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Kinetics of Long-Chain (Sphingoid) Base Biosynthesis in Intact LM Cells: Effects of Varying the Extracellular Concentrations of Serine and Fatty Acid Precursors of This Pathway†

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ABSTRACT: Serine palmitoyltransferase (EC 2.3.1.50) catalyzes the condensation of L-serine and palmitoyl-CoA to yield 3-ketosphinganine in the first unique reaction of long-chain (sphingoid) base biosynthesis. The kinetic effects of changing the extracellular concentrations of the precursors for this pathway were studied with LM cells by following the incorporation of L-[3-^{14}C]serine into the long-chain base (i.e., sphinganine and sphingenine) backbones of complex sphingolipids. [^{14}C]Serine was taken up by the cells and rapidly reached steady-state concentrations similar to those of the medium. From the cellular [^{14}C]serine concentrations and specific activities, the apparent V_{max} [14 pmol min^{-1} (10^6 cells)$^{-1}$] and K_m (0.23 mM) values for long-chain base synthesis were determined and found to be essentially identical with those for serine palmitoyltransferase assayed in vitro [i.e., 13 pmol min^{-1} (10^6 cells)$^{-1}$ and 0.27 mM, respectively]. The other precursor, palmitic acid, was also taken up rapidly and increased long-chain base biosynthesis in a concentration-dependent manner. This effect was limited to palmitic acid and matched the known specificity of serine palmitoyltransferase for saturated fatty acyl-CoA's of 16 ± 1 carbon atoms. These studies delineate the influence of extracellular precursors on the formation of the sphingolipid backbone and suggest that the kinetic properties of serine palmitoyltransferase govern this behavior of long-chain base synthesis in intact cells.

Sphingolipids are elaborations of a group of compounds referred to as long-chain (or sphingoid) bases, which encompass sphingenine, sphinganine, 4-D-hydroxysphinganine (phytosphingosine), and homologues of these compounds (Karlsson, 1970).[1] Long-chain base biosynthesis begins with the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine [Snell et al., 1970; for recent reviews, see Kishimoto (1983) and Radin (1984)], which is catalyzed by serine palmitoyltransferase, a pyridoxal 5′-phosphate dependent enzyme. Serine palmitoyltransferase has been thought to be a control point for this pathway because (1) it catalyzes the first unique and committed step, (2) the reaction is essentially irreversible, (3) the reaction is rate limiting because free long-chain bases do not accumulate as biosynthetic intermediates in intact cells (Wang & Merrill, 1986), (4) the activity correlates with the approximate sphingolipid composition of tissues and changes when tissues undergo increased long-chain base biosynthesis (Williams et al., 1984a; Merrill et al., 1985), (5) mechanism-based inhibitors of serine palmitoyltransferase decrease the levels of some complex sphingolipids (Sundaram & Lev, 1985), and (6) other factors that decrease sphingolipid

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[1] The nomenclature used in this paper generally conforms to the recommendations of IUPAC/IUB; however, the terms sphingenine and sphinganine have been used for the long-chain bases with and without the 4-trans double bond, without specification of the alkyl chain length. Other frequently used names for sphingenine and sphinganine are sphingosine and dihydrosphingosine, respectively.

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formation, including α-fluoropalmitate (Soltysiak et al., 1984), lipoproteins (Verdery & Theolis, 1982; Chatterjee et al., 1986), and vitamin B6 deficiency (Kurtz & Kanfer, 1973), appear to partially inhibit serine incorporation into long-chain bases.

An additional indication of its role in regulating the rate of long-chain base formation is that kinetic analyses of the enzyme in microorganisms (Di Mari et al., 1971; Lev & Milford, 1981) and microsomal preparations from rat brain (Braun et al., 1970), liver (Williams et al., 1984b), and other tissues (Merrill et al., 1985) have found that the apparent K_m for serine is somewhat less than 1 mM. If a similar serine concentration dependence exists in intact cells, this suggests that substrate availability influences the flux through this pathway since this is the same range over which the serine concentration of plasma and hepatocytes is known to vary pre- and postprandially (Remesy et al., 1983).

