REGULATORY PROPERTIES OF AMP-DEAMINASE FROM LATERAL RED MUSCLE AND DORSAL WHITE MUSCLE OF GOLDFISH, CARASSIUS AURATUS (L.)

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Abstract—1. The enzyme AMP-aminohydrolase (AMP-deaminase, EC 3.5.4.6) has been purified 160-190 fold from 48,000 g supernatantia of lateral red muscle and dorsal white muscle of goldfish. The procedure comprised ammonium sulphate precipitation and cellulose phosphate chromatography.

2. Red muscle and white muscle appeared to contain different isozymes, as indicated by different chromatographic behaviour on cellulose phosphate, a different dependence of activity on pH and a different response to activators.

3. Kinetic and regulatory properties of the enzymes have been examined in the presence of 120 mM KCl and physiological levels of substrate (0.1 mM).

4. The pH-optimum of both enzymes is situated between pH 6.8 and pH 7.0.

5. Both enzymes are activated by ATP and ADP, up to 80% in red muscle, but up to 500% in white muscle.

6. The activating effect of ATP and ADP is significantly reduced by addition of millimolar amounts of Mg²⁺.

7. Within physiological range GTP and inorganic phosphate can act as strong inhibitors.

8. Physiological amounts of alanine have no regulatory effect.

INTRODUCTION

The enzyme AMP-deaminase, which catalyzes the hydrolytic deamination of AMP to IMP and ammonia, has been isolated and purified from skeletal muscles of several fish species (Dingle & Hines, 1967; Makariewicz, 1969; Purzycka-Preis & Zydowo, 1969; Stanikiewicz et al., 1979). The investigations were concerned with extraction and kinetics, but little attention was paid to the regulatory properties and the authors did not discriminate between the lateral red muscle and epaxial white muscle.

Although the physiological function of the enzyme is not yet fully understood, it certainly plays a role in the following aspects of cellular metabolism: the regulation of the relative concentrations of intracellular adenine, hypoxanthine and guanine nucleotide pools (Setlow et al., 1966), the stabilization of the adenylate energy charge during a temporary imbalance between the rates of ATP-production and ATP-consumption (Chapman & Atkinson, 1973; Chapman et al., 1976; Coffee & Solano, 1977) and the deamination of amino acids via the so-called purine nucleotide cycle (Goodman & Lowenstein, 1977).

In the present study the kinetic and regulatory properties of AMP-deaminases from lateral red muscle and dorsal white muscle of goldfish have been examined and compared with those of the enzymes from other animal species. Adenine nucleotides, guanine nucleotides, inorganic phosphate, alanine and magnesium ions have been reported to be effectors of AMP-deaminase (Coffee & Solano, 1977; Kaletha et al., 1976; Ronca-Testoni et al., 1970; Smiley et al., 1967; Wheeler & Lowenstein, 1979). The main object of our research has been the function and regulation of AMP-deaminase in vivo; therefore we have used concentrations of substrate and effectors within the physiological range.

MATERIALS AND METHODS

Animals

Experiments were carried out in summer 1980 with healthy, 3-yr-old goldfish (mean weight 80 g, body length 14 cm). The animals were acclimated to 20°C, normal oxygen levels (PO₂ = 130-160 mmHg) and a 16 hr light period. They were fed daily with Trouvit pellets (Trouw, Putten, The Netherlands).

