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Metabolism of Sphingosine Bases, XI[1]

Distribution and Properties of Dihydrosphingosine-1-phosphate Aldolase (Sphinganine-1-phosphate alkanal-lyase)

By WILHELM STOFFEL, DAC LEKIM and GUIDO STICHT

*Institut für Physiologische Chemie der Universität Köln**

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Summary: The distribution and properties of dihydrosphingosine-1-phosphate aldolase (sphinganine-1-phosphate-alkanal-lyase EC 4.1.2.?) have been described. This enzyme cleaves dihydrosphingosine 1-phosphate into palmitaldehyde and phosphorylethanolamine in a pyridoxal phosphate dependent catalysis. The presence of this enzyme has been shown in all organs investigated so far: liver, brain, heart, muscle, kidney, spleen and lung of the rat, liver and kidney of pigs, beef liver and *Hansenula ciferrii*. It is bound to the membranes of the endoplasmic reticulum and of the mitochondria.

Zusammenfassung: *Stoffwechsel von Sphingosinbasen, XI: Verteilung und Eigenschaften von Dihydrosphingosin-1-phosphat-Aldolase (Sphinganin-1-phosphat-Alkanal-Lyase).* Es wird über die Verteilung und die Eigenschaften der Dihydrosphingosin-1-phosphat-Aldolase (Sphinganin-1-phosphat-Alkanal-Lyase EC 4.1.2.?) berichtet. Dieses Enzym spaltet Dihydrosphingosin-1-phosphat in Palmitinaldehyd und Phosphoryläthanolamin. Es besitzt Pyridoxalphosphat als Coenzym. Das Enzym wurde in folgenden untersuchten Organen nachgewiesen: Leber, Gehirn, Herz, Muskulatur, Niere, Milz und Lunge der Ratte, in Schweineleber und -niere, Rinderleber sowie der Hefe *Hansenula ciferrii*. Das Enzym ist an die Membranen des endoplasmatischen Reticulums und der Mitochondrien gebunden.

Previous studies have demonstrated that sphingosine bases are rapidly degraded in the animal cell and in the yeast *Hansenula ciferrii*[2–4]. Palmitic acid, phosphorylethanolamine and free ethanolamine have been isolated in experiments *in vivo* as degradation products of specifically labeled *erythro-* or *threo*-dihydrosphingosines and of sphingosine. The overall reaction in the breakdown of the 2-amino-**1**,3-dihydroxy-alkane or -4-alkene exhibits no particular chain length specificity. Eicosadihydrosphingosine as well as medium and short chain 2-amino-1,3-dihydroxy-alkanes are rapidly

* Address: Prof. Dr. Dr. W. STOFFEL, Institut für Physiologische Chemie der Universität Köln, D-5 Köln-Lindenthal, Joseph-Stelzmann-Straße 52.

Enzymes:

Dihydrosphingosine-1-phosphate aldolase, sphinganine-1-phosphate alkanal-lyase (EC 4.1.2.?; not yet listed)

Dihydrosphingosine-1-phosphatase, sphinganine 1-phosphohydrolase (EC 3.1.3.?; not yet listed)

Threonine aldolase, L-threonine acetaldehyde-lyase (EC 4.1.2.5)

Allothreonine aldolase, L-allothreonine acetaldehyde-lyase (EC 4.1.2.6)

[1] X. Commun.: W. STOFFEL, G. STICHT and D. KEKIM, this journal **350**, 63 [1969].
[2] W. STOFFEL and G. STICHT, this journal **348**, 941 [1967]
[3] W. STOFFEL and G. STICHT, this journal **348**, 1345 [1967].
[4] W. STOFFEL, G. STICHT and D. LEKIM, this journal **349**, 1149 [1968].

cleaved in experiments *in vivo*[5,6]. All substrates yielded phosphorylethanolamine and the corresponding hydrocarbon fragment with two carbon atoms shorter than the substrate. The degradation reactions were studied in experiments *in vitro* in order to gain insight into the reaction mechanism[7]. We were able to demonstrate that the cleavage of the long-chain bases a) required ATP and b) yielded as the primary products the fatty aldehyde and phosphorylethanolamine. Palmitic acid isolated in the experiments *in vivo* resulted from the oxydation of palmitaldehyde, because our experiments *in vitro* with [3-^{14}C; 3-^{3}H]dihydrosphingosine clearly proved that the ^{3}H/^{14}C ratio in [1-^{14}C; ^{3}H]palmitaldehyde isolated as cleavage product remained unchanged compared with that of the substrate. The main intracellular sites of this degradation are the endoplasmic reticulum and the mitochondria.