Furthermore, serine palmitoyltransferase has a preference for saturated fatty acyl-CoA's of 16 ± 1 carbon atoms in length when assayed in microsomal preparations (Braun et al., 1970; Williams et al., 1984b; Merrill et al., 1985). It is not known whether or not this specificity also governs the types of long-chain bases that are made by intact cells. Most sphingolipids consist mainly of 18-carbon long-chain bases (exceptions include brain gangliosides, for example) (Karlsson, 1970). However, Kulmacz and Schroepfer (1978) found that the long-chain base composition of yeast sphingolipids could be manipulated by changes in the fatty acids added to the culture medium.

In this study, we measured [^{14}C]serine incorporation into long-chain bases by LM cells under different culture conditions to delineate the relationship between the supply of extracellular precursors and their utilization for this pathway. The effects of both serine and different fatty acids on long-chain base formation in intact cells were found to exactly parallel the properties of serine palmitoyltransferase. This suggests that the kinetic properties of serine palmitoyltransferase in vitro are relevant to understanding long-chain base formation in vivo.

Materials and Methods

Materials. Ham's F12 medium and MCDB 301 (prepared minus serine) were purchased from Gibco. All other tissue culture reagents were obtained from Sigma. [^{14}C]Serine (3-^{14}C, 55 mCi/mmol), [9,10-^{3}H]palmitic acid (1.86 Ci/mmol), [1-^{14}C]stearic acid (60 mCi/mmol), [^{3}H]dextran (M_r ca. 70 000), and [^{3}H]water were obtained from Amersham. Sphingolipid standards were purchased from Sigma. *trans*-2-Hexadecenoic acid was obtained from P-L Biochemicals, and other fatty acids were from Sigma. The other reagents, including those used for assays of serine palmitoyltransferase, were the same as have been described in other reports from this laboratory. All chemicals and solvents were of high quality.

Cell Culture. Mouse LM cells (ATCC CCL 1.2) were grown at 37 °C in suspension culture in Ham's F12 medium supplemented with 0.15–0.25 mM fatty acid free bovine serum albumin (Sigma), penicillin G (61 mg/L), streptomycin (100 mg/L), and sodium bicarbonate (1.176 g/L). The bottles were equilibrated with 5% carbon dioxide in air and stirred at approximately 60 rpm. The cells grew to a density of 0.6×10^6 cells/mL. The ability to grow in suspension was dependent on the lot and supplier of bovine serum albumin. Cell numbers were determined by counting the viable cells (usually >95% based on Trypan blue exclusion) in a hemacytometer.

For experiments, cells were removed from the spinner bottle and centrifuged at 600*g* for 5 min, and the pellet was washed thrice with Dulbecco's phosphate-buffered saline (PBS). Unless otherwise noted, they were resuspended at 10^7 cells/mL in serine-free MCDB 301 medium with the appropriate supplements and incubated at 37 °C in a New Brunswick Gyrotory shaker at approximately 100 rpm. Where noted, Ham's F12 medium was substituted for serine-free MCDB 301 (both have similar formulations) when it was not necessary for the medium to be serine free. In all instances, cell viabilities were >95% throughout the experiments.

Measurement of the Rate of Serine Uptake. Uptake was measured by using LM cells grown in glass scintillation vials as described by Englesberg et al. (1976). From 2×10^5 to 4×10^5 cells were incubated for varying times in medium containing 0.1 or 1.0 mM [^{14}C]serine, followed by rapid washes with ice-cold medium without radiolabel. The cells were then either dried and solubilized for scintillation counting of the total [^{14}C]serine incorporated or treated with 1% trichloroacetic acid (TCA), centrifuged, and counted (after extraction of the TCA with water-saturated ether) to estimate free [^{14}C]serine. The cpm were corrected for incomplete removal of the medium by subtracting the time zero cpm, and for losses in extraction and quenching by analyzing a sample spiked with [^{14}C]serine. Examination of aliquots of the acid-soluble radiolabel by paper chromatography (see below) established that >90% of the TCA-soluble radiolabel resided in serine.