Enzyme extraction and purification

The animals were killed by decapitation. After removal of the scales and skin, the lateral red muscle or epaxial white muscle was excised. After excision all operations were carried out at a temperature of 4°C. Muscles were minced to 1 mm³ cubes with a tissue slicer (designed by Dr G. van den Thillart) and subsequently homogenized in 3 vols of phosphate buffer (80 mM K₂HPO₄, 50 mM KCl, 0.75 mM MgCl₂, pH 7.0) using an ILA-mixer (type X-1020) at maximum speed (25,000 rev/min). Homogenates were stirred by magnetic stirring bars during 1 hr, after which undissolved protein was removed by centrifugation (30 min at 48,000 g). The supernatant was decanted in a glass beaker, continuously stirred and solid ammonium sulphate was slowly added during a period of 4 hr to a final concentration of 2.26 M. Precipitated protein was removed by centrifugation (15 min at 20,000 g). The supernatant was discarded and the precipitate redissolved in a small volume of phosphate buffer (80 mM K₂HPO₄, 50 mM KCl, 0.75 mM MgCl₂, pH 7.0) using an ILA-mixer (type X-1020) at maximum speed (25,000 rev/min). Homogenates were stirred by magnetic stirring bars during 1 hr, after which undissolved protein was removed by centrifugation (15 min at 20,000 g). The supernatant was discarded and the precipitate redissolved in a small volume of buffer (50 mM KCl, 50 mM HEPES-K, pH 6.3). The enzyme solution was now applied on the top of a cellulose phosphate column (2.5 x 15 cm), which had been washed with 0.5 N KOH, water, 0.5 N HCl, water, 5 mM EDTA and phosphate buffer (180 mM KCl, 54 mM KH₂PO₄,
35 mM K₃HPO₄, pH 6.5) before use. Unbound protein was washed from the column by elution with 500 ml of the same buffer. In the case of the red muscle enzyme, inactive protein was eluted with 100 ml of a solution of 1 M KCl, 1 mM mercaptoethanol, pH 7.0, while contaminating protein from white muscle extracts was removed with 100 ml of 0.45 M KCl, 1 mM mercaptoethanol, pH 7.0. Finally the enzyme was eluted with 2.25 M KCl, 1 mM mercapto-ethanol, pH 7.0 (red muscle) or 1 M KCl, 1 mM mercapto-ethanol, pH 7.0 (white muscle). The eluate was collected in 2 ml fractions; all elutions were carried out at a flow rate of 0.5 ml/min. The most active fractions were pooled and used as an enzyme solution for kinetic studies.

This purification method is a modification of those described by Smiley et al. (1967) and Purzycka-Preis & Zydowo (1969).

**Enzyme assays**

**Assay A.** When low concentrations of substrate were present (total nucleotide level less than 0.15 mM) activity was measured spectrophotometrically by monitoring the rate of AMP-removal at a wavelength of 265 nm. The assay consisted of 0.75 ml buffer (100 mM histidine-chloride, 150 mM KCl, pH as indicated in the figures), 0.10 ml AMP-solution (1 mM) and either 0.10 ml (red muscle) or 0.01 ml (white muscle) of eluate.

**Assay B.** Because results of assay A are unreliable at nucleotide concentrations higher than 0.15 mM, in these cases a glutamate dehydrogenase-coupled assay as described by Van Waarde (1981) was used. Enzyme assays were carried out in duplicate at an incubation temperature of 25°C. The extinction coefficient as mentioned by Smiley et al. (1967) has been used for the calculation of activities in assay A. All effectors were dissolved in reaction buffer and adjusted to the pH of assay.

**Protein determination**

Protein concentrations were calculated from measurements of optical density at a wavelength of 280 nm. It has been assumed that an extinction of 1.0 at a light path of 1.0 cm corresponds with a protein concentration of 1 mg/ml (Dixon et al., 1979).

**RESULTS**

**Elution profile on cellulose phosphate**

During cellulose phosphate chromatography, AMP-deaminases from lateral red muscle and epaxial white muscle of goldfish showed a different chromatographic behaviour. While white muscle deaminase could be eluted with 1.0 M KCl, red muscle enzyme

![Graph showing elution profile on cellulose phosphate](https://via.placeholder.com/150)

**Fig. 1.** Elution profile of AMP-deaminases on cellulose phosphate. Red and white muscle enzymes were eluted with 2.25 M and 1.0 M KCl respectively, both containing 1 mM mercapto-ethanol pH 7.0. The flow rate was 0.5 ml/min and 2 ml were collected per tube. AMP-deaminase activity in the eluate was determined by assay A described in the Methods section.
AMP-deaminase in goldfish