Both degradation products are very effectively utilized for the synthesis of phospholipids; phosphorylethanolamine for the hydrophilic part of phosphatidylethanolamine and phosphatidylcholine, palmitic acid for the acylation of 3-glycerophosphate in the biosynthesis of ester lipids[1–3,8]. Young rats (10—25 days of age) incorporate the palmitaldehyde liberated from dihydrosphingosine effectively into plasmalogens[9,10].

In this communication we report on the distribution and properties of the enzyme and the further characterization of the lyase reaction, which leads to the cleavage of dihydrosphingosine 1-phosphate.

Results

1) Distribution of dihydrosphingosine-1-phosphate aldolase in the rat liver cell

a) Intracellular distribution

The kinetics of the lyase reaction using [3-^{14}C]*erythro*-DL-dihydrosphingosine 1-phosphate as substrate were measured with the different subcellular fractions of rat liver prepared according to SIEKEVITZ[11] in order to localize the sites of the degradation. Fig. 1 indicates that the major site of the lyase reaction is the endoplasmic reticulum followed by the mitochondrial fraction. The supernatant is essentially free of enzymic activity.

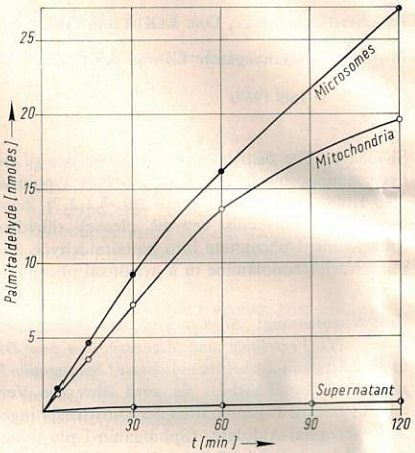

Fig. 1. Kinetics of the dihydrosphingosine-1-phosphate aldolase reaction in cell fractions from rat liver.

The incubation contained in a total volume of 2.5 m*l*: 0.6 μmoles of [3-^{14}C]*erythro*-DL-dihydrosphingosine 1-phosphate (268000 dpm), 5 mg enzyme protein, 1 μmole of pyridoxal phosphate, 20 μmoles of NaF, 250 μmoles of sodium potassium phosphate buffer, pH 7.4. 0.5 m*l* samples from the incubation were analyzed at times indicated for [1-^{14}C]palmitaldehyde and fatty acids as described under Experimental.

The outer and inner mitochondrial membranes and the matrix were isolated according to SOTTOCASA *et al.*[12] and investigated for their ability to degrade dihydrosphingosine 1-phosphate. The inner mitochondrial membrane possesses by far the highest enzymic activity (Table 1), if one takes into account that the mass of inner membranes is about 10—20 times higher than that of the outer membrane.

[5] W. STOFFEL, G. STICHT and D. LEKIM, this journal **350**, 63 [1969].

[6] W. STOFFEL and A. SCHEID, this journal, in preparation

[7] W. STOFFEL, G. STICHT and D. LEKIM, this journal **349**, 1745 [1968].

[8] W. STOFFEL and R. HENNING, this journal **349**, 1400 [1968].

[9] W. STOFFEL, Int. Symp. Chemistry and Metabolism of Sphingolipids, East Lansing, Michigan, 5 May 1969.

[10] W. STOFFEL and D. LEKIM, this journal, in preparat.

[11] P. SIEKEVITZ, Methods in Enzymol. **5**, 61 [1962].

[12] G. L. SOTTOCASA, B. KUYLENSTIERNA, L. ERNSTER and A. BERGSTRAND, J. Cell Biol. **32**, 415 [1967].

able 1. Degradation of dihydrosphingosine 1-phosphate by outer and inner mitochondrial membrane.
ncubation components in a volume of 2 ml: substrate 1 μmole; pyridoxal phosphate 0.5 μmoles; NaF 0 μmoles; phosphate buffer 200 μmoles, pH 7.4; protein as indicated.

