EWA PRUS, JADWIGA PURZYCKA-PREIS, MICHAL WOZNIAK
and MARIUSZ ZYDOWO

PURIFICATION AND REGULATORY PROPERTIES OF PIG KIDNEY
AMP DEAMINASE *

Department of Biochemistry, Medical School,
ul. Dębińska 1, 80-211 Gdańsk, Poland

AMP deaminase (EC 3.5.4.6) from pig kidney was purified about 1200-fold
by chromatography on cellulose phosphate. The enzyme showed a sigmoid-shaped
substrate saturation curve which was converted to hyperbolic by addition of
ATP. The ATP-activated enzyme was sensitive to phosphatidylcholine-containing
liposomes which caused a further increase of activity by lowering the $S_{\text{m},c}$
value and increasing $V_{\text{max}}$. In the absence of ATP, the enzyme was not
sensitive to phosphatidylcholine-containing liposomes.

Phosphatidate-containing liposomes exerted an inhibitory effect both in the
presence and absence of ATP. In the presence of ATP phosphatidate was
a non-competitive inhibitor. Orthophosphate was found to be a competitive
inhibitor of AMP deaminase from pig kidney. When the phosphatidylcholine/
phosphatidic acid ratio in liposomes was 2.7, AMP deaminase was activated,
whereas when this ratio dropped below 2.1, liposomes exerted a non-competitive
inhibitory effect.

Direct deamination of AMP in the kidney by AMP-deaminase (EC 3.5.4.6)
has been shown several years ago to accompany the indirect deamination
of this compound after its dephosphorylation (Żydowo, 1960). The participation
of this enzyme in the formation of ammonia excreted in urine has been
suggested (Żydowo, 1960; Bogusky et al., 1976). This suggestion is
supported by the adaptability of the enzyme activity in rat kidney to
acidosis (Żydowo et al., 1962), and by the fact that the activity of AMP
deaminase in the excretory organs of lower ammonotelic vertebrates is very
high (Makarewicz & Żydowo, 1962). Although AMP deaminases from several
sources have been extensively investigated (for review see Stankiewicz,
1978), little attention has been paid so far to the enzyme from the kidney.

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In this report kinetic and regulatory properties of AMP deaminase isolated from pig kidney are presented and the inhibitory effect of phosphatidate-containing liposomes is described.

MATERIALS AND METHODS

Tissue. Pig kidneys were transported from slaughter house in ice as soon as possible after death of the animal. In the laboratory, excessive fat and connective tissue were removed, the kidneys were dissected and homogenized.

Purification procedure. AMP deaminase from pig kidney was purified by cellulose phosphate chromatography essentially as described by Smiley et al. (1967) for the skeletal muscle enzyme, with slight modifications: All the steps were performed at 4°C instead of at room temperature, the extraction was carried out by stirring the homogenate for 12 h, and the absorption on cellulose phosphate was prolonged to 1 h. A summary of the purification procedure is given in Table 1. The enzyme obtained was stable for at least 1 month when stored at 4°C; it showed optimum activity at pH about 6.5 (Fig. 1) in potassium succinate buffer. The enzyme was very specific: no deamination of 2'-AMP, 3'-AMP, cyclic 3',5'-AMP, dAMP, ATP, CMP, CTP, adenosine-5'-phosphoroamidate, adenosine 5'-sulphate or adenosine could be detected even on incubation of the enzyme with these compounds for 60 min.

Enzyme assays. AMP deaminase activity was measured by estimating ammonia produced with the use of phenol-hypochlorite reagent as described by Chaney & Marbach (1962). The incubation mixture contained in the final volume 0.5 ml: 100 mm-potassium succinate buffer, pH 6.4, 150 mm-KCl, 5 - 10 μg enzyme protein, AMP and other additions as indicated in legends to the Figures. The incubation was carried out for 10 min at 30°C; the reaction was started by the addition of enzyme solution, terminated by the addition of phenol reagent, and the ammonia liberated was determined. When the enzyme activity was assayed in the crude extract, the reaction mixture contained 4 - 5 mg of protein, therefore the incubation was terminated by the addition of an equal volume of 15% (w/v) trichloroacetic acid, the solution was neutralized by KOH, and ammonia was estimated in an aliquot of a clear supernatant.