Table 1. Purification of AMP-deaminase from goldfish muscles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (ml)</th>
<th>Activity (assay A)</th>
<th>Yield (%)</th>
<th>Protein concentration (mg/ml)</th>
<th>Specific activity (assay A)</th>
<th>Purification factor</th>
<th>Maximum velocity (µmoles/min/mg protein, ADP-activated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral red muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenate supernatant</td>
<td>42.9</td>
<td>0.118</td>
<td>100</td>
<td>24.7</td>
<td>5.1</td>
<td>1 ×</td>
<td></td>
</tr>
<tr>
<td>Cellulose-P eluate</td>
<td>16.7</td>
<td>0.051</td>
<td>16.8</td>
<td>0.067</td>
<td>816.4</td>
<td>160 x</td>
<td>8.1</td>
</tr>
<tr>
<td>Dorsal white muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenate supernatant</td>
<td>81.0</td>
<td>0.360</td>
<td>100</td>
<td>10.2</td>
<td>34.1</td>
<td>1 ×</td>
<td></td>
</tr>
<tr>
<td>Cellulose-P eluate peak 1</td>
<td>29.8</td>
<td>0.260</td>
<td>26.6</td>
<td>0.047</td>
<td>5369.6</td>
<td>157 x</td>
<td>234</td>
</tr>
<tr>
<td>Cellulose-P eluate peak 2</td>
<td>8.0</td>
<td>0.350</td>
<td>9.6</td>
<td>0.052</td>
<td>6533.2</td>
<td>192 x</td>
<td>270</td>
</tr>
</tbody>
</table>

10.9 g (lateral red muscle) and 19.5 g (dorsal white muscle) of tissue have been used as starting material.

was more firmly bound and elution required high ionic strength (2.25 M KCl).

As shown in Fig. 1, the red muscle enzyme was eluted as a single peak. Fraction Nos 23–29 were pooled and used as an enzyme solution for kinetic studies. The efficiency of purification is indicated in Table 1. The enzyme solution proved to be fairly stable, losing less than 20% of its activity during 2½ weeks of storage at 4°C in 2.25 M KCl, 1 mM mercapteto-ethanol, pH 7.0, while the regulatory properties were not lost.

White muscle enzyme was eluted as a double peak (Fig. 1). Fraction nos 43–53 and 57–60 were pooled and indicated as white muscle deaminase "peak 1" and "peak 2" respectively. During storage in 1 M KCl, 1 mM mercapteto-ethanol pH 7.0 white muscle enzyme showed a similar stability as that of red muscle.

During prolonged storage (2½ months at 4°C) both the white and red muscle enzymes lost very much of their activity. Because of this long-term instability, all measurements were carried out within 2 weeks (red muscle) or 3 weeks (white muscle) after purification.

pH-optimum

As depicted in Fig. 2, red muscle deaminase shows a very broad pH-optimum, no change of activity between pH 6.2 and 7.2 being detectable. White muscle deaminase shows a much narrower pH-optimum, situated at pH 6.8–7.2. Both peaks of white muscle deaminase showed a similar substrate curve and a similar sensitivity to effectors. For the sake of clarity, results are only presented for peak 1.

Substrate curve

In the presence of 120–130 mM KCl, both enzymes show Michaelis–Menten kinetics with respect to AMP. Apparent $K_m$ and maximum rate of ammonia production of red muscle deaminase show much lower values than those of white muscle enzyme (Tables 1 and 2). $K_m$'s are lowered by ADP.

Influence of effectors on activity

The influence of effectors on activity was examined in the presence of 120 mM KCl and physiological concentrations of AMP (0.1 mM; Van den Thillart et al., 1980).

1. ATP

ATP proved to have a biphasic effect on goldfish muscle deaminase (Fig. 3). Very low levels of ATP inhibit and millimolar amounts activate the enzyme. Maximal inhibition of red and white muscle enzymes occurs at ATP-concentrations of 100 µM and 5 µM respectively. Physiological levels of ATP activate the red muscle enzyme for about 50%, while the white muscle enzyme is activated for about 300%. Half-maximal activation of red and white muscle deaminases occurs at ATP-concentrations of 1.3 mM and 0.33 mM respectively. By addition of Mg$^{2+}$ the activating effect is decreased; this decrease is much more pronounced at higher Mg-concentrations (see Table 3).

2. GTP

GTP is a strong inhibitor of goldfish muscle deaminases, half-maximal inhibition being observed in the presence of 10 µM GTP (Fig. 4). By millimolar amounts of GTP both the red muscle enzyme and white muscle enzyme are inhibited for about 95%. Addition of Mg$^{2+}$ to the reaction mixture has little influence on the inhibition pattern.