	Mitochondrial					
	membranes				matrix	
	outer		inner			
Protein [mg]	0.4	0.6	2.0	5.0	1.1	3.3
Palmitaldehyde formed [dpm]	480	600	1530	2370	172	505

) Distribution in organs of the rat

Using the same substrate concentrations and reaction conditions the organs listed in Table 2 were screened for enzymic activity. Homogenates were prepared and their enzymic activities related to rat liver homogenate. Rat liver contains the highest aldolase activity followed by spleen and kidney.

Table 2. Distribution of dihydrosphingosine-1-phosphate aldolase in different organs of the rat.
Incubation components in 2.0 ml: protein (homogenate) 10 mg; substrate 0.1 μmole; pyridoxal phosphate 0.5 μmole; NaF 20 μmoles; phosphate buffer 200 μmoles, pH 7.4. Incubation for 2 h at 37°C. The incubation with liver protein yielded 9.8 nmoles (9.8%) of palmitaldehyde. Pig kidney, pig liver and beef liver showed enzymic activities comparable with the rat organs.

Organ	Relative activities [%]
Liver	100
Spleen	87 ± 2
Kidney	55 ± 3
Brain	34 ± 3
Lung	32 ± 1
Heart	30 ± 3
Muscle	11 ± 2

Hansenula ciferrii, which is known to secrete large amounts of acetylated phytosphingosine[13], also degrades dihydrosphingosine and phytosphingosine. *In-vivo* studies have shown, that radioactive palmitic acid and 2-hydroxypalmitic acid can be isolated when [3-^{14}C]dihydrosphingosine or phytosphingosine serve as substrates[4]. We therefore included this yeast in our *in-vitro* studies. Incubation of [3-^{14}C]dihydrosphingosine 1-phosphate with the microsomal fraction of this yeast, which proved to be the most active subcellular fraction, led to a measurable degree of degradation as determined by measurement of the radioactivity of palmitaldehyde formed during the incubation. However, rat liver microsomes proved to be ten times as active as *Hansenula cif.* microsomes. Table 3 summarizes the relationship between the yield in the cleavage reaction and the concentration of *Hansenula cif.* microsomal protein.

Table 3. Degradation *in vitro* of dihydrosphingosine 1-phosphate by *Hansenula ciferrii* microsomal protein. The conditions of the incubations are the same as described in Table 2.

Hansenula cif. microsomes [mg]	Degradation [%]
1.1	1.35
2.2	1.51
4.4	1.96
8.8	2.86
13.2	2.74
Rat liver microsomes [mg]	
5.0	19.05

2. Reaction conditions for and properties of the enzyme dihydrosphingosine-1-phosphate aldolase

a) Characterization of reaction products

The results described in the previous section led us to use the microsomal fraction of rat liver as the enzyme source for our further studies.
When a batch incubation of [3-^{14}C]*erythro*-DL-dihydrosphingosine 1-phosphate (2 μmoles) was carried out under optimal conditions, which are described in the following sections, and inactive palmitaldehyde was added after the incubation, radioactive [1-^{14}C]palmitaldehyde (0.165 μmole) was isolated in 8.2% yield and characterized as its crystalline semicarbazone (mp. 104.5°C from heptane (uncorrected), lit. 107°C). This derivative had constant specific radioactivity over four recrystallizations. The same experiment was carried out with [3-^{3}H;3-^{14}C]*erythro*-DL-dihydrosphingosine1-phosphate. [1-^{3}H;1-^{14}C]Palmitaldehyde was obtained

[13] L. J. Wickerham and F. J. Stodola, J. Bacteriol. **80**, 484 [1960].

in 10% yield. The $^3H/^{14}C$ ratio of the substrate was 24, that of the semicarbazone of [1-^{3}H; 1-^{14}C]palmitaldehyde formed in the aldolase reaction ranged between 23 and 25.8 after four recrystallizations from heptane. This conclusively demonstrated that palmitaldehyde was formed as the primary reaction product representing C_3 to C_{18} of dihydrosphingosine. Phosphorylethanolamine liberated as the other fragment represents C_1 and C_2 with its functional groups intact as demonstrated with [1-^{14}C]dihydrosphingosine 1-phosphate previously[5,6].

b) Cofactor requirement of the lyase reaction

In our initial studies on the *in vitro* degradation of dihydrosphingosine 1-phosphate whole mitochondria which were prepared isotonically were used as the enzyme source[5]. The cleavage reaction suggested pyridoxal phosphate as coenzyme of the lyase due to the similarity of the enzymic reaction to that of threonine and allothreonine aldolases[14–17]. Pyridoxal phosphate did not significantly stimulate the reaction. However, when we used the dialysed microsomal enzyme fraction or the isolated outer and inner mitochondrial membranes pyridoxal phosphate significantly stimulated the reaction. This is shown in Fig. 2.

