Note

[Agr. Biol. Chem., Vol. 28, No. 11, p. 807~808, 1964]

Studies on Conjugated Lipids

Part XVIII.* Enzymatic Synthesis of the Sphingosine Base

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Received June 18, 1964

Recent studies¹⁻⁴⁾ show that serine and the 16-carbon fragment, palmityl CoA or palmitic aldehyde, are the two precursors in the enzymatic synthesis of the animal sphingosine base, sphingosine and dihydrosphingosine. The present paper describes that the C₁₆ unsaturated aldehyde is also active as the precursor of the sphingosine base.

Labeled serine, palmitic aldehyde and the microsomal fraction of rat brain were mixed,²⁾ and free sphingosine and dihydrosphingosine formed during incubation were separately isolated by the thin-layer chromatographic technique as previously reported.⁴⁾ Radioactivity of the respective sphingosine base being periodically measured, sphingosine was observed to appear prior to dihydrosphingosine, as shown in Fig. 1, in contradiction to the view from the other laboratory¹⁾ where isolation of the sphingosine base was carried out by the paper-chromatographic procedure which was found to be inadequate for separation of sphingosine from dihydrosphingosine.⁵⁾

Each tube contained 150 μ moles of phosphate buffer (pH 7.8), 6 μ moles of MgSO₄, 0.5 μ mole

FIG. 1. Time Course of Enzymatic Formation of Sphingosine and Dihydrosphingosine.

of MnCl₂, 20 μ moles of Nicotinamide, 2 μ moles of erythro sphingosine, 1 μ mole of pyridoxal phosphate, 2.3 mg of Tween 20, 2 μ moles of DL-serine-3-C¹⁴ (500,000 counts/min/ μ mole), 0.3 μ mole of palmitic aldehyde, and 1 ml of microsomal fraction sedimented between 20,000 $\times$ g and 100,000 $\times$ g from 20% homogenate of 15~20 days-old rat brain. The final volume was 2 ml. Tubes were incubated at 37°C.

Since sphingosine was initially formed without an addition of unsaturated precursor to the reaction mixture, it was conceived that a partial

* Part XVII, Y. Fujino, S. Ito and T. Negishi, Research Bulletin of Obihiro Zootechnical University, in preparation.

1) R.O. Brady and G.J. Koval, *J. Biol. Chem.*, **233**, 26 (1958).

2) R.O. Brady, J.V. Formica and G.J. Koval, *ibid.*, **233**, 1072 (1958).

3) Y. Fujino and I. Zabin, *This Journal*, **26**, 267 (1962).

4) Y. Fujino and I. Zabin, *J. Biol. Chem.*, **237**, 2069 (1962).

5) S. Ito, Y. Fujino and T. Negishi, A communication presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Sapporo, July 19~21, 1964.

TABLE I. REACTIVITY OF 2-HEXADECENAL AS
SUBSTRATE

Incubation system	Radioactivity of sphingosine base
Complete	520 cpm
Complete, —palmitic aldehyde	20
Complete, —palmitic aldehyde, +2-hexadecenal	580

The complete incubation system was similar to that shown in Fig. 1. Tubes were incubated at 37°C for 2.5 hours.

unsaturation of palmitic aldehyde was involved in the course of reaction. Thus, reactivity as substrate of 2-hexadecenal, prepared⁶⁾ from

6) H.E. Carter, F.J. Glick, W.P. Norris and G.E. Phillips, *J. Biol. Chem.*, **170**, 285 (1947).

sphingosine by degradation with periodate, was tested in comparison with that of palmitic aldehyde, hexadecanal. Both aldehydes were detected to have about the same activity, the unsaturated aldehyde being rather active than the saturated one, as shown in Table I.

These results demonstrate that not only palmitic aldehyde but also 2-hexadecenal was responsible for the enzymatic synthesis of the sphingosine base as the long-chain precursor.