon breast muscle extract, which yielded only one form. The two leg muscle enzyme isoforms manifested similar kinetic and regulatory properties. They were activated by very low concentration of potassium ions and demonstrated similar patterns of pH and effector dependence. At pH 6.5, as well as at other pH values tested, ADP and ATP slightly stimulated, whereas GTP and orthophosphate inhibited the two molecular forms of pigeon leg muscle enzyme. Surprisingly, the molecular form of AMP deaminase present in pigeon breast muscle was inhibited by ATP at all pH values tested.

The kinetic and regulatory properties of the three molecular forms of pigeon skeletal muscle AMP deaminase examined do not resemble those which have been described for pigeon heart muscle enzyme.

AMP deaminase (EC 3.5.4.6; AMP aminohydrolase), the enzyme catalyzing an irreversible deamination of AMP to form IMP, is widely distributed in animal tissues. In skeletal muscle, AMP deaminase activity is much higher than in other tissues including heart and smooth muscle [1].

Thus far, AMP deaminase extracted from rabbit and rat skeletal muscle was studied most throughly. It has been shown that the activity of the enzyme is regulated by many low molecular weight allosteric modifiers, among them potassium, ADP, ATP, GTP and orthophosphate playing the most important role [2].

In 1975 Ogasawara et al. [3] reported the existence of multiple molecular

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forms of AMP deaminase in brain, liver, lung and spleen, but of one
only in the kidney, heart and skeletal muscle. In the same year Raggi
et al. [4] described conditions at which rabbit and rat skeletal muscle
enzymes could be separated into two different isoforms. In rabbit white
muscle, all of AMP deaminase activity was accounted for by a form eluted
with 0.6 M KCl (isoform B), whereas in the red muscle two peaks of
activity have been detected. The form eluted with 0.4 M KCl (isoform A)
included a great majority of the total enzyme activity, but it displayed
a lower substrate affinity than the form eluted with 0.6 M KCl. Interestingly,
the forms A and B detected in rabbit red skeletal muscle were released from
the phosphocellulose column at the same ionic strength as the heart
and white skeletal muscle enzyme, respectively. The studies of Ogasawara
et al. [5] confirmed not only chromatographic but also immunological
and kinetic diversity of the two red muscle isoforms (Red I and Red II)
and their identity with the heart and white skeletal muscle AMP deaminase,
respectively. However, the studies performed by Solano & Coffee [6] indicated
clearly that AMP deaminases from these two tissues differ one from another
both in their regulatory and chromatographic properties.

In this paper, the kinetic and regulatory properties of three molecular
isoforms of AMP deaminase from pigeon skeletal muscle are described.
The differences between their properties and those described recently for
pigeon heart muscle enzyme [7] are also discussed.

MATERIALS AND METHODS

Isolation of the enzyme. Pigeons (Columbia livia) were bought from com-
mercial sources. AMP deaminase from breast and leg muscles was isolated
by chromatography on phosphocellulose, essentially according to the procedure
of Smiley et al. [8]. After the enzyme had been adsorbed on the column
it was washed with 0.4 M KCl and then eluted by a linear gradient
between 0.4 M and 0.2 M KCl. The active fractions were pooled, concentrated
in polyethylene glycol and dialysed against 100 mM imidazole/HCl buffer,
ph 6.5, containing 5 mM KCl. The enzyme preparations obtained were
purified 100 - 200 fold. The breast muscle isoform (isoform A) was released
from the column at approximately 0.7 M KCl and displayed a specific
activity about 12 μmol of substrate decomposed (×mg⁻¹×min⁻¹), while
the two leg muscle isoforms were released from the column at approximately
0.5 M (isoform B) and 0.6 M (isoform B2) KCl, and displayed specific
activities of 3 and 5 μmol of substrate decomposed (×mg⁻¹×min⁻¹),
when measured at 0.075 mM AMP at 30°C.

Enzyme assay. The enzyme activity was estimated spectrophotometrically
according to Kalckar [9], by monitoring for 2 min either the decrease
in absorbance at 265 nm or the increase in absorbance at 285 nm. The rate of adenylate deamination was measured on a Specord UV-Vis spectrophotometer fitted with a constant temperature cell housing. The changes in absorbance were registered for 2 min. The initial, linear part of the curve, corresponding to not more than 1 min was taken for calculation of the initial velocity of the reaction. To determine the exact values of intermediate substrate concentrations the calculation method of Waley [10] was applied. The reaction mixture in the final volume of 3 ml contained 50 mM imidazole/HCl buffer, pH 6.5, 100 mM KCl, substrate (0.025 - 1.5 mM AMP) and where indicated — effectors. After the temperature (30°C) had been equilibrated, the reaction was started by addition of the enzyme preparation (about 3 μg of protein). The blank cuvette contained all the additions except the substrate.