At the optimal coenzyme concentration (10^{-4}M) the yield of product formed increased 6fold. On the other hand deoxypyridoxine phosphate inhibited the enzyme. The yield was reduced to 50% at a concentration of deoxypyridoxine phosphate of 1.5×10^{-3}M. Fig. 3 visualizes the inhibition of the lyase by increasing concentrations of deoxypyridoxine phosphate.

c) Inhibition of sphinganine-1-phosphate hydrolase

When dihydrosphingosine 1-phosphate was incubated with the microsomal or mitochondrial fractions of rat liver a rapid hydrolysis of the phosphomonoester bond was observed yielding free dihydrosphingosine and phosphate. This phosphatase, the properties of which will be described in a sub-

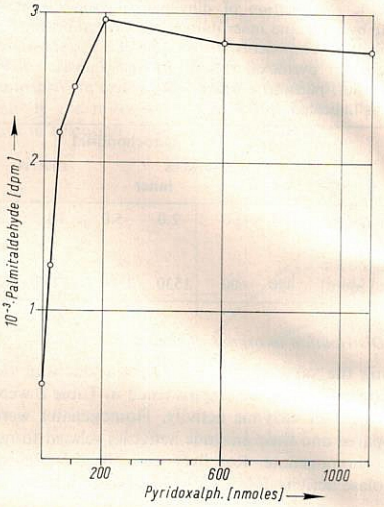

Fig. 2. Cofactor requirement of dihydrosphingosine-1-phosphate aldolase. Incubation components in a volume of 2.0 ml: substrate 0.1 μmole; NaF 20 μmoles; sodium potassium phosphate buffer 200 μmoles, pH 7.4; microsomal enzyme 5.0 mg; pyridoxal phosphate as indicated; 2 h at 37°C.

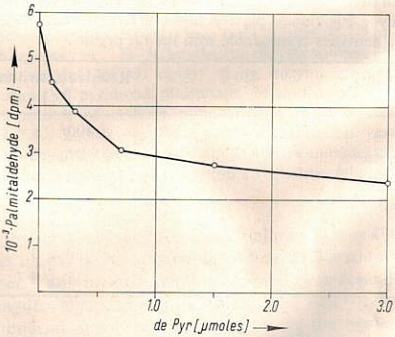

Fig. 3. Inhibition of dihydrosphingosine-1-phosphate aldolase by increasing concentrations of deoxypyridoxine phosphate (de Pyr). Incubation components in a total volume of 2.0 ml: substrate 0.1 μmole; NaF 20 μmoles; pyridoxal phosphate 0.5 μmole; microsomal protein 5.0 mg; phosphate buffer 200 μmoles, pH 7.4. Preincubations with concentrations of deoxypyridoxine phosphate as indicated.

[14] A. E. BRAUNSTEIN and G. Y. VILENKINA, Doklady Akad. Nauk. UsSSR [Ber. Akad. Wiss. Usbek. SSR] **66**, 1243 [1949].

[15] S. LIN and D. M. GREENBERG, J. gen. Physiol. **38**, 181 [1954].

[16] M. A. KARASEK and D. M. GREENBERG, J. biol. Chemistry **227**, 191 [1957].

[17] L. I. MALKIN and D. M. GREENBERG, Biochim. biophysica Acta [Amsterdam] **85**, 117 [1964].

sequent paper[18], was effectively inhibited by sodium fluoride at a 2×10^{-3}M concentration (Fig. 4). The lyase, however, was not impaired at all.

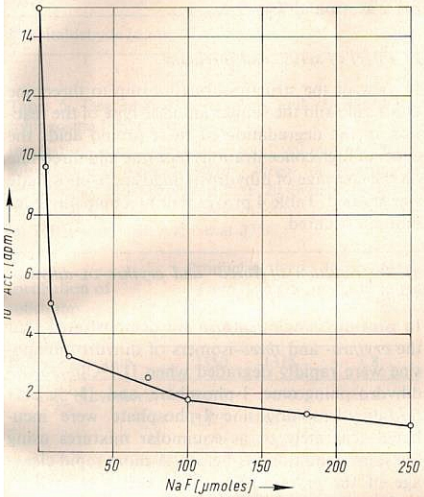

Fig. 4. Inhibition of dihydrosphingosine-1-phosphate hydrolase by sodium fluoride. Incubation components in a total volume of 2.0 ml: [3-^{14}C]dihydrosphingosine 1-phosphate 0.1 μmole; NaF as indicated; pyridoxal phosphate 0.5 μmole; phosphate buffer 120 μmoles; microsomal protein 5.0 mg; 2 h at 37°C. Ordinate: Hydrolyzed [3-^{14}C]dihydrosphingosine 1-phosphate.

