

Regulation of *de novo* sphingolipid biosynthesis and the toxic consequences of its disruption

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Abstract

Complex sphingolipids are 'built' on highly bioactive backbones (sphingoid bases and ceramides) that can cause cell death when the amounts are elevated by turnover of complex sphingolipids, disruption of normal sphingolipid metabolism, or over-induction of sphingolipid biosynthesis *de novo*. Under normal conditions, it appears that the bioactive intermediates of this pathway (3-ketosphinganine, sphinganine and ceramides) are kept at relatively low levels. Both the intrinsic activity of serine palmitoyltransferase (SPT) and the availability of its substrates (especially palmitoyl-CoA) can have toxic consequences for cells by increasing the production of cytotoxic intermediates. Recent work has also revealed that diverse agonists and stresses (cytokines, UV light, glucocorticoids, heat shock and toxic compounds) modulate SPT activity by induction of SPTLC2 gene transcription and/or post-translational modification. Mutation of the SPTLC1 component of SPT has also been shown to cause hereditary sensory neuropathy type I, possibly via aberrant oversynthesis of sphingolipids. Another key step of the pathway is the acylation of sphinganine (and sphingosine in the recycling pathway) by ceramide synthase, and up-regulation of this enzyme (or its inhibition to cause accumulation of sphinganine) can also be toxic for cells. Since it appears that most, if not all, tissues synthesize sphingolipids *de novo*, it may not be surprising that disruption of this pathway has been implicated in a wide spectrum of disease.

Introduction

Complex sphingolipids are composed of highly bioactive backbones (sphingoid bases and ceramides) that can cause cell death when the amounts are elevated by turnover of complex sphingolipids, disruption of normal sphingolipid metabolism, or, as is becoming ever more apparent, induction of

sphingolipid biosynthesis *de novo*. Because the participation of sphingolipid turnover in cell regulation has been discussed extensively elsewhere [1–3], this article focuses on sphingolipid intermediates that are formed via the biosynthetic pathway.

Sphingolipid biosynthesis actually produces several bioactive intermediates that parallel the 'mediators' formed from complex sphingolipid turnover: ceramides, sphingoid bases (sphingosine and sphinganine), and sphingoid base 1-phosphates [3,4] (Figure 1). Hence, in addition to the enzymes of sphingolipid turnover that participate in cell signalling (sphingomyelinases, ceramidases, etc.), there are at least six key enzymes in the *de novo* pathway that form/remove these compounds: serine palmitoyltransferase (SPT), which catalyzes the first reaction of the pathway; ceramide synthase, the enzyme responsible for trapping sphinganine as dihydroceramides; dihydroceramide desaturase, which converts relatively biologically innocuous dihydroceramides to ceramides; and enzymes such as sphinganine kinase, sphingomyelin synthase, glucosylceramide synthase, etc. which remove or modify these intermediates.

Serine palmitoyltransferase

SPT is a pyridoxal 5'-phosphate-dependent enzyme that condenses serine with palmitoyl-CoA to produce 3-ketosphinganine (Figure 1). For mammals and yeast, two gene products (termed SPTLC1 and SPTLC2, or sometimes SPT1 and SPT2) are necessary for activity [5] and appear to be associated physically [6]. A third has also been identified in yeast [7]. Relatively little is known about the structure of this enzyme due to its membrane association; however, the recent finding of a soluble, homodimeric SPT from *Sphingomonas* [8] should greatly facilitate structural and mechanistic studies.

The regulation of SPT activity and its role in controlling *de novo* sphingolipid synthesis are only beginning to be understood. One of the first factors that was reported to affect SPT activity was the availability of its substrates, which appear to be present in cells at approximately the apparent K_m

Key words: apoptosis, toxicity, xenobiotic, genetic disease, signal transduction.

Abbreviations used: SPT, serine palmitoyltransferase.

