D-Serine inhibits serine palmitoyltransferase, the enzyme catalyzing the initial step of sphingolipid biosynthesis

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Abstract Serine palmitoyltransferase (SPT), responsible for the initial step of sphingolipid biosynthesis, catalyzes condensation of palmitoyl coenzyme A and L-serine to produce 3-ketodihydrosphingosine (KDS). For determination of the stereochemical specificity of the amino acid substrate, a competition analysis of the production of [^{3}H]KDS from L-[^{3}H]serine was performed using purified SPT. D-Serine inhibited [^{3}H]KDS production as effectively as non-radioactive L-serine, whereas neither D-alanine nor D-threonine showed any significant effect. Incubation of purified SPT with [palmitoyl 1-^{14}C]palmitoyl coenzyme A and D-serine did not produce [^{14}C]KDS, while the control incubation with L-serine did. These results suggest that D-serine competes with L-serine for the amino acid recognition site of SPT, but that D-serine is not utilized by this enzyme to produce KDS.

Key words: D-Serine; Serine palmitoyltransferase; Sphingolipid

1. Introduction

Sphingolipids are ubiquitous constituents of membrane lipids in mammalian cells, and are also distributed widely in other animals, plants, and microbes [1]. Sphingolipid biosynthesis is initiated by condensation of L-serine with palmitoyl coenzyme A (CoA) to generate 3-ketodihydrosphingosine (KDS), which is a reaction catalyzed by serine palmitoyltransferase (SPT), a pyridoxal phosphate-dependent enzyme embedded in the endoplasmic reticulum ([2–4] and references therein). The SPT enzyme consists of two subunits, 53-kDa LCB1 and 63-kDa LCB2, with a 1:1 stoichiometry [4]. The LCB2 subunit, but not the LCB1, has a conserved motif, in which a lysine residue has been speculated to bind to a pyridoxal phosphate group [5–10], although both subunits require expression of the SPT activity [5,6,10–12]. Substrate specificity analysis with a purified enzyme has shown that SPT preferentially uses palmitoyl CoA as the acyl CoA substrate and specifically serine as the amino acid substrate [4].

Recently, D-serine has been demonstrated to exist naturally in the brain [13,14], in which sphingolipids are relatively abundant as compared with other tissues [15,16]. Thus, in the present study, we examined the stereochemical specificity of the amino acid substrate for SPT, and showed that D-serine is a competitive inhibitor of L-serine in the SPT reaction.

2. Materials and methods

2.1. Materials

L-Serine, D-alanine, D-threonine, and palmitoyl CoA were purchased from Sigma, and D-serine was from Peptide Institute Inc. (Osaka, Japan). L-[^{3}H(G)]serine (20 Ci/mmol) and [palmitoyl 1-^{14}C]palmitoyl CoA (55 mCi/mmol) were from American Radiolabeled Chemicals.

2.2. Purification of SPT

The LY-B strain is a Chinese hamster ovary (CHO) cell mutant lacking the endogenous LCB1 subunit [11], and LY-B/FHcLCB1 is an LY-B transformant stably expressing a FLAG- and His_6-peptide tagged version of the hamster LCB1 protein [4]. SPT was purified from LY-B/FHcLCB1 cells, as described previously [4]. In brief, membranes prepared from LY-B/FHcLCB1 cells were solubilized by a non-ionic detergent sucrose monolaurate, and then active SPT was purified from the solubilized membrane fraction to homogeneity by affinity peptide chromatography.

2.3. Assay of SPT activity

Purified SPT (25 ng protein) was incubated in 200 μl of a standard SPT reaction buffer (50 mM HEPES–NaOH buffer (pH 7.5) containing 5 mM EDTA, 5 mM dithiothreitol, 50 μM pyridoxal phosphate, 25 μM palmitoyl CoA, and 0.1 mM L-[^{3}H(G)]serine (50 mCi/mmol)) at 37°C for 10 min. After stopping the reaction, lipids were extracted and the radioactivity of the [^{3}H]KDS that formed was measured, as described previously [2]. Radioactivity extracted from enzyme-minus controls was regarded as a background control.