LM Cell Volume and Serine Concentrations. A centrifugal filtration method (Palmieri & Klingenberg, 1979; Stevens et al., 1985) was employed to measure the aqueous volume of LM cells in suspension. The cells (1×10^6) were incubated for 1 h at 37 °C in 1 mL of medium containing [^{3}H]dextran or [^{3}H]water. To rapidly separate the cells from the medium, an aliquot was centrifuged through a layer of oil into 1.6 M perchloric acid (PCA). The cpm in an aliquot of the PCA layer were determined by scintillation counting and corrected for quenching. The cpm from [^{3}H]dextran were used to estimate the extracellular medium that accompanied the cells (about 20% of the total volume) and to derive the cellular aqueous volume from the total [^{3}H]water. For comparison, the diameter of the cells was estimated by using a calibrated hemacytometer.

The concentration and specific activity of the [^{14}C]serine were determined by incubating suspended LM cells in media containing varying concentrations of serine or [^{3}H]dextran for 30 min (pilot experiments determined that isotopic equilibrium had been reached) and separating the cells and media by centrifugal filtration as described above. The radiolabel in free serine was determined by counting an aliquot of the lower perchloric acid solution with correction for quenching, and for the extracellular serine (from [^{3}H]dextran, as described above). Aliquots were applied to Whatman 1 chromatography paper which was air-dried and developed (descending) with 1-butanol/acetic acid/water (85:15:5 v/v/v). Greater than 90% of the total radiolabel in the perchloric acid soluble fraction comigrated with serine. The total serine mass was measured with a 119Cl amino acid analyzer (Beckman Instruments, Palo Alto, CA) according to Vega et al. (1969) after the pH of the deproteinized sample was adjusted to approximately 1.7 with 2 N NaOH. The specific activity was estimated as the cpm in serine divided by the total free serine concentration; cellular concentrations were calculated by dividing the cell-associated serine concentration by the cell volume.

Analysis of Long-Chain Base Biosynthesis. The cells were incubated for 2 h in varying concentrations of serine as described above, then 25 μg each of sphingenine and sphinganine carriers was added, and the radiolabeled long-chain bases were

extracted and quantitated as described previously (Wang & Merrill, 1986). Since all of the radiolabel resides in complex (amide-linked) sphingolipids, this involves hydrolysis of the chloroform extracts in acid (Gaver & Sweeley, 1965) followed by thin-layer chromatography (TLC) using silica gel H chromatoplates (Brinkmann) developed with chloroform/methanol/2 N ammonium hydroxide (40:10:1 v/v/v). The long-chain bases were visualized by autoradiography, and the radiolabel was determined by scintillation counting with the appropriate quench corrections. The conditions for acid hydrolysis were critical; otherwise, significant amounts of the 3-methyl ethers could also be seen on the autoradiograms and on the TLC plates as ninhydrin-positive degradation products of unlabeled sphinganine and sphingenine carriers. When this occurred, the results were discarded.

Fatty Acid Uptake and Long-Chain Base Biosynthesis. For estimates of fatty acid uptake, cells in glass scintillation vials were incubated at 37 °C with [^{3}H]palmitic acid or [^{14}C]stearic acid (at 1 μM and 1 mM, respectively, prepared as the 1:1 complexes with fatty acid free bovine serum albumin) in F12 medium. After varying times, the medium was removed rapidly, and the cpm were determined as described above.

Long-chain base biosynthesis in the presence of different fatty acids was determined by adding the indicated fatty acids at 1 mM as the 1:1 complexes with bovine serum albumin.

Analyses of Long-Chain Base Levels of Cells Grown in High and Low Serine Concentrations. Long-chain base amounts were determined by analysis of the dinitrophenyl (DNP) derivatives using reverse-phase high-performance liquid chromatography. Lipid extracts were acid hydrolyzed; then the DNP derivatives were prepared according to Braun and Snell (1968). The derivatives were dissolved in methanol/5 mM potassium phosphate (pH 7.0) (90:10), injected onto a 2.5 × 25 cm C18 column (Isco), and eluted isocratically with this same solvent. The derivatives were detected at 360 nm with an Isco V4 detector and compared to standards prepared from sphinosine (which eluted at 9 min), sphinganine (11.2 min), and phytosphingenine (7.5 min). Sphingenine was the major species, with smaller amounts of sphinganine and 4-D-hydroxysphinganine and other homologues.

