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PIG THYROID AMP DEAMINASE

PURIFICATION AND SOME PROPERTIES

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SUMMARY: AMP deaminase from pig thyroid gland was purified about 500-fold using phosphocellulose adsorption chromatography. The purified enzyme had a pH optimum of about 7.0. The kinetics of AMP deamination was sigmoid-shaped. The values of the cooperativity coefficient n_H and the substrate concentrations required to reach half maximum valocity $S_{0.5}$ calculated from the Hill equation were 1.65 and 5.63 mM respectively. The activity of the thyroid enzyme is regulated by adenine and guanine nucleotides, inorganic phosphate and potassium ions.

INTRODUCTION

AMP deaminase (AMP aminohydrolase EC 3.5.4.6) catalyzes irreversible deamination of AMP to IMP and ammonia. AMP plays an important role in controlling the purin biosynthesis and interconversion, glycolysis, gluconeogenesis in cells basides acting as a substrate (1). An alteration of the concentration of AMP or IMP through the action of the deaminase, could conceivably result in changing the rates of nucleic acid biosynthesis and of other reactions (1,2). The AMP deaminase is widely distributed in mammalian tissues and has been studied intensively (3-14). Recently we reported the occurrence and subcellular localization of AMP deaminase in the pig thyroid gland (15). The enzyme from the thyroid has not been purified nor has its regulation by effectors been studied. In this paper, partial purification and some properties of the purified enzyme are reported.

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Table I PURIFICATION OF AMP DEAMINASE FROM PIG THYROID GLAND

For experimental details see the text. The starting Material was 250 g of the frozen thyroid.

Fraction	Volume (ml)	Protein (mg/ml)	Total activity (μmol/min)	Specific activity (μmol/min/mg protein)	Yield (%)	Purification (-fold)
Homogenate	1050	15.40	48.7	0.003	100	1.00
Extract	950	14.60	44.1	0.0032	90.5	1.07
Phospho-cellulose P-11 eluate	25	0.72	28.6	1.58	58.7	526.0

MATERIALS AND METHODS

Materials. Various nucleotides and nucleosides were obtained from Sigma Chemical Co., cellulose phosphate P_{11} from Whatman, and other reagents from POCH Gliwice (Poland). The pig thyroid glands were obtained from the slaughterhouse. After the removal of the outer membrane and fat, the thyroid glands were kept frozen at -20°C.

Enzyme assay. The activity of AMP deaminase was assayed by determination of ammonia liberated from AMP (16). The incubation mixture contained in a final volume of 0.5 ml, 0.143 M veronal buffer pH 7.0, 5 mM 5'-AMP and the enzyme. The reaction was usually carried out at 37° for 10 min and was stopped by adding 1.5 ml deproteinizing agent. In the control experiment a deproteinizing agent was added to the mixture at 0 time. Ammonia was assayed after centrifugation in the 1 ml samples.

Protein concentration were determined by the method of Lowry et al. (17) using bovine albumin as a standard.

Purification of the enzyme. AMP deaminase was prepared by the procedure described by Smiley et al. (4) with certain modifications. The enzyme was adsorbed on phosphocellulose and then washed on a Büchner funnel with 0.18 M KCl in 0.09 M phosphate buffer pH 6.5. When no more protein was eluted in the wash, the cellulose phosphate was transferred to a column and the enzyme was eluted with 0.45 M KCl in 0.09 M phosphate buffer pH 6.5, containing 1 mM mercaptoethanol.
Polyacrylamide gel electrophoresis was performed by the method of Davis (18).

RESULTS

Purification of AMP deaminase. AMP deaminase from the pig thyroid gland was purified over 500-fold by chromatography on phosphocellulose (Table I). The purified enzyme had a specific acti-

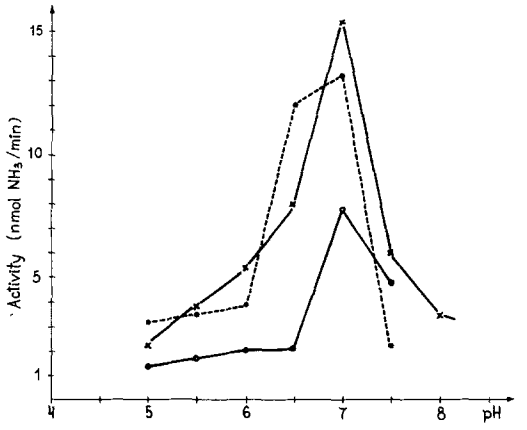
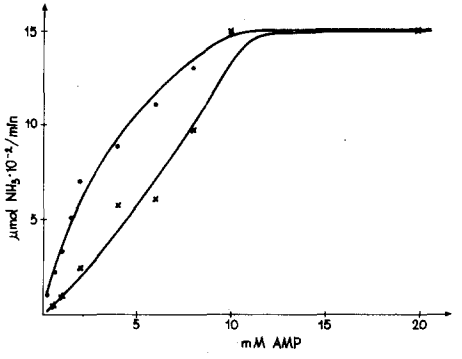


