Utilization of different fatty acyl-CoA thioesters by serine palmitoyltransferase from rat brain

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Abstract Serine palmitoyltransferase (EC 2.3.1.50) catalyzes the first unique reaction of sphingolipid biosynthesis. Activities were determined with different fatty acyl-CoA substrates to describe the range of long-chain bases that could be made by rat brain microsomes. The activities were greatest with palmitoyl-CoA and palmitelaidoyl-CoA, followed by fully saturated homologs differing from these by only one carbon atom, and diminished considerably as the alkyl-chain length increased or decreased, or with the presence of a *cis*-double bond. These characteristics explain the predominance of long-chain bases with 18 carbon atoms in brain sphingolipids, and account for the minor variants such as the C_{17}- and C_{20}-long chain bases.—**Merrill, A. H., Jr. and R. D. Williams.** Utilization of different fatty acyl-CoA thioesters by serine palmitoyltransferase from rat brain. *J. Lipid Res.* 1984. **25:** 185–188.

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Serine palmitoyltransferase (EC 2.3.1.50) catalyzes the condensation of palmitoyl-CoA and L-serine to yield 3-ketosphinganine. This compound is the first unique and committed intermediate of sphingolipid biosynthesis. The 3-ketosphinganine is subsequently modified to provide the predominant long-chain bases of most brain sphingolipids: sphinganine, sphingosine, and 4-D-hydroxysphinganine (phytosphingosine). Various homologs of these compounds, such as the icosasphingosine of brain gangliosides, are also found and would be expected to be synthesized by this enzyme via the utilization of different fatty acyl-CoA's. Studies of this reaction using mouse brain microsomes (1) found activities with palmitoyl-CoA which were more than twice those obtained with stearoyl-CoA, and no activity was observed with oleoyl-CoA or lignoceroyl-CoA. A similar analysis with rat brain microsomes (2) reported activity only with palmitoyl-CoA. Since recent studies of serine palmitoyltransferase from *Bacteroides melaninogenicus* (3) and rat liver[2] have demonstrated activity with a broad range of fatty acyl-CoA thioesters, we have reinvestigated this phenomenon with rat brain microsomes to resolve these discrepancies.

EXPERIMENTAL

Chemicals

Radioactive [G-^{3}H]L-serine was purchased from ICN Radiochemicals and was routinely purified by chromatography on a column of Dowex 50W-X8, hydrogen form, from Bio-Rad Laboratories to remove a chloroform-soluble impurity, and diluted with unlabeled L-serine to a specific activity of 25 mCi/mmol. The [9,10-^{3}H(N)]palmitoyl-CoA was synthesized enzymatically (4) from radiolabeled palmitic acid (New England Nuclear, Boston, MA) which had been diluted to a specific activity of 30 mCi/mmol. Unlabeled palmitoyl-CoA, stearoyl-CoA, and oleoyl-CoA were purchased both from Sigma, St. Louis, MO and PL Biochemicals, Milwaukee, WI. The other fatty acyl-CoA's and biochemicals were obtained from Sigma. Econofluor was purchased from New England Nuclear; organic solvents were obtained from various suppliers and were reagent grade or better.

Animals

Female buffalo rats (80–99 g) were obtained from Harlan Industries, Walkerstown, MD. They were fed Purina chow ad libitum until 18 hr before their use for experimentation, when only water was provided. The animal quarters were maintained on a 12-hr cycle of light and dark beginning at 7:00 AM.

Preparation of rat brain microsomes

Fasted rats (150–225 g) were killed by decapitation between 8:00 and 10:00 AM and the brains were removed

Abbreviations and nomenclature: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; DTT, dithiothreitol. Serine palmitoyltransferase has also been called 3-ketodihydrosphingosine (or 3-ketosphinganine) synthetase. The names used here and the lipid nomenclature follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature.

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[2] Williams, R. D., E. Wang, and A. H. Merrill, Jr. 1984. *Arch. Biochem. Biophys.* **228.** In press.

and placed on ice. To the weighed brains was added 4 ml of 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (pH 7.4 at 4°C), 0.25 M sucrose, and 5 mM EDTA (pH adjusted to 7.4 with NaOH) per g of brain, and the suspension was homogenized by six strokes of a TenBroeck glass homogenizer. The homogenate was centrifuged for 15 min at 18,000 *g* and the resulting supernatant was centrifuged for 1 hr at 100,000 *g*. The microsomal pellet was resuspended in 50 mM HEPES (pH 7.4 at 4°C), 5 mM EDTA, and 5 mM dithiothreitol (DTT) at 2 ml per g of brain, and after homogenization, centrifuged at 100,000 *g* for 1 hr. The "washed" microsomes were resuspended in the same buffer containing 20% (w/v) glycerol at 0.5 ml per g of brain, and stored frozen in aliquots at −80°C. No loss of activity was observed with up to 6 months of storage.