In Vitro Assay of Serine Palmitoyltransferase. Cells were disrupted by sonication and assayed as we have described previously for Chinese hamster ovary cells (Merrill, 1983). Palmitoyl-CoA was added at 150 μM because this was found to be the maximum concentration that could be used without substrate inhibition.

Unless otherwise noted, all the data presented reflect means ± of SD triplicate analyses.

RESULTS

Long-Chain Base Biosynthesis by Cells Assayed in Varying Concentrations of [^{14}C]Serine and Palmitic Acid. The rate of formation of sphingenine and sphinganine by intact cells depended on the extracellular concentrations of both of the precursors for this pathway. As the concentration of [^{14}C]-serine was varied, the cpm in long-chain bases increased in a manner resembling a simple saturation curve with an apparent K_m of 0.6 mM and a V_{max} of 3 pmol min^{-1} (10^6 cells)$^{-1}$, assuming that the cpm of intracellular serine were similar to those of the medium (see below). Addition of palmitic acid also increased the incorporation of radiolabel from [^{14}C]serine into long-chain bases (Figure 1), but saturation behavior was not observed, probably because concentrations higher than 1 mM could not be studied without loss of cell viability.

The simplest explanation for the different rates of long-chain base synthesis is that they reflect variations in serine palmitoyltransferase activity as the concentrations of its substrates are altered. This hypothesis was explored in more detail as follows.

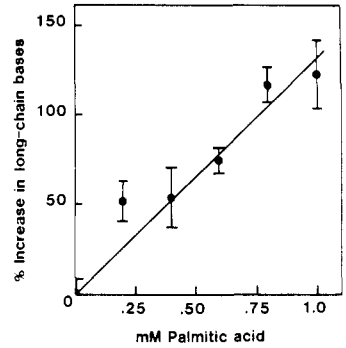

FIGURE 1: Effect of varying the palmitic acid concentration of the culture medium on long-chain base synthesis by LM cells. LM cells (2.5×10^6) were incubated for 2 h in MCDB 301 medium containing [^{14}C]serine and varying concentrations of a palmitic acid–bovine serum albumin complex (1:1). The cpm in sphinganine and sphingenine were added to give total long-chain bases and expressed as the percent increase above the amount formed in F12 alone.

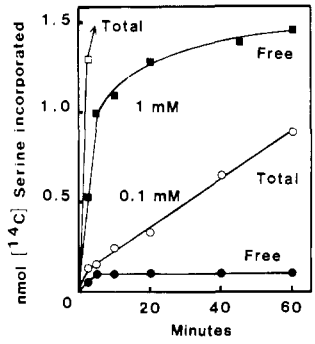

FIGURE 2: Appearance of free and total [^{14}C]serine in LM cells. Approximately 3.3×10^5 cells in glass scintillation vials were incubated at 37 °C with medium containing 0.1 or 1 mM [^{14}C]serine. At the times shown, the medium was removed rapidly, and aliquots were counted for total cpm or separated by paper chromatography for free [^{14}C]serine.

Serine Uptake by LM Cells. The reported K_m for serine (0.7 mM) with rat liver microsomes (Williams et al., 1984a), which is similar to that found with other sources (Braun et al., 1970; Di Mari et al., 1971; Williams et al., 1984b; Merrill et al., 1985), is similar to the apparent K_m observed with intact LM cells. However, since other factors (i.e., transport) would also contribute to this behavior, both the rate of uptake and the cellular levels of [^{14}C]serine were measured (Figure 2). There was continuous uptake of radiolabel by the cells (Figure 2, open symbols), most of which was incorporated into acid-insoluble products. At both low (0.1 mM) and high (1 mM) concentrations, the uptake was very rapid (i.e., nanomoles per minute per 10^6 cells) compared to the rate of long-chain base formation (picomoles per minute per 10^6 cells). The amount of radiolabel in free serine (Figure 2, closed symbols) also increased rapidly, reaching a steady state within 10–20 min at both concentrations, and persisted for 4–6 h (not shown). The cellular levels of free [^{14}C]serine appeared to be proportional to the extracellular concentration.