Table 2. Affinity of goldfish AMP-deaminases for AMP

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_m$ without effectors (mM)</th>
<th>$K_m$ with 1.3 mM ADP (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red muscle</td>
<td>0.88</td>
<td>0.34</td>
</tr>
<tr>
<td>White muscle</td>
<td>1.83</td>
<td>1.04</td>
</tr>
</tbody>
</table>

The effect of substrate concentration on activity was measured with assay B as described in the Methods section.
3. **ADP**

ADP proved to have a biphasic effect on goldfish muscle AMP-deaminase (Fig. 5). At concentrations below 1 mM, an activation is observed, but reaction rates are lowered when the ADP-concentration is increased above 2 mM. Half-maximal activation of red and white muscle deaminases occurs at ADP-levels of 0.33 mM and 0.09 mM respectively. Physiological levels of ADP activate the red muscle enzyme for about 50%, while the white muscle enzyme is activated for about 500%. By addition of Mg\(^2+\) the activating effect is decreased; this decrease is even more pronounced at higher Mg-concentrations (see Table 3).

4. **Inorganic phosphate**

Inorganic phosphate is a powerful inhibitor of goldfish muscle deaminases, causing maximally about 95% inhibition, with apparent affinity constants of 4.7 mM (red muscle) and 2.2 mM (white muscle) respectively (Fig. 6).
Fig. 3. Effect of ATP on AMP-deaminase activity in the presence and absence of Mg\(^{2+}\). Assays were performed at a substrate level of 0.1 mM AMP in the presence and absence of 4 mM (red muscle) or 1 mM (white muscle) of MgCl\(_2\). Assay B, as described in the Methods section, was used throughout.

**Table 3. Influence of effectors on AMP-deaminase activity in the presence of a physiological adenylate pool**

<table>
<thead>
<tr>
<th>Effector</th>
<th>Without Mg</th>
<th>With Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red muscle</td>
<td>White muscle</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5 mM CrP</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td>0.4 mM Asp</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>0.3 mM GTP</td>
<td>76</td>
<td>86</td>
</tr>
<tr>
<td>3.3 mM Ala</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>50 mM Pi</td>
<td>57</td>
<td>124</td>
</tr>
<tr>
<td>pH changed (to 7.8)</td>
<td>131</td>
<td>194</td>
</tr>
</tbody>
</table>

Activities were measured with assay B at a physiological adenylate pool (red muscle: 3.50 mM ATP, 0.30 mM ADP, 0.11 mM AMP, energy charge 0.93; white muscle: 5.50 mM ATP, 0.80 mM ADP, 0.05 mM AMP, energy charge 0.93; see van den Thillart et al., 1980) in the presence or absence of 10 mM (red muscle) or 17 mM (white muscle) of Mg\(^{2+}\), being the total Mg-concentration of goldfish muscles (Internal Report, Department of Physiology).
5. Alanine

Although alanine causes a slight inhibition of white muscle deaminase at higher concentrations (5-10 mM), both the red and white muscle enzymes are not influenced by alanine addition in physiological amounts (0-5 mM; Fig. 6).

Influence of effectors on activity in the presence of a physiological adenylate pool

The influences of several effectors on the activity have also been examined in the presence of a physiological adenylate pool. The pool size and composition data were derived from van den Thillart et al. (1980), those on total Mg$^{2+}$-levels in goldfish muscles from an internal report of our department.

As depicted in Table 3, in the presence of an adenylate pool without Mg$^{2+}$ physiological amounts of CrP, aspartate and alanine have no significant influence on the activity of both enzymes, while GTP causes some inhibition. By a change in pH from 7.0 to 7.8 reaction rates are increased, which was rather unexpected. By addition of inorganic phosphate the red muscle enzyme is inhibited, while white muscle deaminase seems to be activated. These effects were reproducible and are not due to a change in pH, because all effector solutions were adjusted to the pH of assay.