3. Results

3.1. D-Serine inhibits SPT

Using assays to determine whether the production of [^{3}H]KDS from 0.1 mM L-[^{3}H]serine with purified SPT was inhibited by excess amounts of non-radioactive competitors, we have recently shown that neither L-alanine, L-threonine, L-serinamide, D,L-serinol, nor L-serine methylester serves as a strong competitor against the production of [^{3}H]KDS, indicating that all of the amino, carboxyl, and hydroxyl groups of L-serine are responsible for the substrate recognition of the enzyme [4]. In the present study, we used a competition assay to examine the stereochemical specificity of the amino acid substrate for SPT activity. When 5 mM D-serine was used as a competitor, [^{3}H]KDS formation was inhibited by 90%, similar to the result with 5 mM non-radioactive L-serine (Fig. 1). D-Alanine and D-threonine, however, were ineffective (Fig. 1A). A dose-response analysis determined that a concentration caused 50% inhibition (IC_{50}) of D-serine for [^{3}H]KDS

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Abbreviations: CoA, coenzyme A; KDS, 3-ketodihydrosphingosine; SPT, serine palmitoyltransferase; CHO, Chinese hamster ovary; IC_{50}, the concentration which caused 50% inhibition

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production was 0.3 mM, which was almost identical to the IC_{50} of L-serine (Fig. 1B). This unexpected nature of SPT was not due to a possible partial denaturation of the enzyme during purification, because similar inhibitory effects of D-serine were observed when intact membranes from wild-type CHO-K1 and bovine brain cells were used as enzyme sources (data not shown). This effect of D-serine was also not due to contaminated L-serine, which unavoidably exists in commercially available D-serine to a certain extent, because the level of L-serine in the D-serine reagent used was less than 0.6% (mol/mol) (personal communication from the manufacturer, Peptide Institute Inc.). D-Serine obtained from a different supplier (Sigma) also inhibited SPT activity (data not shown), although the level of L-serine in the D-serine reagent from Sigma was unknown.

To test whether the inhibition mode by D-serine was competitive with L-serine, the influence of D-serine on double reciprocal kinetic plots of KDS formation from L-serine was examined. Compared with plots obtained at various L-serine concentrations in the absence of D-serine, addition of 0.3 mM D-serine to the SPT reaction mixture did not cause any reduction of the apparent V_{max} value, but caused a change in the slope of the plots (Fig. 1C), suggesting that D-serine inhibited the SPT reaction in a competitive manner to L-serine.