The concentration dependence of the initial rates of [^{14}C]serine uptake (measured over minutes) was examined in more detail using cells centrifuged through oil (Table I, first and second columns) and did not exhibit saturation over this concentration range. In addition, the concentration dependence of the cellular levels of total free serine (determined by amino acid analysis) was determined at the steady state (i.e., after 30 min). The amount increased 7-fold as the extracellular

Table I: [^{14}C]Serine Uptake and Concentrations for LM Cells

serine added (mM)	initial rate of serine uptake[a] [nmol min^{-1} (10^6 cells)$^{-1}$]	steady-state serine amount[b] (nmol/10^6 cells)	cellular serine concn[c] (mM)	cellular sp act.[d] (cpm/nmol)
0.1	0.8 ± 0.1	0.54 ± 0.08	0.17	5450 ± 330
0.25	1.7 ± 0.1	0.98 ± 0.06	0.31	7906 ± 403
0.5	3.3 ± 0.3	1.6 ± 0.06	0.50	10914 ± 714
0.75	5.3 ± 0.1	2.4 ± 0.1	0.75	10945 ± 605
1.0	6.3 ± 0.5	3.0 ± 0.1	0.94	12335 ± 425
1.25	8.0 ± 0.4	3.7 ± 0.1	1.16	12293 ± 668

[a] Uptake rates were determined by measuring the amounts of radiolabel in the cells after incubation for 2 min followed by rapid centrifugation of the cells through an oil layer. [b] Determined after the amount of free [^{14}C]serine reached steady state (at 30 min) by separation of the cells from the medium by centrifugation through an oil layer and quantitation of the total serine by amino acid analysis. [c] Calculated from a cellular volume of 3.2 ± 0.8 μL/10^6 cells. [d] Calculated from the cpm in free [^{14}C]serine and the amount of labeled and unlabeled serine in the cell.

serine concentration was varied between 0.1 and 1.25 mM (Table I, third column). Both of these results agreed with the initial findings with [^{14}C]serine uptake (cf. Figure 2 and Table I); hence, these findings suggest that the rate of serine uptake and equilibration of cellular serine concentrations with the extracellular media are too rapid to influence the incorporation of radiolabel into long-chain bases, which occurs over a longer time course (2 h).[2] Furthermore, the agreement between experiments using cells attached to scintillation vials (which are rapidly but repeatedly washed to remove extracellular serine) and cells centrifuged through oil ensures that the serine is associated with intact cells.

The aqueous volume of the LM cells was measured so that the cellular serine concentrations could be estimated. On the basis of the uptake of [^{3}H]water, the volume was 3.2 ± 0.8 μL/10^6 cells (mean ± SE, n = 5), which was consistent with the diameter of these cells (17–20 μm). With this volume, the cellular serine concentrations were calculated to range from 0.17 to 1.16 mM (Table I, fourth column).

At 0.1 and 0.25 mM serine in the medium, the cellular concentrations were greater than the extracellular medium, which indicates that the cells contribute a significant amount of additional serine from de novo synthesis. At 0.5 mM and above, the concentrations in the cells and medium were essentially identical. The contribution of unlabeled serine by the cells was also reflected in the specific activity of the cellular [^{14}C]serine (Table I, fifth column). Again, at 0.5 mM and above, the specific activities were essentially identical with the added [^{14}C]serine (11 mCi/mmol) and about twice that at 0.1 mM, where there was significant dilution of the radiolabel with endogenous serine.

Long-Chain Base Biosynthesis at Different Intracellular Concentrations of Serine. These specific activities were used to calculate the actual picomoles of long-chain bases formed at different cellular serine concentrations.[3] It is apparent that some of the concentration dependence of long-chain base synthesis was due to variation in the specific activity of the precursor pool; however, even when these corrections were made, long-chain base biosynthesis exhibited saturation behavior (Figure 3) with an apparent V_{max} of 14 pmol min^{-1} (10^6 cells)$^{-1}$ and a K_m of 0.23 mM (estimated by Lineweaver–Burk plots of the data).