Calculation of the kinetic parameters. The kinetic parameters of the reaction (the half-saturation constant (S₀₅,) and the cooperativity coefficient (1/n₅₀) were computed using a program written in BASIC, based on the flow diagram described by Atkins [11]. The fundamental part of this program is a subroutine which performs for a given, preliminary value of V₅₀, a series of iterative linear regression steps. In the course of these iterations, the logarithmic form of the Hill equation was fitted directly to the experimental kinetic data by minimizing the sum of squares of residuals. Protein was determined spectrophotometrically [12].

Reagents. 5'-AMP (free acid from equine skeletal muscle, type V), ADP, ATP (Na⁺ salts), GTP (Li⁺ salt) as well as 2'-deoxyadenosine 5'-monophosphate (dAMP), adenosine 2'-monophosphoric acid (2'-AMP), adenosine 3'-monophosphoric acid (3'-AMP), adenosine 5'-diphosphoribose (ADPR), adenosine 5'-diphosphoglucose (ADPG) and l-alanine were supplied by Sigma Chemical Co. (St. Louis, U.S.A.). Adenosine was purchased from Boehringer (Mannheim, F.R.G.) and cellulose phosphate from Whatman (Maidstone, England). All other chemicals were from POCh (Gliwice, Poland).

RESULTS

As it may be seen from Fig. 1, the chromatography of the extract obtained from pigeon leg muscle displayed two well-separated, symmetrical peaks of AMP-deaminase activity, indicating the presence of two chromatographically different isofoms of the enzyme. In contrast to leg muscle, the extract obtained from pigeon breast muscle emerged from the column as one, assymetrical peak of activity.

In the presence of 100 mM potassium chloride (the physiological concentration of K⁺ ion in the muscle tissue), without other effectors added, the reaction catalysed by all three molecular forms investigated followed
a regular, hyperbolic kinetics (not shown). The half-saturation constants and cooperativity coefficients calculated for each of these isoforms are presented in Table 1.

Figure 2 illustrates the velocities of the reaction catalysed by pigeon skeletal muscle AMP deaminase isoforms as a function of potassium ions concentration. It may be seen from this Figure, that at the potassium ions concentration as low as 5 mM the three AMP deaminase isoforms examined represented high, nearly maximum activities. The further increase of potassium ions concentration did not influence significantly the activities of these isoforms. This is true especially in the case of isoform B2, the activity of which started to decrease below the initial level at concentration of potassium ions higher than 30 mM. The same was observed for the two other isoforms at the concentration of about 50 mM (isoform B1) or 80 mM (isoform A).

The influence of some important effectors (ADP, ATP, GTP and orthophosphate) of skeletal muscle AMP deaminase on the activity of the three molecular isoforms of pigeon skeletal muscle enzyme, measured at low, approximately physiological [13] concentration of AMP is presented in Table 2.

**Table 1**

*The values of kinetic parameters for molecular isoforms of pigeon skeletal muscle AMP deaminase*

The data are means of three experiments. The values given in brackets represent S.E.

<table>
<thead>
<tr>
<th>Molecular form of the enzyme</th>
<th>$K_{m}$ (mM)</th>
<th>$n_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoform A</td>
<td>0.16 (0.02)</td>
<td>1.0</td>
</tr>
<tr>
<td>Isoform B1</td>
<td>0.18 (0.03)</td>
<td>0.9</td>
</tr>
<tr>
<td>Isoform B2</td>
<td>0.11 (0.02)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table 2**

*The influence of some effectors on the activity of molecular isoforms of pigeon skeletal muscle AMP deaminase*

The substrate (AMP) concentration was 50 μM. $v_{0}$ is the reaction velocity measured in the presence of 100 mM potassium chloride only (control conditions).

<table>
<thead>
<tr>
<th>Molecular form of the enzyme</th>
<th>Relative velocity of the reaction ($v/v_{0}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td></td>
<td>5 μM</td>
</tr>
<tr>
<td>Isoform A</td>
<td>0.9</td>
</tr>
<tr>
<td>Isoform B1</td>
<td>1.0</td>
</tr>
<tr>
<td>Isoform B2</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Fig. 1. The elution profile of breast (a) and leg (b) skeletal muscle AMP deaminase from a phosphocellulose column. The fractions (3 ml) between the vertical lines were pooled for further concentration. For experimental conditions see text.
Fig. 2. The effect of potassium chloride concentration on the activity of three isoforms of AMP deaminase isolated from pigeon skeletal muscles. Breast isoform A (○), leg isoform B1 (△) and B2 (▽). AMP concentration was 50 μM.

As may be seen from this Table, the observed effects are rather moderate. Generally, the two isoforms extracted from leg muscle exhibited a similar pattern of regulation. These isoforms are slightly stimulated by ADP and ATP, and inhibited by GTP and orthophosphate. The pigeon breast muscle isoenzyme was inhibited not only by GTP and orthophosphate, but also by ATP. Alanine (not shown), which at high, non-physiological 5 mM concentration [14] was shown to inhibit the chicken skeletal muscle AMP deaminase, had no effect on the activities of the three pigeon skeletal muscle isoforms.