By addition of the total amount of Mg$^{2+}$ normally present in goldfish muscles red and white muscle deaminases are inhibited for 56% and 83%, respectively. In the presence of Mg$^{2+}$ neither CrP, aspartate and alanine, nor GTP have pronounced effects on activity. By a change in pH from 7.0 to 7.8, however, reaction rates are very much increased. By addition of inorganic phosphate the red muscle enzyme is not influenced, while white muscle deaminase is activated.
Fig. 5. Effect of ADP on AMP-deaminase activity in the presence and absence of Mg\(^{2+}\). Assays were performed at a substrate level of 0.1 mM AMP in the presence and absence of 4 mM MgCl\(_2\). Assay B, as described in the Methods section, was used throughout.

Table 4. Substrate specificity of goldfish AMP-deaminases

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative rate of deamination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red muscle isozyme</td>
</tr>
<tr>
<td>0.1 mM AMP</td>
<td>1.000</td>
</tr>
<tr>
<td>0.1 mM 2-deoxy AMP</td>
<td>0.047</td>
</tr>
<tr>
<td>0.1 mM ado</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Activities were measured with assay B as described in the Methods section.
AgEN VAN WAARDE and FANJA KEBE

0.7
0.6
0.5
0.4
0.3
0.2
0.1

Red muscle

White muscle

Effector concentration (mM)

Fig. 6. Effect of inorganic phosphate and alanine on AMP-deaminase activity. Assays were performed at a substrate level of 0.1 mM AMP. Assay A as described in the Methods section was used throughout.

Substrate specificity

Some data on the substrate specificity of goldfish muscle AMP-deaminases are presented in Table 4. Both the red and white muscle enzyme proved to be able to deaminate 2-deoxy-5'AMP at relatively slow rates, but adenosine deaminase activity was undetectable.

DISCUSSION

Lateral red muscle and epaxial white muscle of goldfish contain different isozymes of AMP-deaminase, as indicated by their different chromatographic behaviour on cellulose phosphate (Fig. 1), the different shape of their pH-optimum (Fig. 2) and their different response to activators (Figs 3 and 5). The red muscle enzyme of rabbits was less firmly bound to cellulose-phosphate than the one from white muscle (Solano & Coffee, 1978), however, the contrary is observed in goldfish (Fig. 1). The enzyme from goldfish red muscle shows an unusual behaviour by its very tight binding to cellulose phosphate, which is not observed with any isozyme in rat or rabbit (Ogasawara et al., 1975, Solano & Coffee 1978).

As shown in Figs 3–6 and Table 5, AMP-deaminases from red muscle and white muscle of goldfish show identical responses to inhibitors (with exception of the apparent affinity for inorganic phosphate), but very different activation patterns. White muscle deaminase shows both a much higher affinity for ATP and ADP and a much stronger activation by these adenylates than the enzyme from red muscle (Table 5). This implies that the white muscle enzyme can be modulated over a much wider dynamic range of activities (from 95% inhibition to 100% activation) than the enzyme from red muscle (from 95% inhibition to 80% activation), which is consistent with the activity patterns of both muscles, the white muscle preferentially being used during burst swimming and red muscle during sustained swimming. Therefore the enzyme activity in white muscle should be modulated much faster and adjusted to a much greater range of variations in energy demand than that in red muscle.

The experimental results recently described by Meyer & Terjung (1979) may be correlated with different regulatory properties of AMP-deaminases from fast and slow muscles. These authors electrically stimulated fast-twitch (gastrocnemius) and slow-twitch (soleus) muscles of anesthetized rats to a comparable tension development. In fast muscle progressive decreases in total adenine nucleotide content were balanced by equivalent increases in IMP and NH₃ levels, but in slow muscle, although a small decrease of the adenylate pool could be observed, this was not accompanied by any increase in IMP or NH₃, probably because the small change in adenylate levels could not bring about a significant increase in the rate of AMP-deamination, while white muscle deaminase is much more sensitive to variations in effector concentration.