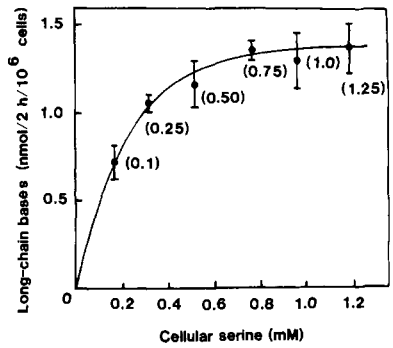

FIGURE 3: Long-chain base formation at varying concentrations of cellular serine. LM cells were incubated in [^{14}C]serine at the concentrations shown in parentheses, and the cellular concentrations (shown as the x axis) and specific activities (see Table II) were determined and used to calculate the amounts of long-chain bases (sphinganine plus sphingenine) formed.

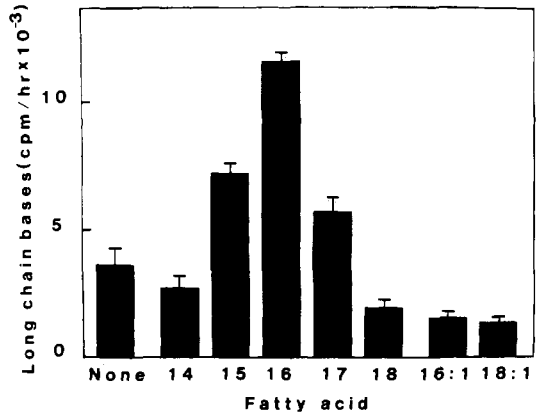

FIGURE 4: Long-chain base biosynthesis by LM cells in the presence of different fatty acids. LM cells (2.5 × 10^6) were incubated for 2 h in MCDB 301 medium containing [^{14}C]serine alone ("none") or plus 1 mM complexes (1:1) of bovine serum albumin with different fatty acids having the alkyl chain lengths shown. 16:1 and 18:1 represent unsaturated fatty acids with double bonds at positions 2 (i.e., *trans*-hexadecenoic acid) and 9 (i.e., oleic acid). The cpm in sphinganine and sphingenine were added to give total long-chain bases.

Activities of Serine Palmitoyltransferase in LM Cells in Vitro. These results were compared with the activity of serine palmitoyltransferase assayed in vitro. Assays of this enzyme in broken LM cells yielded an apparent V_{max} of 13 pmol min^{-1} (10^6 cells)$^{-1}$ and a K_m for serine of 0.27 mM, which are essentially identical with the results obtained with whole cells. On the basis of these results, the simplest explanation for the effects of the variation of long-chain base synthesis in intact cells as the intra- (and extra-) cellular serine concentration increases is that this directly reflects titration of serine palmitoyltransferase with this substrate.

Effects of Fatty Acids. Similar studies of the effects of palmitic acid are not as straightforward because this precursor must be converted to the CoA thio ester before utilization. Furthermore, even if the formation of palmitoyl-CoA was measured, the known localization of this molecule in multiple subcellular compartments precludes definitive interpretations about its availability to serine palmitoyltransferase.

[2] Previous studies have shown that long-chain base synthesis from [^{14}C]serine increases continuously between 30 min and 2 h (Merrill & Wang, 1986); hence, incubations were conducted for 2 h to yield a significant number of cpm in the products at even the low serine concentrations.

[3] The specific activity of the long-chain bases synthesized de novo cannot be determined directly because free long-chain bases are not detected as intermediates of the pathway (Merrill & Wang, 1986); hence, the long-chain bases obtained after acid hydrolysis will also reflect preexisting sphingolipids. However, since the cellular specific activity of [^{14}C]serine rapidly reaches a steady state, one can assume that long-chain bases made de novo over the longer time course of these experiments will have a similar specific activity unless a separate serine pool is used by this pathway.