In the experiment on the effect of liposomes, 25 - 50 μl of enzyme solution containing 5 - 10 μg protein was preincubated for 1 h at 4°C with 250 μl of liposomes suspension containing the amount of phospholipids indicated in the Figures. In these cases the reaction was started by the addition of an appropriate amount of AMP solution, to make up the final volume of the incubation mixture to 0.5 ml.

Preparation of liposomes. Liposomes containing different amounts of phospholipids were prepared as described previously (Purzycka-Preis et al., 1978).
Analytical procedures. Protein was determined either by the method of Lowry et al. (1951) or by the method of Warburg and Christian as described by Layne (1957). Phospholipid phosphorus after mineralization was estimated according to Gomori as described by Power (1953).

Reagents. Adenosine, AMP, ADP, ATP, adenosine 2'-phosphate, adenosine 3'-phosphate, dAMP, CMP, CTP were purchased from Sigma Chem. Co. (St. Louis, U.S.A.). Cyclic AMP was from Schuchardt (München, F.R.G.), cellulose phosphate P-11 from Whatman (Maidstone, England). Egg lecithin was obtained from Serva (Heidelberg, F.R.G.), phosphatidic acid ex egg lecithin from Koch-Light (Colnbrook, England). Adenosine 5'-sulphate A grade and adenosine 5'-phosphoromidate were obtained from Calbiochem (San Diego, Calif., U.S.A.). All other chemicals were of highest analytical grade obtainable from P.O.Ch. (Gliwice, Poland).

RESULTS AND DISCUSSION

AMP deaminase from pig kidney was purified 1200-fold with a yield of 42% by a one step procedure (Table 1). The purified enzyme showed a broad pH optimum at 6.5 (Fig. 1) and displayed a sigmoid-shaped substrate saturation curve similar to that obtained for the AMP deaminase isolated from pig heart (Purzycka-Pris et al., 1978) and human heart (Kaletha et al., 1979). As may be seen from Fig. 2, ATP was a positive effector of pig kidney AMP deaminase, exerting a mixed (\(V, K_m\)) type activation, characterized by lower half-saturation substrate concentration (\(S_{0.5}\)) i.e. from 4.5 mm to 1.9 mm, and by the increased maximum velocity by about 40%. The addition of phosphatidylcholine-containing liposomes to the ATP-activated enzyme caused a further decrease of \(S_{0.5}\) to 1.2 mm and an increase of \(V_{\text{max}}\) from 3.2 to 4.5 μmol NH₃ per minute. In the absence of ATP, the phosphatidylcholine-containing liposomes had no effect on the AMP deaminase activity.

![Fig. 1. The pH-dependence of pig kidney AMP deaminase activity. For experimental details see text.](image-url)
Fig. 2. Substrate saturation curve of AMP deaminase from pig kidney. Incubations were carried out as described in the text, without any additions (×), and in the presence of: 5 mM-APT (○); 375 nmol of phosphatidylcholine-containing liposomes (△); and 5 mM-ATP plus 375 nmol of phosphatidylcholine-containing liposomes (●).

Table 1

Purification of adenylate deaminase from pig kidney

For experimental details see the text.

<table>
<thead>
<tr>
<th>Extract Elute from phosphocellulose column</th>
<th>Total protein (mg)</th>
<th>Total activity (μmol NH₃ × min⁻¹)</th>
<th>Specific activity (μmol NH₃ × min⁻¹ per mg protein)</th>
<th>Yield (%)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 000</td>
<td>85</td>
<td>0.005</td>
<td>100</td>
<td>1200</td>
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<tr>
<td></td>
<td>6</td>
<td>36</td>
<td>6</td>
<td>42</td>
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</table>

When, however, the enzyme had been preincubated with the liposomes containing phosphatidic acid instead of phosphatidylcholine, inhibition was observed both in the presence and absence of ATP (Fig. 3). Phosphatidate-containing liposomes did not affect the $S_{0.5}$ value either in the presence or in the absence of ATP. As may be seen from the Dixon plot presented in Fig. 4, phosphatidate was found to exhibit a purely non-competitive inhibitory effect on the ATP-activated AMP deaminase with the $K_i$ value of 4.5 μM. Orthophosphate, which is known to inhibit AMP deaminase from pig heart (Purzycka-Preis et al., 1978), inhibited also the pig kidney enzyme (not shown), but the inhibition was competitive and the $K_i$ value was 3 mM.