Assay of serine palmitoyltransferase

Assays were conducted in a volume of 0.1 ml which contained (final concentrations are given): 0.1 M HEPES (pH 8.3 at 25°C), 5 mM DTT, 2.5 mM EDTA (pH 7.4), 50 μM pyridoxal 5'-phosphate, 1 mM [^{3}H]serine, 0.2 mM palmitoyl-CoA or other fatty acyl-CoA, and varying amounts of enzyme (20 to 100 μg). The reaction was initiated by adding the enzyme, and allowed to proceed for 10 min at 37°C. It was terminated by adding 0.1 ml of 0.5 N NH_4OH; 25 μg of sphinganine was added as carrier, and the radiolabeled products were recovered by organic solvent extraction (5). Until the extraction, all procedures were conducted in dim light. Half of the chloroform extract was transferred to a scintillation vial, evaporated to dryness, and the radioactivity was measured using Econofluor. The efficiency of extraction was found to be greater than 90%; the counting efficiency was approximately 40%.

Other procedures

Protein was determined by a modification of the Lowry method (6) with bovine serum albumin as the standard. The rate of palmitoyl-CoA hydrolysis during serine palmitoyltransferase assays was estimated by omitting [^{3}H]serine from the assay and substituting [^{3}H]palmitoyl-CoA. After varying time intervals, aliquots were added to an equal volume of 1% $HClO_4$ and applied to paper chromatograms (Whatman No. 1) which were developed with 1-butanol–acetic acid–water 5:2:3 (v/v/v). The [^{3}H]palmitate and [^{3}H]palmitoyl-CoA were identified by comparison with standards and were extracted from the paper and quantitated by liquid scintillation counting using Econofluor. From 62 to 77% of the initial radiolabel was recovered as these two compounds; the recovery of [^{3}H]palmitoyl-CoA when applied to the chromatograms alone was approximately 94%. The thioesterase activity was calculated from the rate of loss of [^{3}H]palmitoyl-CoA.

RESULTS AND DISCUSSION

Under the assay conditions described under Experimental, product formation was proportional to the reaction time for up to 15 min, and the rates with palmitoyl-CoA were proportional to the enzyme concentration over the entire range used. These conditions were also met when other fatty acyl-CoA's were used in assays containing 20–60 μg of microsomal protein. Radiolabel was found exclusively in the expected chloroform-soluble products, when analyzed by thin-layer chromatography (8), since the synthesis of the other serine-containing phospholipid, phosphatidylserine, requires divalent cations (2). Some radiolabel, due mainly to contaminants of the [^{3}H]serine, was found when assays were conducted without added fatty acyl-CoA. These cpm were subtracted from the cpm obtained with complete assay mixtures to correct for this background (this correction was insignificant, except with the poorest substrates).

The serine palmitoyltransferase activities obtained with different fatty acyl-CoA thioesters are shown in **Fig. 1.** Palmitoyl-CoA yielded the greatest activity, which was

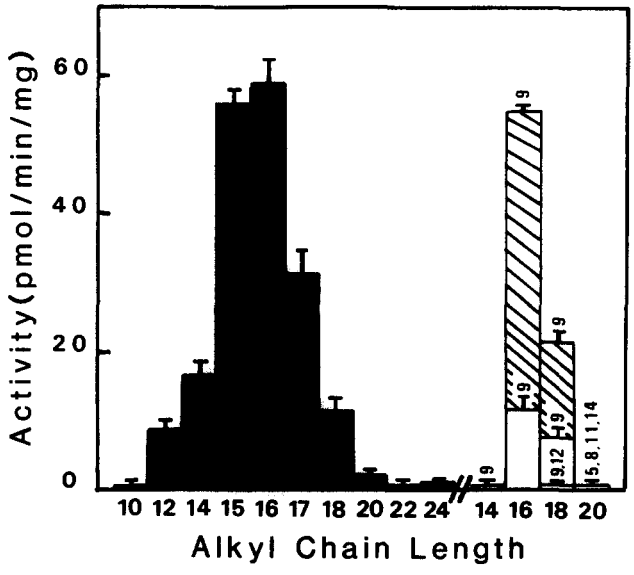

Fig. 1. Serine palmitoyltransferase activities with different fatty acyl-CoA thioesters. Assays were conducted as described under Experimental with fatty acyl-CoA's having the alkyl chain lengths shown by the numbers. Fully saturated fatty-acyl CoA's are grouped on the left; unsaturated fatty acyl-CoA's are indicated by numbers representing the positions of the double bond(s). All double bonds are *cis*, except for palmitelaidoyl-CoA, which is shown by a slashed bar over palmitoleoyl-CoA (C 16:1, *cis*-Δ^9), and elaidoyl-CoA, which is shown over oleoyl-CoA (C 18:1, *cis*-Δ^9).

