SOME PROPERTIES OF ADENINE AMINOHYDROLASE FROM *Escherichia coli*

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There are several pathways of adenine metabolism in *Escherichia coli* cells. One pathway is the conversion of adenine into adenosine monophosphate (AMP) by means of the enzyme adenine-phosphoribosyl transferase (AMP-pyrophosphorylase). The second pathway of adenine metabolism is associated with its deamination by the enzyme adenase (adenine aminohydrolase), which catalyses the reaction of hydrolytic deamination of adenine with the formation of hypoxanthine and ammonia.

The first mention of adenine aminohydrolase activity in *E. coli* cells appeared in the publications of Lutwak-Mann [8], and later these observations were confirmed by other workers [4, 11]. Interest in this enzyme is not accidental, for it plays an important role in purine metabolism in various bacteria and yeasts.

The object of the present investigation was to study some properties of the adenine aminohydrolase in *E. coli* cells, for existing publications contain few data on the properties of this enzyme.

EXPERIMENTAL METHOD

The experiments were carried out on a biomass of an *E. coli* culture grown in a fermenter (Marubishi, Japan) on nutrient broth with glucose (1%) and adenine (10 mg/100 ml), pH 7.2-7.4. The inoculum, which was cultured overnight at 37°C in a flask under stationary conditions, was transferred to the fermenter, where the culture was grown for 4 h at 37°C with aeration. The biomass was harvested on a separator or by centrifugation, using the culture in the logarithmic phase of growth. The harvested biomass was washed first with distilled water, then twice with 0.025 M sodium-phosphate buffer, pH 7.0, and freeze-dried. The sonicated extract from the freeze-dried powder was used as the source of enzyme.

Experimental samples with a total volume of 2.5 ml contained 7.5 μmoles adenine, 0.1M sodium-phosphate buffer, pH 7.0, and the source of enzyme. The samples were incubated for 90 min. In the experiments to determine the substrate specificity of the adenase, aza-adenine, 6-chloropurine, 6-iodopurine, 6-mercaptopurine, 6-methylaminopurine, and 2,6-diaminopurine were added instead of adenine. It was shown previously that in the case of 2,6-diaminopurine, guanine is formed by the enzyme reaction, and this was determined by the method described previously [5]. Adenine aminohydrolase activity was determined by a spectrophotometric method [2], based on measurement of the optical density of the hypoxanthine thus formed, and by a colorimetric method based on measurement of the quantity of ammonia liberated by means of Nessler's reagent.

Specific activity of adenine aminohydrolase was expressed in micromoles hypoxanthine formed per milligram protein. Protein was determined by Lowry's method [9].

EXPERIMENTAL RESULTS

The results of the study of the substrate specificity of adenine aminohydrolase from *E. coli* are given in Table 1. The preferred substrates were adenine and 2,6-diaminopurine. 6-Mercaptopurine was not a substrate. The data in Table 1 show that adenase has broad specificity and acts on many compounds with structural similarity. In each case the enzyme removes the group substituted in position 6 of the purine ring, i.e., the enzyme can remove chlorine, iodine, and methyl groups and replace them by hydroxyl groups.

A study of the effect of metals on adenase activity (Table 2) showed that Cd^{++}, Co^{++}, Cu^{++}, and Al^{+++} inhibit enzyme activity; a marked inhibitory action was observed when these cations were added to the incubation sample in the form of chlorides (except Hg^{++}).

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TABLE 1. Substrate Specificity of Adenase

Substrate	Specific adenase activity, μmoles hypoxanthine formed per mg protein	Activity, %
Adenine	0,113	100
2,6-Diaminopurine*	0,06	53,1
6-Iodopurine	0,035	30,9
Aza-adenine	0,023	20,3
6-Chloropurine	0,025	22,1
6-Methylaminopurine	0,016	14,1
6-Mercaptopurine	Not decomposed	Not decomposed

*Specific activity in μmoles guanine formed per mg protein.

TABLE 2. Effect of Metallic Ions on Adenase Activity

Cation 0.75 μM	Inhibition, %	
	chlorides	sulfates
Cd^{2+}	100	100
Co^{2+}	100	73,8
Cu^{2+}	100	64
Al^{3+}	100	—
Mn^{2+}	0	28
Mg^{2+}	0	0
Ba^{2+}	0	—
Ca^{2+}	0	—
Hg^{2+}	25,7	100

Legend. 0) Not inhibited.

The experiments also showed that adenase from *E. coli* is stable on keeping. The freeze-dried powder can be kept for several months at −4°C without loss of adenase activity. Heat treatment of the adenase for 15 min at 60, 70, and 80°C led to inactivation of the enzyme. When the enzyme preparation was heated for 15 min at 50°C activity was reduced by 50%, but when heated for 5 min it was inhibited by only 20%.

A previous study showed that exogenous adenine induces adenase formation in yeast [10]. Adaptive growth on adenine has also been observed in the case of *Salmonella typhimurium* [12]. However, in the case of adenase from *Pseudomonas aeruginosa* and *Ps. testosteroni,* adenine inhibits cell growth [1, 3].

The present experiments with adenase from *E. coli* showed that addition of adenine and its analogs, such as aza-adenine, 6-chloropurine, and 2,6-diaminopurine, to the growth medium intensified cell growth and increased adenase activity by 30, 43, 29, and 83% respectively, whereas 6-iodopurine inhibited enzyme activity.

The experiments thus showed that adenase from *E. coli* has wide substrate specificity, just as in the case of other bacterial and yeast adenases [5-7, 13], and like the other bacterial adenases it has a pH-optimum between 6.5 and 8.0, it is stable on keeping, and is inhibited by Cd^{++}, Co^{++}, Cu^{++}, and Al^{+++}; after disintegration of the cell culture all the adenase was found in the soluble fraction separable by centrifugation.

LITERATURE CITED

1. G. P. A. Bongaerts, J. L. Sin, A. L. Petters, et al., Biochim. Biophys. Acta, 499, 111 (1977).
2. H. M. Calckar, J. Biol. Chem., 167, 429 (1947).
3. N. Franke and G. H. Hahn, Z. Physiol. Chem., 301, 90 (1955).
4. S. Friedman and J. S. Gots, J. Biol. Chem., 201, 125 (1953).
5. J. Guern, M. Doree, J. Leguay, et al., C. R. Acad. Sci. (Paris), Ser. D., 275, 377 (1972).
6. R. C. Hartenstein and J. F. Fridovich, J. Biol. Chem., 242, 740 (1967).
7. R. G. Howell and J. F. Fridovich, J. Biol. Chem., 242, 493 (1967).
8. C. Lutwak-Mann, Biochem. J., 30, 1405 (1936).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. A. H. Roush, Arch. Biochem., 50, 510 (1954).
11. M. Stephenson and A. R. Trim, Biochem., J., 32, 1740 (1938).
12. V. Schatz, Naturwissenschaften, 51, 121 (1964).
13. Sakai Takuo Jun and Hong Ki, J. Ferment. Technol., 56, 257 (1978).