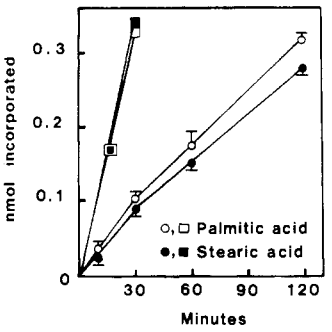

FIGURE 5: Uptake of palmitic acid and stearic acid by LM cells. The 1:1 complexes of bovine serum albumin and [^{3}H]palmitic acid (open symbols) or [^{14}C]stearic acid (closed symbols) at 1 μm (circles) or 1 mM (squares) were incubated with approximately 2 × 10^5 LM cells for the times shown, and the amount of radiolabel associated with the cells was determined.

Instead, the known preference of this enzyme for fatty acyl-CoA's with alkyl chain lengths of 16 ± 1 carbon atoms (Braun et al., 1968; Williams et al., 1984a,b; Merrill et al., 1985) was used to evaluate the mechanism of the stimulation of long-chain formation. As shown in Figure 4, palmitic acid (C16:0), followed by pentadecanoic acid (C15:0) and heptadecanoic (C17:0) acid, caused an increase in the rate of long-chain base formation compared to that without supplementation (Figure 4, "none"). Stearic (C18:0) and myristic (C14:0) acids and fatty acids with a cis double bond, such as oleic acid (C18:1), decreased long-chain base synthesis below that in the presence of no exogenous fatty acids. Although *trans*-2-hexadecenoic acid (C16:1) has been found to be a sphingenine precursor in yeast (Di Mari et al., 1971), it did not appear to be utilized by a cell-free preparation from oysters (Hammond & Sweeley, 1973) and was inhibitory for long-chain base formation from [^{14}C]serine in LM cells. Fatty acid free bovine serum albumin alone also caused slight inhibition (35%, not shown); however, albumin had no effect if fatty acids were added just before extraction, and this was ascribed to interference with recovery of the long-chain bases. In addition, fatty acids were not observed to alter total [^{14}C]serine uptake by LM cells nor to inhibit serine palmitoyltransferase in vitro.

Fatty Acid Uptake. Although this response was identical with the known fatty acyl-CoA specificity of serine palmitoyltransferase, it might also reflect differing rates of fatty acid uptake by LM cells. To test this possibility, the uptake rates of two fatty acids that stimulated (i.e., palmitic acid) and inhibited (i.e., stearic acid) long-chain base formation were compared (Figure 5). The rates were essentially identical for these two fatty acids at both high (1 mM) and low (1 μM) concentrations. Furthermore, both fatty acids can apparently be converted to the CoA thio esters because their incorporation into total lipid cellular lipids (i.e., cpm in total lipid extracts) was also identical (not shown) despite a 6-fold difference in long-chain base synthesis in media containing these two compounds (Figure 5). This agreed with previous reports that the fatty acyl-CoA synthetases do not exhibit this degree of specificity (Tanaka et al., 1979), in contrast to serine palmitoyltransferase.

Long-Chain Base Amounts and Composition of Cells Grown in Different Media. In light of these findings about the effects of the precursors of this pathway, two additional experiments were conducted in an attempt to determine whether or not varying the extracellular precursors alters the long-chain base composition of LM cells.

Cells were grown in media containing 2 mM serine versus the level in F12 medium alone (0.1 mM). This resulted in a small increase (30%) in the amounts of total long-chain bases obtained after acid hydrolysis (Table II). There was no obvious increase in a specific sphingolipid subclass, such as sphingomyelin or mono- and diglycosylceramides (data not shown), the major sphingolipids of L cells (Yogeeswaran et al., 1973). Since the differences were small, further studies of this effect will require more detailed analyses of individual sphingolipids than lies within the scope of this initial investigation. However, these results suggest that changes in the rate of long-chain base formation de novo can influence the amounts of these molecules in cells.

Table II: Long-Chain Base Content of LM Cells Grown in High and Low Serine[a]

expt	long chain bases (nmol/10^6 cells) at serine concn (mM) of 0.1	2	% increase
1	3.2 ± 0.1	3.8 ± 0.5	19
2	3.8 ± 0.4	5.3 ± 1.6	39
av	3.5	4.6	30

[a] LM cells were grown in F12 medium containing the final concentrations of serine shown, then the lipids were extracted and acid hydrolyzed, and the total amounts of long-chain bases were determined by reverse-phase high-performance liquid chromatography as described in the text.