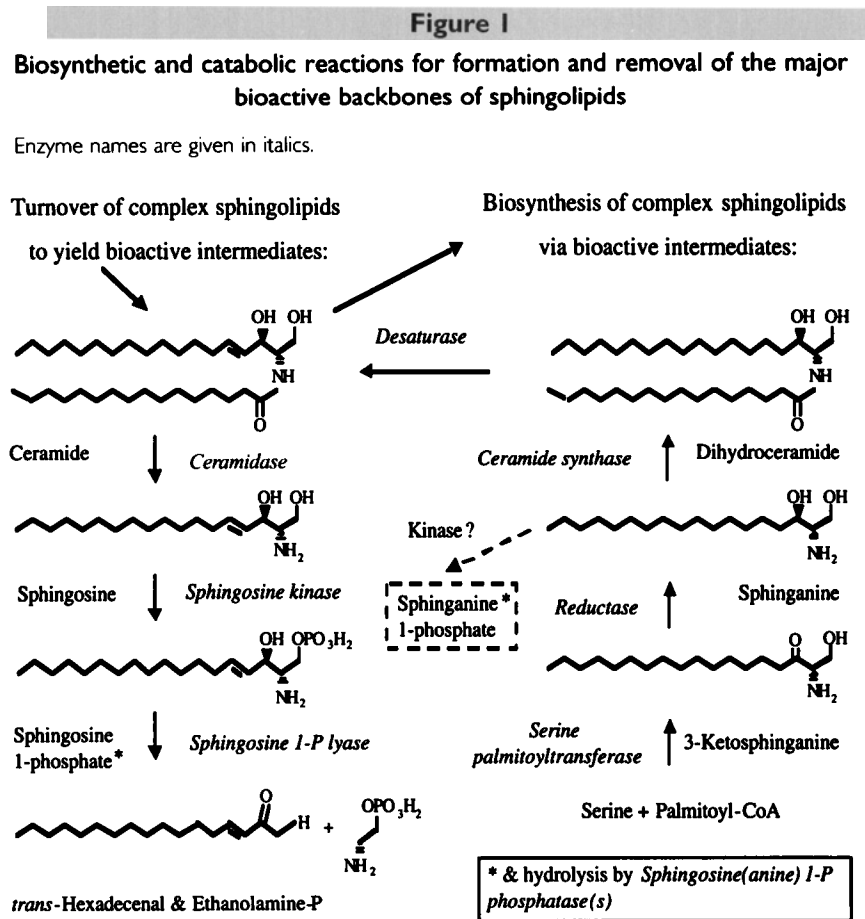
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for this enzyme [9]. Another is the selectivity of SPT for fatty acyl-CoA with 16 ± 1 carbon atoms [10], which probably accounts for the prevalence of long-chain bases of 18 carbon atoms (16 from palmitoyl-CoA and 2 from serine) in most sphingolipids. The required cofactor pyridoxal 5'-phosphate is bound by SPT with an apparent K_d of approx. $1 \mu\text{M}$ [10], and activity is reduced when animals are fed diets deficient in pyridoxine [11].

SPT is inhibited by a number of synthetic and naturally occurring agents. As is the case with many pyridoxal 5'-phosphate-dependent enzymes, it undergoes active-site-dependent ('suicide') inhibition with β -halo-alanines and other aldehyde-reactive compounds such as cycloserine [12,13]. These compounds have been used in studies of cells in culture and *in vivo* (for example, L-cycloserine has been shown to depress the level of sphingolipids in atheromatous plaque) [14]; however, several more potent and selective inhibitors have been isolated recently from microorganisms [sphingofungins, lipoxamycins and myriocin (ISP-1)] [15]. Screens for additional, naturally occurring [16] and synthetic [17] analogues are underway. These inhibitors have been

valuable in identifying the roles of *de novo* biosynthesis in sphingolipid-mediated cell death. However, care must be taken in using the less specific inhibitors [18].

Sphingoid base synthesis can be suppressed by addition of lipoproteins or free sphingoid bases to cells in culture [19,20], perhaps by down-regulation of SPT by sphingoid base 1-phosphates [20]. Regulation at a transcriptional level has been observed with a number of agents, including endotoxins and cytokines [21], UVB irradiation [22], retinoic acid [23], corticosteroids (J. M. Carton, D. C. Argentieri, D. K. Perry, X.-J. Ma, M. Erlander, H. Q. Guo and D. J. Uhlinger, unpublished work) and phorbol esters (Y. Uchida, S. Murata, S. C. Linn, Y. Hirabayashi, A. H. Merrill, Jr and W. M. Holleran, unpublished work). Somewhat paradoxically, we have found that exogenous addition of sphinganine or sphingosine (or poorly phosphorylated sphingoid base analogues, such as 1-deoxy-5-hydroxy-sphinganine) can increase expression of the SPTLC2 gene, but at concentrations near their toxicity and, therefore, may reflect a stress response (L. M. Andras, S. C. Linn, E. M. Keane