Fig.1 Optimum pH for the activity of pig thyroid AMP deaminase. The reaction mixture contained 5 mM 5'-AMP, the enzyme and buffer in a final volume of 1 ml. • - - - • 0.05 M imidazole buffer, x——x 0.143 M veronal buffer, o——o 0.066 M phosphate buffer. Each point represents the mean from three experiments performed in duplicate.

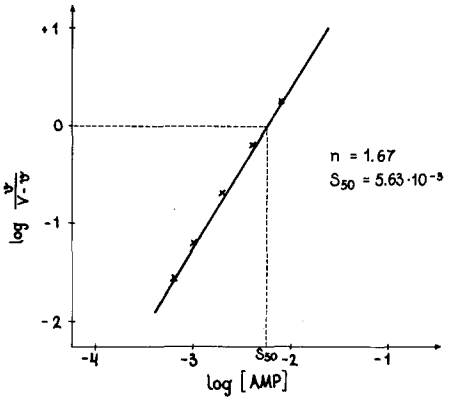
vity of 1.5 - 2 μmoles AMP deaminated per min/mg of protein. The enzyme thus obtained was not homogeneous, electrophoretic tests on polyacrylamide gel giving 3 bands. The enzyme was stored at a temperature of -20°C, since at a temperature of 4° C it lost a considerable amount of its activity after 24 hours' storage. Other methods of purifying the thyroid enzyme were tried such as salting out with ammonium sulphate, chromatography on DEAE cellulose and on Sephadex G-100, and affinity chromatography on octyl sepharose CL 4 B but they did not give satisfactory results.

Effect of pH. The effect of pH on the AMP deaminase activity is shown in Fig. 1. The optimum pH was found to be about 7.0.

Kinetic properties. In the absence of ATP the kinetics of AMP deamination was sigmoid-shaped (Fig.2). When ATP was present in the reaction medium, the sigmoid-shaped curve was transformed to hyperbolic. The values of the cooperativity coefficient n_H and the substrate concentration required to reach half-maximum velo-



(2)



(3)

Fig.2 Initial velocity of pig thyroid AMP deaminase as a function of substrate concentration. Assays were performed in 0.143 M veronal buffer pH 7.0 in absence of ATP x——x, or presence of 2 mM ATP. Each point represents the mean from three experiments performed in duplicate.

Fig.3 Hill plot of log v/V-v versus log S.

city S_{50} calculated from the Hill equation were 1.67 and 5.65 mM respectively (Fig.3).

Substrate specificity. The substrate specificity of the thyroid enzyme is given in Table II. The thyroid AMP deaminase catalyzed the deamination of 5'-AMP as the preferred substrate and had no activity towards 2'-AMP and adenine, inconsiderable activity towards ADP and ATP and negligible activity towards 3'-AMP and adenosine.

Table II SUBSTRATE SPECIFICITY OF PIG THYROID AMP DEAMINASE

Assays were carried out in the presence of 5 mM concentrations of the substrate in 0.1 M veronal buffer pH 7.0.

Substrate	Relative velocity
5'-AMP	1.00
2'-AMP	0.00
3'-AMP	0.03
adenosine	0.02
adenine	0.00
ADP	0.13
ATP	0.07

Table III EFFECT OF ADENINE AND GUANINE NUCLEOTIDES ON PIG THYROID AMP DEAMINASE ACTIVITY.

The activity was estimated in 0.143 M veronal buffer pH 7.0. Relation activity calculated assuming rate in the presence of 5 mM AMP as 1.00

Nucleotide added	Concentration of nucleotide added	Relative activity
None	---	1.00
ATP	1 μM	0.75
ATP	1 mM	1.85
ADP	1 μM	0.27
ADP	1 mM	1.38
GTP	1 μM	1.12
GTP	1 mM	0.58
GDP	1 μM	1.08
GDP	1 mM	0.81

Effects of adenine and guanine nucleotides and ortophosphate. The effect of adenine and guanine nucleotides on thyroid deaminase activity depends on their concentration (Table III). Adenine nucleotides in a concentration of 1 mM activate deaminase, ATP being more effective than ADP. In concentrations of 1 μM the adenine nucleotides inhibit deaminase activity. ADP is a stronger inhibitor than ATP. The reverse was the case as regards guanine nucleotides. In concentrations of 1 mM they inhibit deaminase activity and in concentrations of 1 μM slightly activate it. Ortophosphate has an inhibiting effect on deaminase activity (Table IV). In a concentration of 10 mM it inhibits deaminase by approximately 50%.