On investigating the influence of phospholipid bilayers of different composition on the kinetics of adenylate deamination catalysed by the pig heart
enzyme it has been found that the negatively charged liposomes composed of phosphatidic acid and phosphatidylycholine at the ratio 4:13 exhibit an activating effect (Purzycka-Preis et al., 1978). Therefore, liposomes containing a mixture of phosphatidate and phosphatidylycholine of different molar ratios were prepared and their influence on the activity of pig kidney AMP deaminase was investigated (Fig. 5). The liposomes containing 2.7 times more phosphatidylycholine than phosphatidate showed an activating effect; when this ratio dropped to 2.1:1, an inhibitory effect was observed which became more pronounced with the increase of phosphatidate proportion.

AMP deaminase is one of the enzymes of purine nucleotide cycle operating in several tissues (Lowenstein, 1972); it may be involved in the maintenance of adenylate energy charge (Chapman & Atkinson, 1973), it may also contribute to the removal of AMP from the adenine nucleotide pool, thus preventing production of adenosine. The last compound is known as an agent influencing renal blood flow and glomerular filtration rate.
Fig. 4. Dixon plot of the inhibitory effect of phosphatidic acid on the ATP-activated pig kidney AMP deaminase. Incubations were carried out as described in the text at AMP concentration of: 3 mm (□); 5 mm (○); and 7 mm (△).

Fig. 5. The influence of liposomes containing different proportions of phosphatidate and phosphatidylcholine on the activity of ATP-activated pig kidney AMP deaminase. Incubation mixtures contained 10 mm-AMP, 5 mm-ATP, 150 mm-KCl, 100 mm-potassium succinate buffer, 2.5 µg enzyme protein and 356 - 410 nmol of phospholipids differing in the phosphatidate/phosphatidylcholine molar ratio as indicated. For other details see text. Activity of the control without liposomes was taken as 100.
(Osswald, 1975). Hence, precise regulation of the activity of kidney AMP deaminase is of great physiological importance. The regulatory properties of this enzyme presented in this paper are similar to those of the analogous enzyme from the heart but different from those of the skeletal muscle enzyme (Purzycka-Preis et al., 1978). Especially interesting are the effects of phospholipid bilayers frequently used as a model that may mimic the effect of natural membranes. Kidney AMP deaminase is localized in the cytoplasm (Żydowo, 1960) and one may assume that under variable circumstances it would associate with, or dissociate from, intracellular membranous structures. This may not only speed up and enhance the effect of ATP and other effectors but may also bring about either activation or inhibition, depending on the chemical composition of the phospholipid constituents of the membrane.

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REFERENCES


Oczyszczenie i właściwości regulacyjne dezaminazy adenylanowej z nerki świń

Streszczenie

Dezaminazę AMP z nerki świń oczyszczone około 1200 razy metodą chromatografii na fosfocelulozie. Stwierdzono, że enzym wykazuje sigmoidalną krzywą wysycenia substratem, która ulega zmianie na hiperboliczną w obecności ATP.

Liposomy zawierające lecytynę aktywowały enzym w obecności ATP przez obniżenie półwysycającego stężenia substratu i zwiększenie szybkości maksymalnej działania enzymu. Enzym nie wykazuje wrażliwości na liposomy lecytynowe w nieobecności ATP. Liposomy zawierające kwas fosfatydowy hamowały aktywność enzymu w obecności i nieobecności ATP. Stwierdzono, że w obecności ATP kwas fosfatydowy hamował aktywność enzymu w sposób niekompetencyjny. Kwas ortofosforowy hamował aktywność dezaminazy AMP w sposób kompetencyjny.

Gdy stosunek fosfatydylcholiny do kwasu fosfatydowego w liposomach był równy 2.7, dezaminaza AMP ulegała aktywacji pod ich wpływem. Jeżeli ten stosunek spadał poniżej 2.1, liposomy wywierały niekompetencyjny efekt hamujący.

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