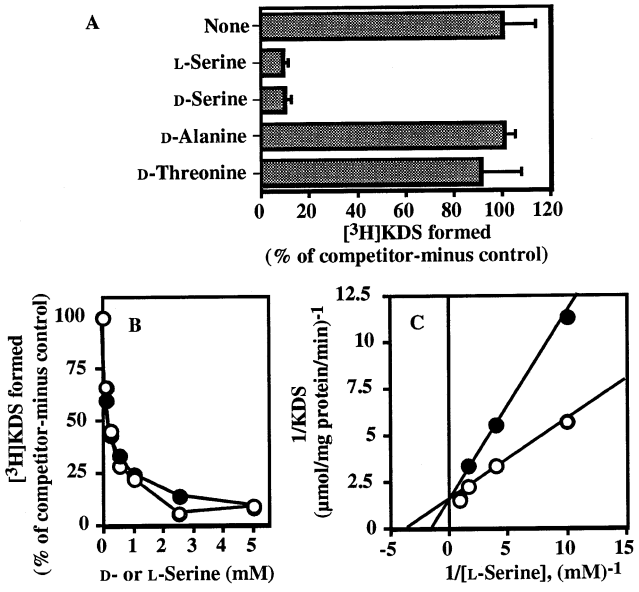

Fig. 1. Effects of D-amino acids on the formation of [^{3}H]KDS from L-[^{3}H]serine. A: Purified SPT (25 ng protein) was incubated in the SPT reaction buffer containing 0.1 mM L-[^{3}H]serine (50 mCi/mmol) and 5 mM each of the non-radioactive competitors indicated at 37°C for 10 min. The radioactivity of the [^{3}H]KDS that formed was measured as described in Section 2. Data from three experiments are shown as the percentage of the mean radioactivity of [^{3}H]KDS formed in the absence of the competitors. B: Purified SPT (25 ng protein) was incubated in the SPT reaction buffer containing 0.1 mM L-[^{3}H]serine (50 mCi/mmol) and various concentrations of non-radioactive D-serine (closed circles) or L-serine (open circles) at 37°C for 10 min. Data are shown as the percentage of radioactivity of [^{3}H]KDS formed without addition of non-radioactive serine. C: Purified SPT (25 ng protein) was incubated in the SPT reaction buffer containing various concentrations of L-[^{3}H]serine in the presence (closed circles) or absence (open circles) of 0.3 mM D-serine at 37°C for 10 min. Data obtained are shown as double reciprocal plots.

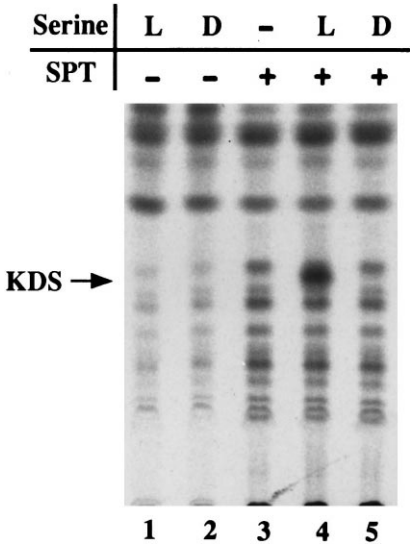

Fig. 2. SPT did not produce KDS from palmitoyl CoA and D-serine. Purified SPT (40 ng protein) was incubated in 200 μl of 50 mM HEPES–NaOH (pH 7.5) containing 25 μM [palmitoyl-1-^{14}C]-palmitoyl CoA (55 mCi/mmol), 50 μM pyridoxal phosphate, 5 mM EDTA, and 5 mM dithiothreitol in the presence or absence of 1 mM L- or D-serine at 37°C for 10 min. As an enzyme-minus control, vehicle buffer was added in place of the enzyme. Radioactive lipids were then extracted and separated by TLC with a solvent of chloroform/methanol/2 *N* ammonia (80/20/2, by volume). Radioactive lipids on the TLC plate were visualized by image analysis. The mobility of the standard [^{14}C]KDS, which was formed by the SPT reaction with L-[U-^{14}C]serine (158 mCi/mmol; Amersham Pharmacia Biotech) is shown by the arrow. Lanes 1 and 2, 1 mM L-serine and D-serine, respectively, without the enzyme; lanes 3–5, no serine, 1 mM L-serine, and 1 mM D-serine, respectively, with purified SPT.

3.2. D-Serine is not a productive substrate of SPT

To address the question whether D-serine serves as a substrate of SPT to produce KDS, purified SPT was incubated with 25 μM [palmitoyl 1-^{14}C]palmitoyl CoA in the presence of 1 mM D- or L-serine, and radioactive lipid products of the reaction were analyzed by TLC. When incubated with D-serine, the radioactive lipid patterns were the same as those of the negative control without any serine (Fig. 2, lane 3 vs. 5), although a radioactive compound co-migrating with standard KDS was produced when incubated with L-serine (Fig. 2, lane 4). These results demonstrated that D-serine is not a productive substrate of SPT.

