ENZYMIC FORMATION OF DEOXYADENOSINE 3',5'-PHOSPHATE[1]

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The wide-spread occurrence as well as the multiple biological functions of adenosine 3',5'-phosphate (cyclic 3',5'-AMP) has been well established (Sutherland, 1962). However, the presence of the deoxy-analogue of this cyclic nucleotide in nature has not so far been described.

In the preceding report, a partially purified enzyme obtained from the extracts of Brevibacterium liquefaciens was shown to catalyze the formation of adenosine 3',5'-phosphate from adenosine triphosphate in the presence of magnesium ion and pyruvate (Hirata and Hayaishi, 1965). Further studies have revealed that the enzyme preparation also catalyzes the conversion of deoxyadenosine triphosphate to deoxyadenosine 3',5'-phosphate, although GTP, ITP, UTP, CTP, TTP, dGTP, dUTP, and dCTP did not serve as substrate to any detectable extent under the conditions employed. With a

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partially purified enzyme preparation, both deoxyadenosine- and adenosine triphosphates were found to be utilized as substrate at approximately the same rate.

Cells of B. liquefaciens were grown and the extracts were prepared as previously described (Hirata and Hayaishi, 1965). The enzyme was purified about 50-fold by the treatment with hydroxylapatite, followed by chromatography on a DEAE-Sephadex column[3/]. Assay of enzyme activity was carried out with the use of adenosine triphosphate as substrate through the steps of enzyme purification.

The incubation mixture (2.0 ml), containing 6 μmoles of deoxyadenosine triphosphate[4/], 20 μmoles of lithium pyruvate, 200 μmoles of $MgSO_4$, 200 μmoles of Tris-HCl, pH 9.0, and 240 μg of the enzyme protein, was incubated for 60 minutes at 33°. After the reaction was terminated by the addition of 0.5 ml of N HCl, the mixture was passed through a charcoal column (1.0 x 0.9 cm). The reaction products were then eluted from the column with 0.5 N NH_4OH-ethanol (1:1) and the eluate was concentrated under reduced pressure at 30°. When an aliquot of the solution was chromatographed on Whatman No. 3 MM filter paper with 1 M ammonium acetate-ethanol (30:75) as the solvent, a new ultraviolet absorbing compound (S) was detected (Fig. 1). The R_F values of the product were the same as those of authentic deoxyadenosine 3',5'-phosphate synthesized chemically (Drummond et al., 1964) in four solvent systems (Table I). The yield of deoxyadenosine 3',5'-phosphate recovered from paper chromatogram was approximately 2 μmoles as estimated by the ultra-

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3/ Details of the purification procedure will be published elsewhere.

4/ A product of the Sigma Chemical Company. It was found to be practically free of adenosine triphosphate as examined by paper chromatography using saturated ammonium sulfate-1 M sodium acetate-iso-propanol (80:18:2). R_F values are: adenosine triphosphate 0.45, deoxyadenosine triphosphate 0.35.

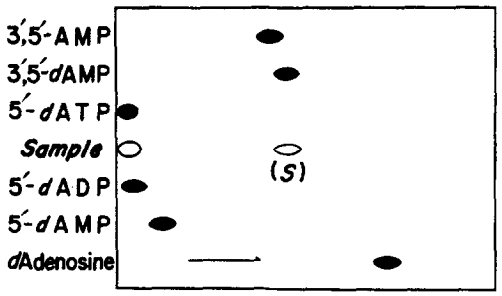

Fig. 1. Ascending paper chromatography was carried out on Whatman No. 3 MM filter paper with 1 M ammonium acetate-ethanol (30:75) as the solvent for 20 hours at room temperature.

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violet absorbance at 260 mμ (Drummond et al., 1964). The ultraviolet absorption spectra of the compound (S) were essentially identical with those of adenosine 3',5'-phosphate at pH 7.0 and 2.5. When it was subjected to acid treatment for 60 minutes at 50° in N HCl, no detectable hydrolysis was observed, while equimolar amounts of adenine and orthophosphate were produced when treated for 60 minutes at 100° in a sealed tube.

The identity was further confirmed by the behavior of the product (S) in the enzymatic digestion processes: the treatment of the compound with a rabbit brain 3',5'-cyclic nucleotide phosphodiesterase (Drummond and Perrott-Yee, 1961) resulted in the formation of deoxyadenosine 5'-phosphate, and subsequent digestion of the latter with a snake venom phosphomonoesterase produced deoxyadenosine. These adenine derivatives thus produced were identified by paper chromatography developed with four solvent systems listed in Table I. Deoxyadenosine 5'-phosphate and adenosine 5'-phosphate were separated on paper with 1.2 M ammonium acetate-ethanol-NH_4OH (16:50:4) containing 0.16 M sodium borate; R_F values were 0.15 and 0.02, respectively.

Another set of experiment was carried out to investigate the

stoichiometry and to see if pyruvate was metabolized during the reaction. When 3.2 µmoles of deoxyadenosine triphosphate, 0.8 µmole of lithium pyruvate, 40 µmoles of $MgSO_4$, 40 µmoles of Tris-HCl (pH 9.0) and 150 µg of enzyme protein, in 0.4 ml were incubated for 60 minutes at 33°, 0.65 µmole of deoxyadenosine 3',5'-phosphate[5/] and 0.66 µmole of pyrophosphate[6/] were produced. During the course of incubation, about 0.04 µmole of pyruvate disappeared, as determined by the enzymatic procedure (Kornberg, 1955), both in the presence and absence of deoxyadenosine triphosphate.

Table I

R_F values of nucleotides and related compounds

Paper chromatography was conducted by the ascending technique at room temperature on Whatman No. 3 MM filter paper for 20 hours,

	Solvent Systems			
Compound	I	II	III	IV
dATP	0.15	0.06	0.03	0.35
dADP	0.18	0.08	0.05	0.29
dAMP	0.33	0.14	0.12	0.25
d-adenosine	0.66	0.70	0.75	0.11
3',5'-dAMP	0.31	0.53	0.45	0.09
compound (S)	0.31	0.53	0.45	0.09
3',5'-AMP	0.27	0.50	0.41	0.12

I. n-butanol-acetate-water (5:2:3), II. iso-propanol-ammonia-water (7:1:2), III. ethanol-1 M ammonium acetate (75:30), IV. saturated ammonium sulfate-1 M sodium acetate-iso-propanol (80:18:2).

5/ Deoxyadenosine triphosphate and deoxyadenosine 3',5'-phosphate were separated on a Dowex 1- x 8 column (0.9 x 4.0 cm) by a gradient between 0.05 N LiCl and 0.25 N LiCl in 0.003 N HCl as the eluting system, and were determined by the absorbance at 260 mµ.

6/ Pyrophosphate was determined by measuring the orthophosphate according to Fiske and SubbaRow (1925) after hydrolysis with yeast pyrophosphatase (Heppel and Hilmoe, 1951).

Although we have not as yet isolated deoxyadenosine 3',5'-phosphate from this microorganism, the present findings suggest that the deoxy-analogue of cyclic 3',5'-AMP may occur in nature. Work in this area, as well as the elucidation of physiological significance of this phenomenon is currently in progress.

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