In rat tissues three types of AMP-deaminase have been described (Ogasawara et al., 1975). Isozyme A was found in skeletal muscle, isozyme B in liver and kidney, while type C occurs in the heart. Other tissues (e.g. brain, lung and spleen) contain a mixture of isozymes B and C. In the absence of nucleoside triphosphates, skeletal muscle deaminase shows a rather high affinity for AMP (0.35–1.0 mM). The enzyme is inhibited by ATP and GTP; this inhibition may partially be relieved by addition of ADP (Smiley et al., 1967; Ronca-Testoni et al., 1970; Coffee & Solano, 1977; Wheeler & Lowenstein, 1979). Liver deaminase has a much lower affinity for AMP (6 mM). This isozyme is activated by ATP and ADP, while adenylate activation may be strongly diminished by the addition of GTP (Lee & Wang, 1968; Smith & Kizer, 1969; Ogasawara et al., 1975). The isozyme from heart shows an intermediate affinity for its substrate (3 mM) and is also activated by ATP and ADP, while GTP-addition has little effect on observed reaction rates (Barsacchi et al., 1979; Kaletha & Skladanowski, 1979; Kaletha et al., 1979). All isozymes are inhibited by inorganic phosphate.
When data on nucleotide regulation of goldfish muscle AMP-deaminases and literature values for muscle deaminases from other vertebrates are compared, goldfish enzymes appear to have unique properties.

Goldfish muscle enzymes show regulatory properties which are comparable to those of rat liver isozyme, although their affinity for AMP is much higher. It has been reported by Makarewicz (1969), that skeletal muscle of the elasmobranch fish *Raia clausata* also contains an AMP-deaminase with unusual properties. Elasmobranch muscle enzyme was found to be activated by ATP, though GTP had no effect on the reaction rate, so that the regulatory properties of this enzyme resemble those of the isozyme from rat heart, although its affinity for AMP is higher. We may conclude that skeletal muscles of fish seem to contain AMP-deaminases which differ from the muscle isozyme of higher vertebrates by the fact that they are activated by ATP instead of inhibited.

Biphasic effects of ATP on AMP-deaminase activity have also been observed in rat muscle by Wheeler & Lowenstein (1979), but in the rat ATP is always inhibitory, even at millimolar concentrations.

Ogasawara et al. (1975) have shown that the three AMP-deaminases from rat tissues differ not only in kinetic and regulatory properties, but are also immunologically distinct and show a different substrate specificity. When data on substrate specificity of enzymes from goldfish muscles (Table 4) are compared to those of rat tissues (Ogasawara et al., 1975) the goldfish muscle enzymes prove to resemble the rat liver isozyme in this respect also. Both the goldfish muscle isozymes and the rat liver isozyme show much lower relative rates of deoxy-AMP deamination than the enzyme from rat muscle.

When data in Table 3 are compared with those of Figs 3–6 it is obvious that in the presence of a physiological adenylate pool effectors have a different influence on AMP-deaminase activity than in its absence. By the presence of millimolar amounts of adenylate GTP-inhibition is very much relieved, both in red and in white muscle. Inorganic phosphate inhibition is partially relieved in red muscle and completely abolished in white muscle. In the presence of adenylates Mg$^{2+}$-ions have a striking inhibitory effect. Wheeler & Lowenstein (1979) also found a decrease of ADP-activation and ATP-inhibition upon addition of Mg$^{2+}$ to rat muscle deaminase. These authors explained their data by the assumption that the "chelated forms" of the nucleotides have no regulatory effect. If this hypothesis is correct, we should expect a lowering of the apparent affinity of AMP-deaminase for ATP and ADP by the addition of Mg$^{2+}$-ions to the reaction mixture. Such an effect was actually found by Wheeler & Lowenstein (1979), but it could not be observed in goldfish, where only the maximum velocity and not the apparent affinity of the enzyme for its activator is lowered by Mg$^{2+}$-addition. Therefore our data cannot be explained by the assumption that only the unchelated forms of the nucleotides activate. In goldfish Mg$^{2+}$-ions have not only an inhibitory effect in the presence of adenylates, but also in their absence (values not shown). Therefore we may conclude that magnesium ions have a direct effect on goldfish muscle AMP-deaminase activity. The effect of pH is dependent on the composition of the adenylate pool. In the absence of ATP and ADP a change of pH from 7.0 to 7.8 is inhibitory (Fig. 2), but in their presence it is activating, both in the presence and absence of Mg$^{2+}$ (Table 3). The effect of pH, however, proved to be also dependent on the composition of the reaction mixture: in the presence of 5 mM inorganic phosphate and a physiological adenylate pool the enzyme loses its sensitivity to changes of pH.