4. Discussion

In the course of the competition assay of the SPT reaction with the purified enzyme, we unexpectedly found that D-serine inhibited [^{3}H]KDS formation with an IC_{50} of ~0.3 mM (Fig. 1B), which is a value similar to the K_m of L-serine for the SPT reaction [4]. The mode of inhibition for [^{3}H]KDS formation by D-serine appears to be competition with L-[^{3}H]serine (Fig. 1C). There was a slight possibility that the inhibition of [^{3}H]KDS production from L-[^{3}H]serine by D-serine was due to a simple dilution effect of non-radioactive L-serine, which might have been produced by isomerization of D-serine during the assay. However, this possibility was eliminated by the observation that incubation of purified SPT with D-serine and radioactive palmitoyl CoA produced no KDS (Fig. 2). These results suggest that D-serine competes with L-serine

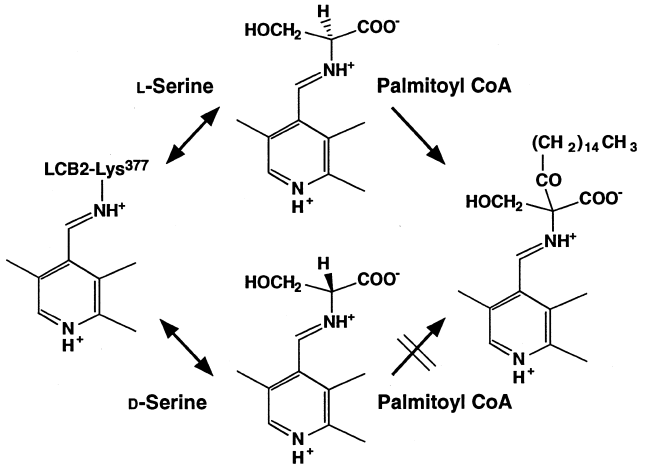

Fig. 3. A postulated mechanism of inhibition of the SPT reaction by D-serine. The Lys residue bound to pyridoxal phosphate in hamster SPT is predicted to be Lys^{377} in the LCB2 subunit [10].

for the amino acid recognition site of SPT, although D-serine is not utilized by SPT to produce KDS.

Recognition of L-serine by SPT is likely to involve Schiff-base formation between the amino acid substrate and a pyridoxal phosphate-group in the enzyme [17,18]. Binding of both enantiomers of a compound to one enzyme is exceptional, but not inconceivable for pyridoxal phosphate-dependent enzymes. For example, serine racemase, a pyridoxal phosphate-dependent enzyme, recognizes both L- and D-serine, and catalyzes the conversion of L-serine to D-serine and vice versa [19]. Thus, the SPT enzyme is presumably able to form Schiff-base complexes with both enantiomers of serine, although in the postulated next reaction, substitution of the α-hydrogen atom of serine by the palmitoyl group of palmitoyl CoA [17,18], may proceed in the Schiff-base complex with L-serine, but not D-serine (Fig. 3).

Sphingolipids synthesized via SPT reaction in intact cells can be metabolically labeled by incubation of the cells with radioactive L-serine [4,10]. Addition of an excess amount (10 mM) of non-radioactive L-serine to a metabolic labeling medium containing L-[^{14}C]serine inhibited formation of radioactive sphingolipids in CHO cells almost completely, whereas 10 mM D-serine caused only slight inhibition (our unpublished observation), probably due to the inefficient uptake of D-serine into cells. However, it has recently been revealed that substantial quantities of D-serine are present in discrete areas of the brain [13,14]. In such specific cell types, D-serine is synthesized from L-serine by serine racemase, a cytosolic enzyme [19,20]. Therefore, our finding that D-serine inhibits in vitro SPT activity raises the previously unrecognized possibility that D-serine affects sphingolipid synthesis in certain cell types enriched by it, although further studies are needed to elucidate the physiological relevance of the inhibition of the SPT reaction by D-serine.

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