Notes

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Investigations on Pantothenic Acid and Its Related Compounds. XVII.[1] Biochemical Studies. (10). Further Studies on the Metabolism of Pantothenylalcohol in Rat Liver

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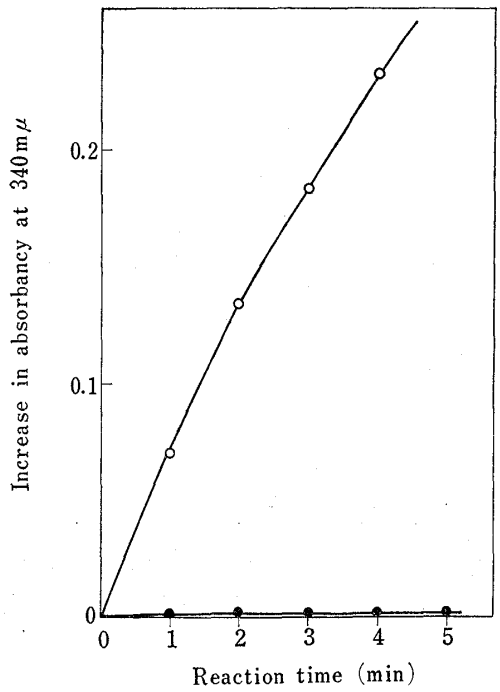

Fig. 1. Enzymatic Oxidation of Pantothenyl Alcohol and Pantothenyl Alcohol 4′-Phosphate by Rat-Liver Alcohol Dehydrogenase

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.

○ : panthothenyl alcohol
● : pantothenyl alcohol 4′-phosphate

Previous report[1] 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 μmoles of ATP, 20 μmoles of $MgSO_4$, and 160 μmoles 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 0.1 M $Ba(OH)_2$ 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 (*Rf* 0.40 in the solvent system of *n*-propanol–ammonia–water, 6:3:1, and *Rf* 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 ($K_i = 5.71 \times 10^{-5}$ 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 1 M pantothenyl alcohol 4′-phosphate or pantothenyl alcohol, 0.1 ml of 1.5×10^{-2} M NAD, 3 ml of 0.1 M 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 mμ 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 ($K_i = 1.43 \times 10^{-2}$ 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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