Fig. 5 demonstrates that below an optimal concentration of NaF (10^{-2}M) the hydrolysis of dihydrosphingosine 1-phosphate leads to a decrease in the yield of palmitaldehyde and that NaF at concentrations between 10^{-2}M to 6×10^{-2}M does not inhibit the lyase.

d) Effect of Triton X-100 on dihydrosphingosine-1-phosphate aldolase

In order to obtain reproducible results in all these reactions it was essential to solubilize dihydrosphingosine 1-phosphate completely. The phosphate ester is barely soluble in water in its acidic form or as alkali salt. A clear aqueous solution can be prepared either in Triton X-100 or as triethanolammonium salt. The lyase was fairly stable towards Triton X-100 and no significant inhibition of the enzymic activity was observed up to a concentration of 15 mg/ml. This is shown in Fig. 6.

[18] G. Sticht and W. Stoffel, in preparation.

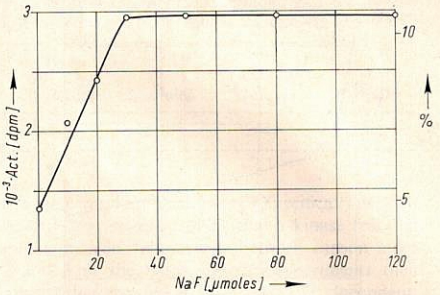

Fig. 5. Effect of sodium fluoride on the dihydrosphingosine-1-phosphate aldolase. Conditions of the incubation as in Fig. 4. Right and left ordinate: [1-^{14}C]-palmitaldehyde formed.

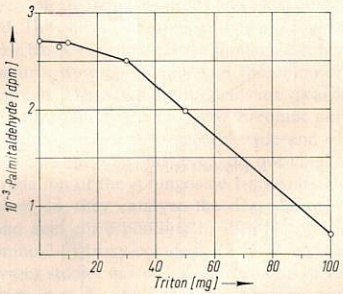

Fig. 6. Effect of Triton X-100 on dihydrosphingosine-1-phosphate aldolase. Incubation components in a total volume of 2.0 ml: substrate 0.1 μmole; microsomal protein 5.0 mg; sodium potassium phosphate buffer 200 μmoles, pH 7.4; Triton X-100 as indicated; 2 h at 37°C.

e) Effect of sulfhydryl reagents

The almost complete inhibition of the enzyme by alkylating reagents of sulfhydryl groups such as *N*-ethylmaleimide, *p*-chloromercuribenzoate and iodoacetamide. (Fig. 7a, b and c) point to the involvement of sulfhydryl group(s) in the catalytic reaction. The inhibitory effect of *p*-chloromercuribenzoate and *N*-ethylmaleimide was more pronounced than that of iodoacetamide.