Effects of alkali ions. The effects of K^+, Na^+, and Li^+ on pig thyroid deaminase are summarized in Table V. At 5 mM AMP, the deaminase is markedly activated by K^+ at 150 mM concentration; Na^+ activating less efficiently. The ions Li^+ under the conditions studied did not affect the thyroid deaminase activity.

Table IV EFFECT OF ORTOPHOSPHATE ON PIG THYROID AMP DEAMINASE ACTIVITY

Reaction mixtures contained 5 mM AMP, 0.143 M veronal buffer pH 7.0, 150 mM KCl and ortophosphate in the concentrations as was indicated.

Concentration of ortophosphate added (mM)	Relative activity
none	1.00
1	0.65
5	0.60
10	0.51

DISCUSSION

The AMP deaminase activity in the thyroid is considerably lower than the activity of that enzyme in the skeletal muscle (4) but, however, AMP deaminase activity in the liver is almost the same as in the thyroid (3,5). Chromatography on phosphocellulose gave a 500-fold purified enzyme. Further purification was extremely difficult because after phosphocellulose chromatography the enzyme quickly lost its activity.

Table V EFFECT OF ALKALI METAL IONS ON THE ACTIVITY OF PIG THYROID AMP DEAMINASE

Activity determined in the presence of 5 mM AMP in 0.143 M veronal buffer pH 7.0. All assays were performed in the absence of ATP.

Addition	Concentration (mM)	Relative velocity
none	---	1.00
KCl	50	1.00
KCl	100	1.10
KCl	150	1.84
NaCl	50	1.00
NaCl	100	1.16
NaCl	150	1.14
LiCl	50	1.00
LiCl	100	1.00
LiCl	150	1.00

AMP isolated from various sources shows differences in substrate affinity and response to cations and ATP. On considering the activation with monovalent cations, the thyroid enzyme is similar to the AMP deaminase from the skeletal muscles and erythrocytes (4,7,11). It is activated to the greatest extent by K^+ ions whereas deaminase from the liver, brain and placenta, have their highest activity in the presence of Li^+(5,8,14). Thyroid deaminase behaves however differently during chromatography on phosphocellulose than does deaminase from the skeletal muscles, that is it binds with phosphocellulose at a lower ionic strength and is eluted by an ionic strength as low as 0.45 M KCl. It should here be noted that the distribution of thyroid deaminase between the sediment and the solution depends on the magnitude of the ionic strength. The higher this strength the more enzyme is to be found in the solution after centrifugation of the homogenate. At the same time, with a greater ionic strength the enzyme is not adsorbed by the phosphocellulose. The passage of the enzyme from the sediment to the solution may be related to the stability and activity of the thyroid enzyme. Shiraki et al. (19) demonstrated that AMP deaminase from the rat muscle was activated by binding to myosin and that this binding might modify the regulatory properties of AMP deaminase. Pipoly et al. (20) showed that the purified human erythrocyte AMP deaminase binds specifically to the cytoplasmic surface of the erythrocyte membrane and that the stability of AMP deaminase is markedly improved by interaction with the membrane. The binding is reversible and the response to alterations of pH, of ionic strength, and ATP and AMP. Like AMP deaminases in animal tissues, thyroid AMP deaminase exhibits typical allosteric properties. Activity of the thyroid enzyme is regulated by potassium ions, inorganic

phosphate and adenine and guanine nucleotides. The activation-inhibition effect of nucleotides indicated that regulation of thyroid AMP deaminase may be dependent upon the nucleotide-guanine ratio rather than absolute concentration of nucleotides.

As Lowenstein suggested (1) AMP deaminase is one of the enzymes of the purine nucleotide cycle which, apart from playing an important role in the regulation and interconversion of nucleotides, may be the pathway of amino acid deamination. Though such a cycle has not been demonstrated in the thyroid, certain facts seem to indicate the occurrence of such a cycle in that tissue, for example the presence of adenylosuccinic acid (21). The preliminary investigations we have carried out on the metabolism on aspartic acid in the thyroid suggests the participation of that amino acid in the purine nucleotide cycle. Studies on this problem are being continued.

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