Notes

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Yasushi Abiko and Masao Shimizu : Investigations on Pantothenic Acid and Its Related Compounds. VIII. Biochemical Studies. (3).*1 Growth Response of *Lactobacillus arabinosus* 17-5 to D-Pantothenic Acid 4′-Phosphate.

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D-Pantothenic acid 4′-phosphate (P-PaA) chemically synthesized in the preceding paper[1] was found to be partially active for the growth of *Lactobacillus arabinosus* 17-5 for which P-PaA had been reported to be inactive.[2,3] In the course of investigations on the biosynthetic pathway of coenzyme A in our laboratory, the product which was formed from D-pantothenic acid (PaA) by the catalysis of rat-liver pantothenate kinase (EC 2.7.1.33) in the presence of ATP was found to be apparently inactive for this organism. The enzymatically phosphorylated product of PaA was reported to be identical with D-pantothenic acid 4′-phosphate.[3,4] The present report deals with the investigation with the aim to clarify these discrepancies of the microbiological activity of P-PaA.

Results and Discussion

Microbiological activity of P-PaA was checked with *Lactobacillus arabinosus* 17-5 (ATCC 8014) as a test organism. The basal culture medium which was obtained from Nissui Seiyaku Co., Ltd. (Tokyo) contained, in double strength, the following substances in 1 liter : casamino acid, 10 g.; L-cystine, 400 mg.; DL-tryptophane, 200 mg.; adenine

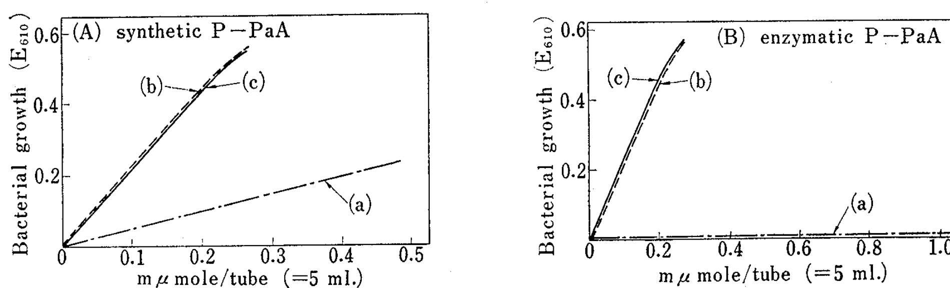

Fig. 1. Responses of *L. arabinosus* 17-5 to Chemically and Enzymatically Prepared D-Pantothenic Acid 4′-Phosphates

L. arabinosus 17-5 was grown on the basal medium supplemented with synthetic P-PaA or enzymatic P-PaA (curves a), or with equimolar amount of phosphatase-digests of them (curves b) at 37° for 17 hours. The D-pantothenate-supplemented medium was used as a control run (curves c).

*1 Part (2). J. Biochem., **61**, 10 (1967)

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sulfate, 20 mg.; guanine hydrochloride, 20 mg.; uracil, 20 mg.; vitamin B_1, 200 μg.; vitamin B_2, 400 μg.; biotin, 0.8 μg.; *p*-aminobenzoic acid, 200 μg.; nicotinic acid, 400 μg.; vitamin B_6, 800 μg.; potassium dihydrogen phosphate, 1 g.; disodium hydrogen phosphate 1 g.; magnesium sulfate 400 mg.; ferrous sulfate 20 mg.; manganese sulfate 20 mg.; sodium acetate, 20 g.; glucose, 40 g. Pantothenate kinase was prepared from rat liver according to the method described elsewhere.[5] Calf intestinal alkaline phosphatase (EC 3.1.3.1) was obtained from Boehringer & Soehne GmbH, Mannheim, Germany.

P-PaA chemically synthesized in our laboratory was partially active for the growth of *L. arabinosus* 17-5 and released the theoretical amount of PaA after digestion with intestinal phosphatase (Fig. 1-A), while the enzymatically prepared P-PaA was practically inactive for this organism before phosphatase digestion and became fully active after phosphatase digestion (Fig. 1-B). The enzymatic P-PaA was prepared by incubating 0.2 μmole of PaA with 10 μmoles of ATP, 5 μmoles of magnesium chloride and 5 units[5] of rat-liver pantothenate kinase in a total volume of 1.0 ml. of 0.04*M* Tris buffer at pH 7.5 and 37° for 1 hour. The reaction mixture was heated in a boiling water bath for 2 minutes, cooled and centrifuged to remove denatured protein precipitate. The resulting supernatant solution was used as a enzymatic P-PaA solution.