Table 3
Deamination of some structural analogues of 5'-AMP by molecular isoforms of pigeon skeletal muscle AMP deaminase

<table>
<thead>
<tr>
<th>Substrate (1 mM)</th>
<th>Relative velocity of the reaction (% of the control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isoform A</td>
</tr>
<tr>
<td>5'-AMP (control)</td>
<td>100</td>
</tr>
<tr>
<td>2'-AMP</td>
<td>0.3</td>
</tr>
<tr>
<td>3'-AMP</td>
<td>3.1</td>
</tr>
<tr>
<td>ADPG</td>
<td>2.8</td>
</tr>
<tr>
<td>ADPR</td>
<td>1.5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.4</td>
</tr>
<tr>
<td>d-AMP</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Fig. 3. The effect of pH on the activity of the reaction catalysed by AMP deaminase isoforms isolated from pigeon skeletal muscles in the absence (×) or in the presence of 25 μM concentration of ATP (▲), ADP (▼), GTP (■) or 2.0 mM orthophosphate (●). The reaction catalysed by the breast isoform A (a), and leg isoforms B1 (b) or B2 (c). AMP concentration was 50 μM.

As seen from Fig. 3, the molecular isoforms of AMP deaminase isolated from pigeon breast and leg muscle preserved their regulatory patterns over almost the whole range of pH values tested. While all of the isoforms displayed their highest activity at pH 6.6 when examined
in the presence of 100 mM potassium chloride only, in the presence of ADP the optimum pH value shifted down to pH 6.2 for isoform A, and up to 7.0 for both B isoforms.

The substrate specificity revealed by the three isoforms of pigeon skeletal muscle AMP deaminase are presented in Table 3.

DISCUSSION

During development, skeletal muscle differentiates into at least three distinct fibre types. These may be designated slow twitch type (type I), fast twitch red (type IIA) and fast twitch white (type IIB). Enzyme analyses have shown that type I fibres show predominantly aerobic metabolism of fats and carbohydrates, whereas type IIB fibres undergo anaerobic glycolysis. Type IIA fibres display both capacities [15, 16].

Skeletal muscle types can also be distinguished on the basis of their staining for AMP deaminase [17]. Although this method is not sufficient to define unambiguously any of the three fibre types, it has been shown that rat slow twitch type fibres contain significantly less AMP deaminase activity than fast twitch type fibres. Little is known about the histological classification of pigeon skeletal muscles. Raggi et al. [18] reported that pigeon leg muscles contain about 25% of AMP deaminase activity of that in the wing muscle. A similar difference has been found during the present study and the specific activities of the enzyme preparations obtained from different muscles may reflect to some extent this fact. On the other hand, it is known that pigeon breast muscle despite its erroneous classification as “white” muscle, is especially rich in mitochondria and represents a highly oxidative type of metabolism. This muscle does not produce significant amount of ammonia, even during strenuous exercise [19]. Intensive ammonia production, the main source of which is AMP deaminase activity [20], is a characteristic feature of muscle showing highly glycolytic metabolism [17].

The existence of chromatographically different isoforms of AMP deaminase in pigeon skeletal muscle is evident from Fig. 1. In contrast to the leg muscle extract, in which two symmetrical activity peaks have been detected (Fig. 1B), the activity present in the extract from breast muscle emerged from the column as one, but asymmetrical peak. This fact may indicate on the presence of molecular subforms of AMP deaminase as the source of the observed heterogeneity in the elution profile of activity from this muscle (Fig. 1A).

Ronca-Testoni et al. [21] reported that pigeon breast muscle AMP deaminase showed high sensitivity activation by potassium ions being almost completely activated by 20 mM KCl. The results presented in this paper confirm this finding, and indicate that the two isoforms from pigeon leg
muscle are even more sensitive to the activatory influence of this cation. At physiological 100 mM potassium ion concentration, which has been found to be necessary for the maximum activation of rabbit and guinea pig muscle enzyme [21], the activities of all the three AMP deaminase isoforms isolated from pigeon skeletal muscle are below their optimum values (Fig. 2). Under the same conditions the modulatory effects of ADP, ATP or GTP were rather moderate and weaker than those observed with the chicken skeletal AMP deaminase [22].

Ogasawara et al. [5] have postulated that the rabbit skeletal muscle AMP-deaminase isoform Red 1 is chromatographically and kinetically identical with the enzyme from cardiac tissue. The results presented in this paper indicate that this seems not to be true for the pigeon — none of the isoforms examined does resemble the kinetic and regulatory characteristics of pigeon cardiac muscle AMP deaminase [7]. Even in the presence of 100 mM potassium chloride, the kinetics manifested by pigeon cardiac enzyme had still a sigmoidal character \((n_\text{H} = 1.7\) \), and the half-saturation constant \((S_{50\%})\) value was as high as 2.8 mM, i.e. manyfold higher than that calculated for each of the three isoforms present in pigeon skeletal muscle (Table 1). The recently published data [23] indicate that also in the case of the rabbit a kinetic dissimilarity between the cardiac and skeletal muscle AMP deaminases does exist.

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REFERENCES