and A. H. Merrill, Jr, unpublished work). Most of the instances of transcriptional activation noted to date appear to involve the SPTLC2 gene because little or no change in SPTLC1 mRNA levels have been observed. Nonetheless, mutations in SPTLC1 have been shown to cause hereditary sensory neuropathy type I, which is the most common hereditary disorder of peripheral sensory neurons [24,25], apparently via overproduction of sphingolipids. Much remains to be discovered regarding the functions and regulation of both of these gene products.

A number of recent studies have uncovered post-translational activation of SPT in yeast in response to etoposide [26] and heat shock [27]. The effects of heat shock in yeast are interesting not only with respect to the signalling pathways that are involved (e.g. changes in amino acid transport [28] and activation of ubiquitin-dependent proteolysis [29]), but also because the long-chain bases that are elevated are predominantly eicosasphinganine (i.e. 20-carbon atoms in length) [30].

Ceramide synthase

The next step in sphingoid base synthesis is the reduction of 3-ketosphinganine by an NADPH-dependent reductase. It is generally believed that reduction is rapid. As far as we are aware, there has been only one, as yet unconfirmed, report of complex sphingolipids containing this 3-keto intermediate [31]. Sphinganine is acylated to dihydroceramides by ceramide synthase, which is also apparently rapid under normal conditions because free sphinganine is detectable but in very low amounts [32].

Ceramide synthase utilizes a variety of fatty acyl-CoAs (palmitoyl-CoA, stearoyl-CoA or very long-chain fatty acyl-CoAs, such as C22:0, C24:0, C24:1 or C26:0) [33] and probably represents a family of isozymes. Because it has not been purified, and the gene has only recently been identified [34], very little is known about the enzymology and regulation of this membrane enzyme.

Ceramide synthase is the target of a number of fungal inhibitors [15,35], of which the most thoroughly studied are the fumonisins [35]. Fumonisins cause diseases of veterinary animals (e.g. equine leukoencephalomalacia and porcine pulmonary oedema) and have been implicated in human cancer [36] and possibly birth defects [37]. By inhibiting ceramide synthase, fumonisins not only block the formation of complex sphingo-

lipids, but also cause the accumulation of sphinganine, which appears to be responsible for most of the deleterious effects of these mycotoxins [38]. Fumonisins have been used to inhibit the induction of apoptosis by a number of agents, as will be discussed below. This is of some concern because these mycotoxins not only inhibit ceramide biosynthesis, but they also elevate sphinganine phosphate [39], which might contribute to, or even account for, the inhibition of apoptosis.

Other reactions

The last step of ceramide synthesis is the insertion of the 4,5-*trans* double bond into dihydroceramide as shown in Figure 1 [40]. This is an important reaction because ceramide, but apparently not dihydroceramides, is active in inducing apoptosis [41]. Little is known about the enzyme that catalyzes this reaction, but a number of sphingolipid desaturases have been cloned from plants [42] and the mammalian gene(s) will probably be identified in the near future. Other modifications, such as hydroxylation to form 4-hydroxy-sphinganine, ceramides with α -hydroxyl-fatty acids, etc., should also be better understood in the near future.