The activity of P-PaA was not constant and was found to vary ranging from 20% to 70% of the activity of equimolar amount of PaA. It was evidenced with the aid of bioautography that the growth-promoting activity of synthetic P-PaA for *L. arabinosus* 17-5 was not due to contamination of PaA in the preparation. The synthetic P-PaA was spotted on Toyo Roshi No. 51-A paper and ascendingly developed in the solvent system of *n*-propanol-ammonium hydroxide-water (6:3:1, v/v) for 17 hours. After air-dried the paper was cut to pieces, which were then separately extracted with water at 100° for 10 minutes. The extract from each paper strip were assayed for the growth-promoting activity for *L. arabinosus* 17-5. The extract from the strip corresponding to the ninhydrin and Hanes-Isherwood[6] reagent positive area (Rf 0.35) was only one that was active for this organism, excluding contamination of PaA which had a Rf value of 0.64 in this system.

Table I. Effects of ATP and Other Substances on the Synthetic D-Pantothenic Acid 4′-Phosphate-dependent Growth of *L. arabinosus* 17-5

Addition	Bacterial growth (E_{610}) in the presence of synthetic P-PaA at the concentration of		
	(A) 4.5 mμmoles/tube	(B) 0.18 mμmole/tube	
			after phosphatase
None	1.20	0.195	0.370
Tris	1.15	—	—
Tris, $MgCl_2$	1.17	—	—
Tris, ATP	0.322	—	—
Tris, $MgCl_2$, ATP	0.295	0.0	0.360
ATP	—	0.0	0.365

L. arabinosus 17-5 was grown on the basal medium supplemented (A) with synthetic P-PaA (4.5 mμmoles/tube) together with or without ATP 0.1 μmole, $MgCl_2$ 0.05 μmole and Tris 2.5 μmoles; and (B) with synthetic P-PaA (0.18 mμmole/tube) together with or without ATP 4 mμmoles, $MgCl_2$ 2 mμmoles and Tris 0.1 μmole at 37° for 17 hours.

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The difference observed between the synthetic compound and enzymatic P–PaA seemed to be caused by the differences in the culture condition. In the experiments demonstrated in Fig. 1, enzymatic P–PaA was checked for its activity in the presence of residual ATP and other materials which were used for the enzymatic phosphorylation of PaA and remained in the enzymatic P–PaA solution tested. When the effects of the components of the enzymatic P–PaA solution on the activity of synthetic P–PaA for the test organism was examined, it was found that ATP decreased the activity of synthetic P–PaA for *L. arabinosus* 17–5 (Table I) and that this effect of ATP was dependent on its concentration (Fig. 2).

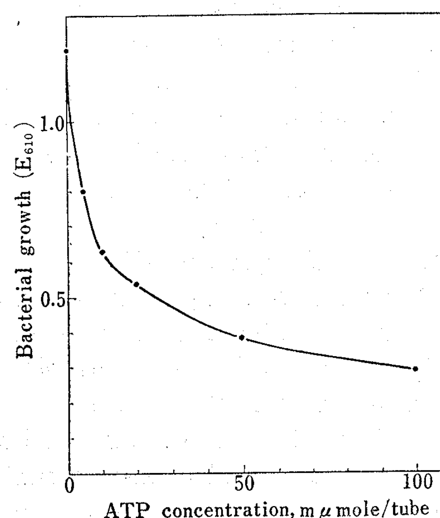

Fig. 2. Effect of ATP Concentration on the D–Pantothenic Acid 4′–Phosphate–dependent Growth of *L. arabinosus* 17–5

L. arabinosus 17–5 was grown on the basal medium supplemented with synthetic P–PaA (4.5 mμmoles/tube) and various amount of ATP at 37° for 17 hours.

On the other hand, enzymatically prepared P–PaA, which was identified with the synthetic compound paper chromatographically, was found to become partially active for *L. arabinosus* 17–5, when it was checked for its activity after separation from contaminating ATP with the aid of paper chromatography in the system of *n*-butanol–acetic acid–water (5:2:3, v/v; Rf 0.53) or ethanol–0.5*M* ammonium acetate, pH 3.8 (5:2, v/v; Rf 0.59).

From these findings, it was concluded that P–PaA was partially active for *L. arabinosus* 17–5 in the culture medium used here and that the activity was inactivated by the presence of ATP. The mechanism by which ATP inhibited apparently the activity of P–PaA for this organism remained unexplained. It has been often observed that the nature of microorganisms varied dependently on the culture condition employed. It would be, therefore, speculated that the difference between the microbiological activity of P–PaA mentioned here and that reported by other investigators[3,4] was probably due to the difference in the composition of the culture medium used. Explanation of these problems awaits further detailed investigation on this organism.

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