Degradation of 5' Adenosine Monophosphate in a Cell-Free System of Escherichia coli

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ABSTRACT. Sonicated cells of Escherichia coli contain an enzyme system degrading 5' adenosine monophosphate (5'AMP) to hypoxanthine. This enzyme system is located in the fraction sedimenting at 20,000 x g. It has a pH optimum at 8.0. In the fraction sedimenting at 20,000 x g the enzyme activity was inhibited by adenosine triphosphate (ATP). Adenosine and adenine are deaminated by this enzyme preparation to inosine and to hypoxanthine, these activities not being inhibited by ATP.

Recently the Ado-3', 5'-P was found to be split by a cyclic phosphodiesterase to 5' AMP in animal tissues (Butcher & Sutherland, 1962) and in Escherichia coli (Braná & Chytil, 1966). In our previous work the appearance of an unidentified compound in the whole homogenate of Escherichia coli was described. As this unidentified compound appeared always in the incubation mixture after the Ado-3', 5'-P had been split, it was assumed to be formed from the 5'AMP produced.

The identification of the unknown compound, the localization of the enzyme system degrading 5' AMP in Escherichia coli cells, and the effect of ATP on this enzyme system was the object of the present study.

MATERIALS AND METHODS

Cultivation of the microorganism and enzyme preparation. Escherichia coli strain C 600 K 12 was cultivated in a phosphate-yeast-glucose medium (Lehman et al., 1958) and treated as described in our previous work (Braná & Chytil, 1966). Cells grown overnight were centrifuged, washed in 0.02 M Tris buffer pH 8.1, resuspended in the Tris buffer and sonicated in a Raytheon 9 kc sonic vibrator for 3 min and then fractionated at 1, 200 x g and 20,000 x g. The whole sonic homogenate, the supernatant and the fraction sedimenting at 20,000 x g were resuspended in 0.02 M Tris buffer and used as enzyme preparations. All preparations were kept frozen at -20°C.

Assay of enzyme activity. The activity of the enzyme system degrading 5' AMP was determined by the method described in our previous work (Braná & Chytil, 1966). The substrate 5' AMP (final concentration 10^{-5} M), MgSO_4 (2 x 10^{-3} M) and the enzyme preparation were incubated at 30°C for the time required, usually 30—60 min. Samples were taken at zero time and at the times indicated, were deproteinized and chromatographed on Whatman No. 3 paper in isopropyl alcohol-ammonia-water (7:1:2). The spots were eluted with distilled water and the absorbance of eluates was measured on the Unicam SP 500 spectrophotometer at 260 and 250 nm.

In the solvent system used the R_F values were about 0.1 for 5' AMP, 0.45 for hypoxanthine and inosine, 0.53 for adenine, 0.59 for adenosine. The separation of
substrates and products was highly satisfactory.

Units of enzyme activity. Activity was expressed as μmoles of product formed in 1 min by 1 mg protein. The amount of the products was calculated using the appropriate extinction coefficients at 250 nm. Protein content was determined by the Folin assay (Lowry et al., 1951).

Isolation of the product of 5'AMP degradation. Enzyme preparation (40 ml), MgSO₄ (8 ml) and 5'AMP (16 ml) at the concentrations described above were incubated in a water bath at 30°C for one hour. The incubation mixture was deproteinized with 0.75 M HClO₄ in 50% ethanol and filtered through a Büchner funnel. The filtrate was adjusted to pH 7.0 with 40% KOH and filtered again. Then 50 ml of the sample were chromatographed in a 3 × 14 cm column of Dowex 2 × 8 (200 to 400 mesh, HCOO⁻ form). After the application of the sample the column was washed with 500 ml of distilled water and then eluted with 1 M HCOOH. The absorbancy of the effluent fraction was read at 260 nm. The fractions containing the absorbing compound were lyophilized and the dried powder was further analyzed.

Analysis of the compound. For the phosphorus content estimation the method of Lowry (Lowry & Lopez, 1946) was used. Before the assay each sample was hydrolyzed with a mixture of 10 n H₂SO₄ and 0.78 n HClO₄ in a sand-bath for 2 h. Ribose was determined by the orcinol method (Meijbaum, 1939). The purine component was identified after acid hydrolysis in 1 N HCl for 30 min at 100°C by paper chromatography, electrophoresis and UV spectrum. The solvent systems used for paper chromatography were isopropylalcohol-ammonia-water (7:1:2), n-butanol saturated with ammonia, and n-butanol-acetic acid-water (4:1:5). Electrophoresis was carried out in 0.02 M phosphate buffer at pH 7.1 and in 0.1 M citrate buffer at pH 3.0 on Whatman No. 1 paper at 400 V/cm.

RESULTS

Localization of the enzyme activity in Escherichia coli cells.

Table 1. Location of the enzyme activity degrading 5'AMP in a cell-free system of Escherichia coli

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Absorbancy at 260 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Nonfractionated sonic homogenate of E. coli cells; protein content 15 mg/ml</td>
<td>0.096</td>
</tr>
<tr>
<td>Supernatant fraction at 20,000 × g; protein content 9.8 mg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Sediment at 20,000 × g; protein content 10 mg/ml</td>
<td>0.255</td>
</tr>
</tbody>
</table>

Preparation of the fractions is described in the text. The sediment at 20,000 × g was resuspended after spinning in 40% of the initial buffer volume. The enzyme assay was performed at pH 8.1 at 30°C. Substrate concentration was 1 mM, magnesium ions at 2 × 10⁻³ M concentration were added in each experiment. The amount of the unidentified compound was estimated at 260 nm.

Fig. 1. Relationship between 5'AMP disappearance and the amount of product formed by the Escherichia coli sediment at 20,000 × g. Experimental conditions are described in Tab. 1. Curve 1 — absorbancy of the substrate; curve 2 — absorbancy of the product. Both absorbing compounds were read at 260 nm.