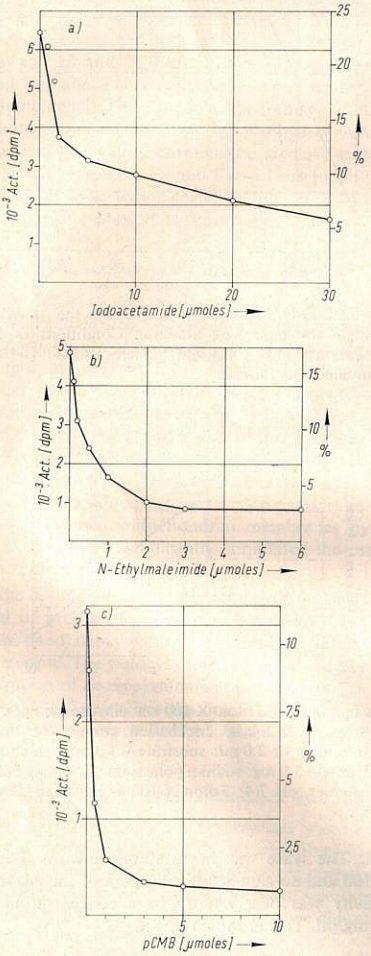

Fig. 7. Effect of SH-reagents on the dihydrosphingosine-1-phosphate aldolase. Reaction components in a total volume of 2.0 ml: dihydrosphingosine 1-phosphate 0.1 μmole; NaF 20 μmoles; pyridoxal phosphate 0.5 μmole; microsomal protein 5.0 mg; tris buffer 200 μmoles, pH 7.4; alkylating reagents as indicated; 2 h at 37°C.
a) Iodoacetamide, b) *N*-ethylmaleimide, c) *p*-chloromercuribenzoate (*p*CMB).
Right and left ordinates: [1-^{14}C]palmitaldehyde formed.

f) Optimal pH

The effect of pH on the rat liver lyase activity was studied with dihydrosphingosine 1-phosphate as substrate under optimal conditions. The optimal pH was around 7.4—7.6.

g) Effect of serine and threonine

In view of the structural relationship to threonine and serine and the similar aldolase type of the reaction in the degradation of these amino acids the effect of high concentrations of serine and threonine on the cleavage of dihydrosphingosine 1-phosphate was studied. Table 4 proves that no competitive inhibition occured.

*h) Degradation of threo- and erythro-*DL*-dihydrosphingosine 1-phosphate*

In previous studies *in vivo* we demonstrated that the *erythro-* and *threo*-isomers of dihydrosphingosine were rapidly degraded when [1-^{14}C]*threo*-DL-dihydrosphingosine 1-phosphate and [3-^{14}C]*erythro*-dihydrosphingosine 1-phosphate were incubated separately or as equimolar mixtures using the same conditions as before. A more rapid cleavage of the *erythro*-isomer has been observed as indicated in Table 5.

i) Reversibility of the aldolase reaction

It was of interest to find out whether the aldolase reaction is reversible. If this should occur the condensation of palmitaldehyde and phosphorylethanolamine would yield dihydrosphingosine 1-phosphate and, after hydrolysis, free dihydrosphingosine. We therefore incubated [1-^{14}C]palmitaldehyde and phosphorylethanolamine in the presence of rat liver microsomes. However neither dihydrosphingosine 1-phosphate nor free dihydrosphingosine could be isolated. This means that the reaction is directed only toward cleavage. Also palmitaldehyde and phosphorylethanolamine did not inhibit the cleavage reaction as shown in Table 6.

Discussion

During the course of our studies on the degradation of long-chain sphingosine bases we observed that 2-amino-1,3-diol alkanes are cleaved in the same fashion, independently of the chain lengths and stereo-chemistry of these substrates, yielding

Table 4. Degradation of [3-^{14}C]*erythro*-dihydrosphingosine 1-phosphate in the presence of serine and threonine. Reaction components in a total volume of 2.0 ml: dihydrosphingosine 1-phosphate 0.1 μmole; NaF 20 μmoles; microsomal protein 5 mg; phosphate buffer 200 μmoles, pH 7.4; DL-serine and DL-threonine as indicated; 2 h at 37°C.

	Addition				
	no addition	Serine 20 μmoles	Threonine 2 μmoles	Threonine 5 μmoles	Threonine 25 μmoles
Palmitaldehyde [dpm]	2208	2890	2717	2841	2348

Table 5. Degradation of *erythro-* and *threo*-dihydrosphingosine 1-phosphate.

Incubation components in a total volume of 2.0 ml: substrate 0.2 μmole (*erythro-*, *threo-* or mixture of *erythro-* and *threo*-isomers); NaF 20 μmoles; pyridoxal phosphate 0.5 μmole; sodium potassium phosphate buffer 200 μmoles, pH 7.4; 2 h at 37°C.

Incubation of substrates	Product derived from isomer *erythro* [dpm]	*threo* [dpm]	Ratio of the degradation of *erythro*/*threo* isomers
Isomers separate	2350	1315	1.8
Mixture of isomers	1670	1190	1.4
	2075	1530	1.35
	1570	940	1.6

Table 6. Incubation of [3-^{14}C]dihydrosphingosine 1-phosphate with aldolase in the presence of palmitaldehyde and phosphorylethanolamine.

Reaction components in a total volume of 2.0 ml: dihydrosphingosine 1-phosphate 0.1 μmole; NaF 20 μmoles; microsomal protein 5 mg; phosphate buffer 200 μmoles, pH 7.4; palmitaldehyde and phosphorylethanolamine as indicated; 2 h at 37°C.