These data make it rather difficult to specify the factors which regulate the activity of goldfish muscle AMP-deaminase in vivo.

Fish muscle contains large amounts (20–40 mM) of orthophosphate which are increased during exercise (Fraser et al., 1966; Nagayama, 1961). Because orthophosphate has little regulatory effect in the presence of physiological amounts of adenylate (Table 3) and orthophosphate levels in fish muscle show a relatively small change during exercise, orthophosphate does not seem to be an important factor in the regulation of goldfish AMP-deaminase activity. The ATP-concentration does not seem to be regulatory either, because physiological levels of ATP are maximally activating. Alanine inhibition has been described for rabbit muscle AMP-deaminase (Kalathia et al., 1976), but unphysiological levels of alanine had to be used before significant inhibition was observed. Similar results are obtained for white muscle AMP-deaminase of goldfish (Fig. 6), while the enzyme from red muscle is not inhibited at all. Creatine phosphate and aspartate also do not appear to have significant regulatory effects (Table 3).

The main factors which regulate the activity of goldfish deaminases in vivo appear to be:

1. The concentration of their substrate AMP, for intracellular AMP-concentrations are much lower than the Michaelis–Menten constant (see Van den Thillart et al., 1980).
2. The concentration of the major activator ADP.
3. The intracellular pH.

<table>
<thead>
<tr>
<th>Activators</th>
<th>Red muscle $K_a$ (mM)</th>
<th>Max. activation (%)</th>
<th>White muscle $K_a$ (mM)</th>
<th>Max. activation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>0.33</td>
<td>77.8</td>
<td>0.09</td>
<td>525.0</td>
</tr>
<tr>
<td>ATP</td>
<td>1.30</td>
<td>52.6</td>
<td>0.33</td>
<td>342.8</td>
</tr>
<tr>
<td>GTP</td>
<td>0.01</td>
<td>96.1</td>
<td>0.01</td>
<td>94–100</td>
</tr>
<tr>
<td>$P_i$</td>
<td>4.68</td>
<td>&gt;91</td>
<td>2.17</td>
<td>95.6</td>
</tr>
</tbody>
</table>

**TABLE 5. Regulatory properties of goldfish muscle deaminases**

When data on nucleotide regulation of goldfish muscle AMP-deaminase activity and literature values for muscle deaminases from other vertebrates are compared, goldfish enzymes appear to have unique properties.
We should expect a positive response of enzyme activity to decreasing energy charge, because a decrease of charge is accompanied by an increase of both the substrate and activator concentration. In the presence of an adenylate pool and energy charge in the physiological range both the red and white muscle enzyme were found to be inhibited for about 85-95% with respect to the maximum velocity, but we would expect the enzyme in the in vivo situation to be inhibited even stronger. It is a well-known fact that most of the ADP in muscle is bound to actin, so that total ADP-levels as obtained by van den Thillart (1980) give a gross over-estimation of the amount of ADP which is available to AMP-deaminase. Free ADP-levels in muscle are reported to be 40-70 nM during rest and after exercise, respectively (Goodman & Lowenstein, 1977; Wheeler & Lowenstein, 1979). A similar situation exists for AMP: the concentration of free AMP in skeletal muscle is likely to be much lower than the total concentration. Goodman & Lowenstein (1977) report that free AMP-concentrations of muscle are in the micromolar range (0.2-1.0 μM). Therefore it appears that in the in vivo situation both substrate and activator are not freely available to the catalytic and effector sites of AMP-deaminase. At such low substrate concentrations the inhibitory effect of GTP may be stronger than shown in Table 3. In fish muscle GTP-concentrations are in the micromolar range (Gras et al., 1967; Jones & Murray, 1960), while levels are decreased during exercise. Therefore the GTP-concentration may be an additional factor in the control of AMP-deaminase activity. The assumptions mentioned above are in accord with previous observations on the adenine nucleotide cycle in goldfish, where it was calculated that in the in vivo situation goldfish muscle deaminases should be inhibited for more than 99% (van Waarde, 1981).

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AMP-deaminase in goldfish