Implications of *de novo* sphingolipid biosynthesis in cell death

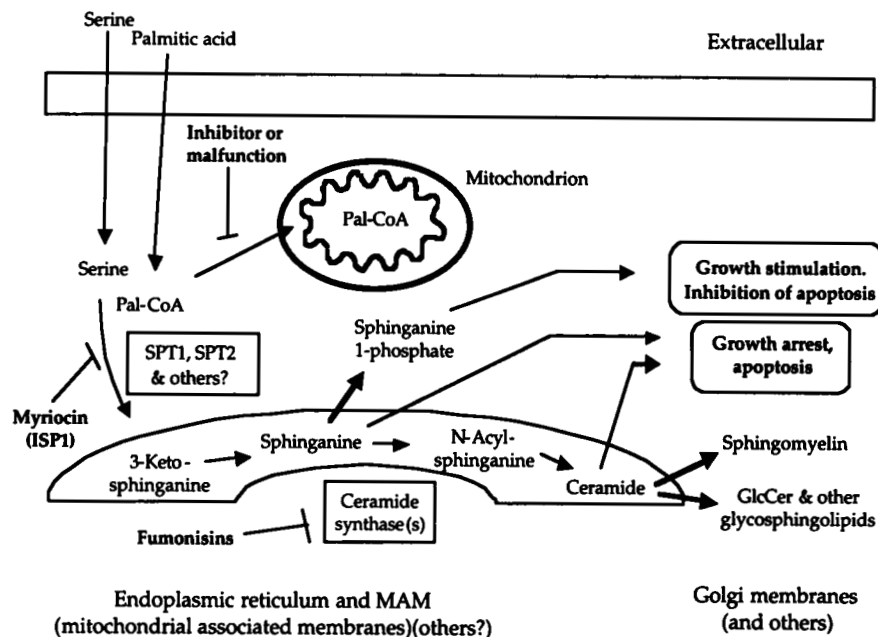
As was mentioned above, the first demonstration of toxicity owing to alteration of *de novo* sphingolipid biosynthesis was the discovery that fumonisins inhibit ceramide synthase [36]. Kolesnick and co-workers [43] subsequently reported that daunorubicin activates ceramide synthase and that *de novo* sphingolipid biosynthesis is critical in daunorubicin-induced apoptosis. Activation of ceramide synthase also appears to be involved in some aspects of phorbol ester- and radiation-induced cell death [44,45]. There are now a large number of agonists and toxic agents (including agents such as angiotensin II and cannabinoids) [46,47] that appear to exert their effects at least in part by increasing sphingolipid biosynthesis.

It is possible that many factors that perturb intermediary metabolism affect sphingolipid biosynthesis and thereby alter the amounts of sphinganine, ceramide, etc. as shown in Figure 2. For example, it has been shown that some aspects of the toxicity of palmitate for cells in culture is due to sphingolipid biosynthesis [48]. In addition, Zucker diabetic fatty rats, which exhibit loss of β -cells by apoptosis, have been shown to have elevated ceramide [49]. Overexpression of SPT

Figure 2

Factors that influence *de novo* formation of the bioactive backbones of sphingolipids

SPT1 and SPT2 are the two components of SPT. The sites of action of commonly used inhibitors (myriocin and fumonisin) are also shown. The subcellular locations of these reactions are indicated only for a general context; there are likely to be other sites where some of these reactions occur. GlcCer, glucosyl-ceramide; Pal-CoA, palmitoyl-CoA.



can also induce apoptosis, as has recently been observed with obese prediabetic *fa/fa* rats [50].

Perspectives

It is not clear why a biosynthetic pathway would produce intermediates with such a diverse spectrum of biological effects and toxicities. One suspects that toxicity reflects severe disruption of 'normal' sphingolipid metabolism and that cells utilize lower amounts of these bioactive intermediates for more subtle regulatory purposes. As often occurs in Nature, pathogenic organisms have developed ways to exploit these pathways for their own purposes, as illustrated by the fumonisins which allow the fungus to kill its host (maize).

Some of the advantages of forming highly bioactive compounds via both complex sphingolipid turnover and *de novo* biosynthesis are: (i) the backbones (sphingoid bases and ceramides) can be increased to higher levels than can be achieved by sphingolipid turnover alone; (ii) the sphingolipid backbone(s) can be formed independently from the cellular status/utilization of complex sphingolipids; (iii) the bioactive compounds may be targeted more directly to the intracellular mem-

branes where they are required to act; (iv) the formation and removal of these species can be integrated with other cell states, such as whether or not the mitochondria are active and utilizing palmitoyl-CoA; and (v) the molecular subspecies (such as the type of fatty acid on the ceramide) can be modified to activate/inhibit downstream targets more selectively.

Understanding these aspects of sphingolipid metabolism should provide new insight into disease and aid in the identification of new pharmacological targets.

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