Notes

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Investigations on Pantothenic Acid and Its Related Compounds. XVII.¹⁾
Biochemical Studies. (10). Further Studies on the Metabolism
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Fig. 1. Enzymatic Oxidation of Pantothenyl Alcohol and Pantothenyl Alcohol 4'-Phosphate by Rat-Liver Alcohol Dehydrogenase

Pantothenyl alcohol or pantothenyl alcohol 4'-phosphate was incubated with rat-liver alcohol dehydrogenase in the presence of NAD at pH 10.0 at 24°. Oxidation of the substrate was presented by the increase in the absorbancy at 340 mμ caused by reduction of NAD.

○ : pantothenyl alcohol
● : pantothenyl alcohol 4'-phosphate

Previous report¹⁾ from our laboratory demonstrated the enzymatic oxidation of pantothenyl alcohol (D-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutylamide, an alcohol analog corresponding to pantothenic acid) to pantothenic acid using a rat-liver extract and identity of the enzyme responsible to the first step of this oxidation with liver alcohol dehydrogenase (EC 1.1.1.1). On the other hand, rat-liver pantothenic acid kinase (EC 2.7.1.33) was demonstrated to possess relatively broad specificity for its substrate: pantothenic acid,³⁻⁵⁾ panteth(e)ine^{4,6)} and pantothenoylcyst(e)ine.⁴⁾ These findings prompted us to examine another possible metabolic route of pantothenylalcohol as a precursor of coenzyme A in rat liver. Can pantothenyl alcohol be phosphorylated by rat-liver pantothenic acid kinase? And can the phosphorylated alcohol, if formed, be oxidized by rat-liver alcohol dehydrogenase? The present report deals with the examination of these possibilities.

Partially purified pantothenic acid kinase (specific activity: 0.56) was prepared from rat liver as described previously.⁴⁾ Rat-liver alcohol dehydrogenase was purified (Sephadex G-150 gel-filtered fraction, specific activity: 8.17) according to the method reported elsewhere.⁷⁾ D-Pantothenyl alcohol 4'-phosphate was synthesized in our laboratory, the synthesis of which will be reported in the following paper.⁸⁾

- 1) Part XVI: Y. Abiko, M. Tomikawa, and M. Shimizu, *J. Vitaminol.* (Kyoto), in press.
- 2) Location: No. 2810, Minamifunabori-cho, Edogawa-ku, Tokyo.
- 3) G.M. Brown, *J. Biol. Chem.*, **234**, 370 (1959).
- 4) Y. Abiko, *J. Biochem.* (Tokyo), **61**, 290 (1967).
- 5) Y. Abiko and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **15**, 884 (1967).
- 6) G.M. Brown and J.J. Reynold, *Ann. Rev. Biochem.*, **32**, 419 (1963).
- 7) M. Tomikawa and Y. Abiko, *J. Biochem.* (Tokyo), in press.
- 8) Y. Hosokawa, M. Tomikawa, O. Nagase, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **17**, 202 (1969).

Four micromoles of pantothenyl alcohol was incubated with 3.36 units of pantothenic acid kinase, 60 μ moles of ATP, 20 μ moles of MgSO_4 , and 160 μ moles of tris(hydroxymethyl)-aminomethane in a total volume of 4 ml at pH 7.4 at 37° for 2 hours. The reaction mixture was heated at 100° for 1 minute to stop the reaction and centrifuged. The resultant supernatant (3.9 ml) was passed through a column of Amberlite IR 120 (H^+). The effluent and washings were combined, adjusted to pH 7.2 with 0.1M $\text{Ba}(\text{OH})_2$ and centrifuged. The supernatant was evaporated to dryness *in vacuo*. The residue was dissolved with a small volume of water and the insoluble material was centrifuged off. The supernatant solution was evaporated and the residue was again dissolved with a smaller volume of water. These operations were repeated three times until a clear solution was obtained. The final solution was passed through a column of Amberlite IR 120 (H^+). The effluent and washings were combined and evaporated. The residue was dissolved in a small volume of ethanol and subjected to paper chromatography. The phosphorylated product was identified with pantothenyl alcohol 4'-phosphate by ascending chromatographies (R_f 0.40 in the solvent system of *n*-propanol-ammonia-water, 6:3:1, and R_f 0.45 in the system of *n*-butanol-acetic acid-water, 5:2:3) and by the descending chromatography in the system of pyridine-*n*-butanol-water-ammonia (70:10:20:1) which distinguished pantothenylalcohol 4'-phosphate from pantothenyl alcohol 3''-phosphate.⁸⁾ Enzymatic phosphorylation of pantothenic acid by the kinase was found to be inhibited competitively by pantothenyl alcohol ($K_i=5.71 \times 10^{-5}\text{M}$), indicating that pantothenic acid and pantothenyl alcohol were competitive substrates of the kinase.

Pantothenyl alcohol 4'-phosphate was examined on its oxidation by rat-liver alcohol dehydrogenase in comparison with pantothenylalcohol. The reaction mixture contained 0.2 ml of 1M pantothenyl alcohol 4'-phosphate or pantothenyl alcohol, 0.1 ml of $1.5 \times 10^{-2}\text{M}$ NAD, 3 ml of 0.1M glycine-NaOH buffer (pH 10.0) containing 0.075% of semicarbazide hydrochloride and 0.2 ml (6.3 units) of rat-liver alcohol dehydrogenase. The increase in the absorbancy at 340 $m\mu$ was recorded at 24°. As shown in Fig. 1, pantothenyl alcohol 4'-phosphate could not be the substrate of the dehydrogenase, while pantothenyl alcohol was rapidly oxidized by this enzyme. Pantothenyl alcohol 4'-phosphate slightly inhibited the oxidation of pantothenyl alcohol by the dehydrogenase in non-competitive manner ($K_i=1.43 \times 10^{-2}\text{M}$).

These results may lead to the conclusion that the presumptive metabolic route of pantothenyl alcohol to coenzyme A mentioned above does not occur because of impossibility of the oxidative conversion of pantothenyl alcohol 4'-phosphate to the corresponding acid.

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