somewhat higher than that exhibited with pentadecanoyl-CoA (C 15:0) or heptadecanoyl-CoA (C 17:0). The activities decreased progressively as the alkyl chain increased or decreased in length. Introduction of a single *cis*-double bond at position 9 (i.e., in palmitoleoyl-CoA) reduced the activity significantly compared to the fully saturated homolog. This effect was not due to the double bond per se, but rather to the steric perturbations created by the *cis*-double bond since the equivalent compound having a *trans*-double bond, palmitelaidoyl-CoA, resulted in activities similar to palmitoyl-CoA. Although activities were consistently lower for the *cis*-unsaturated fatty acyl-CoA's compared to the fully saturated counterparts, the maximal difference was seen with the palmitoyl-CoA versus palmitoleoyl-CoA pair. Similar relationships were observed when these substrates were examined at other fixed concentrations (i.e., 100 μM and 300 μM).

A more detailed kinetic analysis with this microsomal preparation is precluded by the micellar nature of the fatty acyl-CoA's (7) and by the presence of substantial thioesterase activity, which was found to be 9.8 nmol of palmitoyl-CoA hydrolyzed/min per mg of protein under these conditions. Nonetheless, the distribution of activities shown in Fig. 1 is remarkably similar to that obtained with rat liver microsomes,[2] and the partially purified serine palmitoyltransferase from *Bacteroides melaninogenicus* (3) and yeast (8). This consistency and the nearly symmetrical distribution of activities suggest that this enzyme interacts optimally with a fully saturated fatty acyl-CoA of 16 carbon atoms, and accommodates longer and shorter compounds less well. This view explains most of the results of less broad analyses of mouse (1) and rat (3) brain microsomes, except that these authors reported no detectable activity with oleoyl-CoA or stearoyl-CoA, respectively. These discrepancies are undoubtedly due to the greater sensitivity of our assay compared to the previous analyses, which required recovery of the products and their resolution and detection by thin-layer chromatography.

Most sphingolipids contain primarily long-chain bases of 18 carbon atoms, 16 derived from palmitoyl-CoA and 2 from serine, but smaller amounts of long-chain bases of 16 to 22 are carbons also present (9). Brain gangliosides are exceptional, however, because they contain substantial proportions of icosasphingosine and icosaphinganine, 20-carbon homologs, and the proportions of the C_{20}-long-chain bases increase with age (10, 11). The presence of the C_{20}-long-chain bases in brain gangliosides is apparently of functional importance since their physical properties differ from those for gangliosides containing the 18-carbon bases (12).

The range of substrates that were utilized by serine palmitoyltransferase, as well as the relative activities, was in excellent agreement with the long-chain bases found in most brain sphingolipids. This was most apparent in the activity with palmitoyl-CoA, but was equally substantiated by the high activities with pentadecanoyl-CoA, heptadecanoyl-CoA, and stearoyl-CoA. The relatively high activities with the fatty acyl-CoA's with odd carbon numbers correlates with the existence of appreciable amounts of the corresponding long-chain bases (9). Hence, it appears that the presence of these fatty acids in brain in very low amounts (13) is offset by their efficient utilization by serine palmitoyltransferase. This also underscores the potential for changes in the long-chain base composition in brain under conditions that alter the fatty acids, such as the increases in odd-numbered fatty acids found in vitamin B_{12} deficiencies (14).

The major exception was the facile utilization of oleoyl-CoA by the enzyme of rat brain microsomes. The expected long-chain bases, 20-carbon homologs of sphingosine and sphinganine with a double bond between carbons 11 and 12, have not been reported in analyses of sphingolipids, although they might constitute some of the unidentified species in such analyses (9–11).

The relative importance of the biosynthesis of the appropriate long-chain bases versus the selection among the available compounds by subsequent enzymes of sphingolipid biosynthesis is not yet known. The results described here suggest that the types of long-chain bases synthesized de novo closely approximate those found in brain sphingolipids. Whether or not this constitutes a cause and effect relationship, it represents an efficient regulation of the biosynthesis and utilization of these compounds.

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