S-PALMITYL PANTETHEINE AS AN INTERMEDIATE IN THE METABOLISM OF PALMITYL COENZYME A BY RAT LIVER PLASMA MEMBRANE PREPARATIONS

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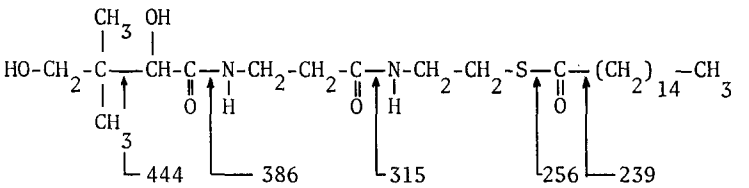
During a study on the biosynthesis of complex lipids in plasma membrane preparations, we observed that a substantial portion of palmityl-^{14}C coenzyme A was converted to a novel metabolic intermediate which we have identified as S-palmityl pantetheine. In the present communication we would like to describe the enzymatic formation, isolation and characterization of palmityl pantetheine.

Rat liver plasma membranes were prepared from liver homogenates according to Neville (1967). Palmityl-1-^{14}C coenzyme A was synthesized enzymatically (Kornberg and Pricer, 1953). From 16 to 18 mg of isolated plasma membranes were incubated with 1.05 μmoles of palmityl-1-^{14}C coenzyme A (2.5 mc/mmole) in 0.1 M Tris buffer, pH 7.4, for 30 minutes at 37°C in a volume of 6.8 ml. The reaction was terminated by the addition of 70 ml of chloroform:methanol (CM, 2:1; v/v) and the aqueous phase removed after centrifugation. The $CHCl_3$ phase was washed once with 35 ml of water and sufficient methanol was added to clear the solution. The extract was then taken to dryness in a flash evaporator and dried over P_2O_5 for several hours. The lipids were dissolved in chloroform and placed on 1 x 10 cm column of silicic acid (Unisil, 200-325 mesh) which had been equilibrated with dry chloroform. The free fatty acids and neutral glycerides were eluted with 100 ml of

$CHCL_3$ which was followed by 200 ml of $CHCl_3$:methanol (CM, 98:2; v/v) which eluted the pantethenyl derivative. This eluate was evaporated and redissolved in 1.5 ml $CHCl_3$. The lipids in this fraction contained from 25 to 35 per cent of the radioactivity which had been added to the incubation mixture in the form of palmityl-^{14}C coenzyme A.

Thin layer chromatography in a variety of solvent systems indicated the presence of only one radioactive compound, but at least seven other lipids could be visualized with iodine vapors. Further purification of the radioactive product was achieved by repeated preparative thin layer chromatography (TLC) on silica gel G plates (Analtech) which were developed in CM 9:1. The radioactive material was confined to an area whose R_f was between 0.45 and 0.55; it was eluted with 4% methanol in $CHCl_3$. Recovery of the radioactive material from preparative TLC was poor and losses were as great as 50 per cent.

The eluate from the final TLC was evaporated and dissolved in hot n-pentane. Upon standing in the cold, a flaky, colorless, semicrystalline material separated out which was recovered by centrifugation, dried in a vacuum desiccator, and then stored in the cold under N_2. Chemical analysis indicated the presence of an ester by the method of Rapport and Alonzo (1955) and the acid (^{14}C) to ester ratio was unity. Chromatography of the hydroxamic acid (Trams, 1968) indicated the presence of only ^{14}C-palmitylhydroxamic acid. Microdeterminations for phosphorus were negative. The presence of nitrogen was shown by the method of Sloane-Stanley (1967). The material exhibited a single absorption maximum at 229 mμ when dissolved in n-pentane. Infrared spectroscopy, using KBr micropellets indicated the presence of an ester (1685 cm^{-1}), amides (1638 and 1530 cm^{-1}), $-CH_2-$, -OH and $-CH_3$ groups. Low resolution mass spectrometry yielded an apparent parent ion with the mass of 514 (palmityl pantetheine M = 516) and mass fragments which are consistent with the S-palmityl pantetheine structure (70, 148, 175, 239, 256, 315, 386 and 444).



Palmityl pantetheine was synthesized chemically from D-pantethine (Sigma) and palmityl chloride and the chromatographic properties were compared with the material obtained from the incubation mixtures. The R_f values in several solvent systems, and the elution patterns from silicic acid columns were identical in all respects for the two compounds.

The sum of these observations led us to conclude that the radioactive product obtained by incubation of membrane fractions with palmityl-^{14}C coenzyme A was S-palmityl pantetheine. Although the S-acyl derivatives of pantetheine (Stern, 1955 and Lynen et al., 1956) have been used in the past as substitutes for acyl coenzyme A esters in various reactions, there has been no reference to the enzymatic formation of long chain acyl pantetheines. Novelli, Kaplan and Lipman (1949) described the liberation of pantothenic acid from coenzyme A by a combination of an intestinal phosphatase with a liver enzyme preparation. Wolff, Dubost and Brignon (1957) have reported the formation of pantetheine from coenzyme A by calf intestinal mucosal phosphatase, but they stated that their preparation was devoid of any 'pantothenic conjugates splitting activity' other than coenzyme A.

We have concluded that the enzymatic formation of S-acyl pantetheine may be characteristic for membrane preparations, since this material was not formed by incubation with other subcellular fractions or by treatment with a variety of snake venom phosphoesterases. Our present information indicates that we are dealing either with a nucleotide pyrophosphatase or with a phosphatase or with a combination of the two. Details of the reactions involved and the possible significance of S-acyl pantetheines in lipid transport through membranes will be discussed in full elsewhere.

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