Similar studies of the effects of different fatty acids were not possible because the LM cells were not viable for long periods of time in fatty acids at 1 mM, even when added as complexes with bovine serum albumin. At the level that they could be added (0.1 mM), there was little effect on the percent of the total long-chain bases found as the 18-carbon homologues in media containing palmitic acid (85%) versus stearic acid (80%).

Discussion

The goal of these experiments was to test relevance of the kinetic properties of serine palmitoyltransferase assayed in vitro to the function of this enzyme in long-chain base synthesis by intact cells. The most definitive finding was that the serine concentration had a major impact on this pathway and that the most likely site of this effect was serine palmitoyltransferase. This finding was in excellent agreement with previous reports relating this enzyme to the regulation of long-chain base formation (as enumerated in the introduction) and represents the first demonstration that the levels of the substrates can contribute to the rate in intact cells.

This provides another example of a frequently encountered principle of enzymology: that the K_m of key enzymes should be similar to the cellular concentrations because it "utilizes most of the catalytic potential of the enzyme, while still maintaining proportional control" (Cleland, 1970). The importance of these observations with a transformed cell line in defined medium is obviously a concern; however, there is preliminary evidence that long-chain base formation by more normal cells (i.e., freshly isolated hepatocytes) is also increased by serine (Messmer & Merrill, 1985). This could account for the observation of Bjerve (1985) that changing the concentration of [^{14}C]serine from 0.2 to 2.25 mM increased the proportion of the radiolabel appearing in sphingomyelin made by hepatocytes from 11% to 42%. Furthermore, it is well-known that serine concentrations in circulation and in tissues vary over 30-fold pre- and postprandially in animals consuming different levels of protein (Remesy et al., 1983).

It may also be significant that palmitic acid increased long-chain base formation whereas most other fatty acids

decreased the rate, possibly because they compete for the cellular CoA pool and decreased endogenous palmitoyl-CoA levels. As well as we are aware, this is the first demonstration that fatty acids can influence long-chain base synthesis in mammalian cells; however, Soltysiak et al. (1984) had earlier found that α-fluoropalmitic acid decreased long-chain base synthesis and suggested that this was due to inhibition of palmitoyl-CoA formation. It would appear that the specificity of this pathway for palmitoyl-CoA in vitro and palmitic acid in intact cells helps account for the predominance of 18-carbon species in most sphingolipids (Karlsson, 1970). This does not preclude the possibility of shifts in the types of sphingoid bases if high proportions of other fatty acids (or low amounts of palmitoyl-CoA) are available, as indicated by the changes in sphingolipid composition of yeast exposed to different exogenous fatty acids (Kulmacz & Schroepfer, 1978), and possibly in hepatomas (Merrill et al., 1986b). The reason for this careful control of the carbon chain length of the long-chain bases is not known; however, the structure of these moieties has been shown to influence the physical properties of some sphingolipids (Yohe et al., 1976).

It is noteworthy that different concentrations of serine and fatty acids affect long-chain base biosynthesis by the same magnitude (i.e., 2–3-fold) as the reported decreases in long-chain base formation by lipoproteins (Verdery & Theolis, 1982; Chatterjee et al., 1986). We have also seen that serum causes a similar decrease in [^{14}C]serine incorporation into long-chain bases in LM cells (data not shown). The presumed explanation for this response is that exogenous long-chain bases decrease the activity of serine palmitoyltransferase, although this has never been shown directly and was not observed in the one instance where examined (Merrill, 1983). It is also plausible that one of the factors identified in this study (such as uptake of unsaturated fatty acids) is responsible for the effects of lipoproteins.

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Registry No. Ser, 56-45-1; sphinganine, 764-22-7; sphingenine, 123-78-4; serine palmitoyltransferase, 62213-50-7; palmitic acid, 57-10-3; pentadecanoic acid, 1002-84-2; heptadecanoic acid, 506-12-7; stearic acid, 57-11-4; myristic acid, 544-63-8; oleic acid, 112-80-1; *trans*-2-hexadecanoic acid, 929-79-3.

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