Incubation mixture	Palmitaldehyde formed [dpm]	[nmoles]
Complete	5740	20.5
+ 5 μmoles phosphoryl-ethanolamine	5740	20.5
+ 5 μmoles palmitaldehyde	5810	20.7
+ 5 μmoles phosphoryl-ethanolamine + 5 μmoles palmitaldehyde	5750	20.5

an aldehyde two carbon atoms shorter than the substrate together with phosphorylethanolamine. The phosphate ester of ethanolamine, which corresponds to the C-1 and C-2 atoms with their functional groups intact, was consistently recovered as the main product as shown by specifically labeled bases. This suggested to us that a kinase reaction might precede the cleavage between carbon atom 2 and 3 of the bases. *In-vitro* experiments then proved that the degradation was ATP dependent. When synthetic [1-^{14}C] and [3-^{14}C]dihydrosphingosine 1-phosphate were incubated with the cleaving enzyme the sole reaction products were [1-^{14}C]palmitaldehyde and [^{14}C]phosphorylethanolamine. The substrate therefore needs first to be phosphorylated. The phosphate esters of the long-chain sphingosine bases exhibited strong similarities when their structures were compared with threonine or allothreonine. These two amino acids are cleaved in a similar fashion by the enzymes threonine and allothreonine aldolase into acetaldehyde and glycine. These two enzymes, however, do not catalyze the degradation of the sphingosine 1-phosphate esters, neither do they catalyze the degradation of the amino acid corresponding to dihydrosphingosine, 2-amino-3-hydroxy-octadecanoic acid, as shown in previous studies *in vivo*[3]. Recent studies of RIARIO-SFORZA *et al.*[19] proved that the threonine and allothreonine aldolases are localized in the supernatant fraction of rat liver. Sphinganine-1-phosphate aldolase however is a structurally bound microsomal and mitochondrial enzyme.

The *in vitro* incubations require sodium fluoride in order to inhibit phosphatases which rapidly hydrolyse the substrate sphinganine 1-phosphate and the coenzyme pyridoxal phosphate. The requirement for pyridoxal phosphate as cofactor becomes apparent when the enzyme preparation is thoroughly washed. Deoxypyridoxine phosphate inhibits competitively the aldolase reaction. The enzyme carries SH-groups essential for its function since a strong inhibition is caused by sulfhydryl reagents such as *N*-ethylmaleimide, iodoacetamide and *p*-chloromercuribenzoate. The enzymic activity of the di-

[19] G. RIARIO-SFORZA, R. PAGANI and E. MARINELLO, Europ. J. Biochem. [Berlin] **8**, 88 [1969].

hydrosphingosine-1-phosphate aldolase is not impaired by Triton X-100 even at concentrations as high as 20 mg/ml, a property which might facilitate the purification of the enzyme.

KEENAN *et al.*[20] also found independently that the aldolase reaction is ATP dependent, requires pyridoxal phosphate and yields palmitaldehyde.

Some further observations should be mentioned with regard to the metabolic pathways of the two fragments phosphorylethanolamine and palmitaldehyde. We have clearly demonstrated that phosphorylethanolamine is rapidly and efficiently utilized in the biosynthesis of the polar group of phosphatidylethanolamine, phosphatidylcholine and sphingomyelin. Palmitaldehyde is utilized in different ways. It is either oxydized to palmitic acid and incorporated as such, or as its elongation product stearic acid, into ester lipids (triglycerides and phospholipids) and into ceramide and sphingomyelin or is rapidly degraded to CO_2. If one compares roughly the rate of the degradation of dihydrosphingosine administered intravenously in our previous experiments and measured by the respiratory $^{14}CO_2$ with the rate of 0.1 nmole · min^{-1} · mg^{-1} of microsomal protein, the physiological role of this cleavage reaction is obvious.

Recently we observed another pathway for the utilization of the palmitaldehyde released from dihydrosphingosine 1-phosphate[9]. When dihydrosphingosine was administered intraperitoneally and intracerebrally to suckling rats during 10 to 21 days of the postnatal period, the palmitaldehyde was incorporated effectively into the plasmalogens of their brains and livers. Details of these studies will be presented in a subsequent paper[10]. If one takes into account both the biosynthesis [21–25] and the degradation of the long chain bases, some major relationships become apparent: 1) serine and palmitate are utilized by a number of transformations *via* dihydrosphingosine for a) the synthesis of the long-chain bases of the ceramide portion of the sphingolipids, and for b) the transformation of serine *via* dihydrosphingosine into phosphorylethanolamine and phosphorylcholine and represents an additional pathway for the synthesis of the polar groups of the phospholipids and sphingomyelin[26]; 2) palmitate finally may form the ester and amide groups of these lipids and 3) the newly synthesized long-chain base may donate carbon atoms 3—18 as palmitaldehyde for the biosynthesis of the alkenyl ether group of the plasmalogens. The scheme (see p. 1241) summarizes this relationship.

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Experimental

Substrates

[1-^{3}H; 3-^{14}C]Dihydrosphingosine (^{3}H: $5.75 \cdot 10^6$ dpm/μmole; ^{14}C: $2.4 \cdot 10^5$ dpm/μmole), [3-^{14}C]dihydrosphingosine (spec. act. $2.4 \cdot 10^5$ dpm/μmole) were synthesized as described before[27]. The [1-^{14}C]dihydrosphingosine and the phosphate esters of the long-chain bases were prepared according to procedures to be published[28]. The [1-^{3}H]palmitaldehyde was prepared from [1,2-$^3H_2$]heptadecen by reductive ozonolysis and purified as its dimethylacetal.

Cell fractionation

Fractionation into mitochondria, microsomes and supernatant was carried out according to SIEKEVITZ[11]. Beef and pig liver and pig kidney were homogenized first with an Ultraturrax, then centrifuged at $1000 \times g$ and $10000 \times g$ for 12 min each. The $10000 \times g$ supernatant was centrifuged at $100000 \times g$ for 60 min. The transparent brown sediment was used as microsomal preparation. *Hansenula ciferrii* was grown as described before and the microsomal fraction isolated by ultracentrifugation[4].

Incubations

Incubations were composed as described in the legends to tables and figures. They were carried out with constant shaking in a WARBURG apparatus under aerobic conditions. The incubations were stopped by the addition of a chloroform/methanol 2:1 mixture which was also used for the lipid extraction.

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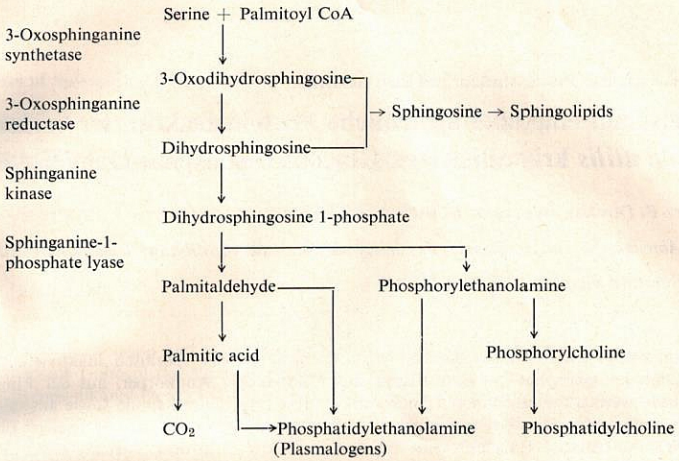

ig. 8.
Metabolic relationships between substrates and products of the biosynthesis and degradation of sphingosine bases.

he extraction process was repeated twice. The two hases were cleared by centrifugation. The chloroform hase was immediately taken to dryness at 30°C in a tream of nitrogen. The residue was transesterified and he acetals of aldehydes formed by refluxing with 5% ydrochloric acid in dry methanol.

Chromatography

he fatty acid methyl esters and dimethylacetals were eparated by thin-layer chromatography, solvent: 1,2-ichloroethane. The radioactive bands were monitored y the thin-layer chromatogram scanning technique sing either the Packard radiochromatogram scanner, model 7201 or that of the Fa. Berthold, model LB 2722. The radioactive bands were recovered by the technique of GOLDRICK and HIRSCH[29] and the compounds eluted with ether. The eluate was concentrated under vacuum. Aliquots were counted in a Packard Tricarb liquid-scintillation counter, model 3380.

The radioactive dimethylacetals were identified by radio-gas liquid chromatography, stationary phase EGS 15% on Kieselgur, column temperature at 160°C, column length 200 cm, argon flow rate 60 ml/min. Radioactive bands were collected discontinuously.

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