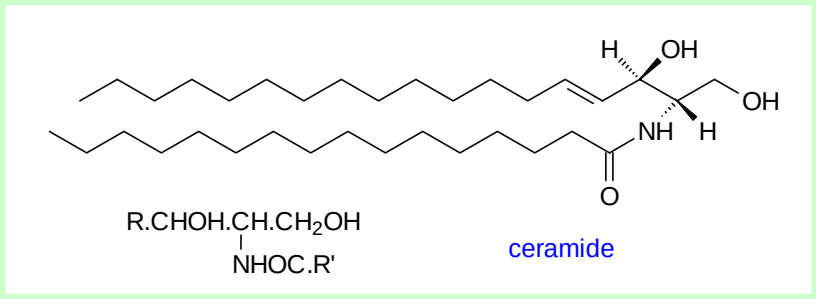
CERAMIDES

STRUCTURE, OCCURRENCE, BIOSYNTHESIS AND ANALYSIS

1. Structure and Occurrence

Ceramides consist of a **long-chain or sphingoid base** linked to a fatty acid via an amide bond. They are rarely found as such at greater than trace levels in tissues, although they can exert important biological effects. Ceramides are formed as the key intermediates in the biosynthesis of all the complex sphingolipids, in which the terminal primary hydroxyl group is linked to carbohydrate, phosphate, *etc*. Unlike the sphingoid precursors, they are not soluble in water and are located in membranes where they participate in raft formation (see below).



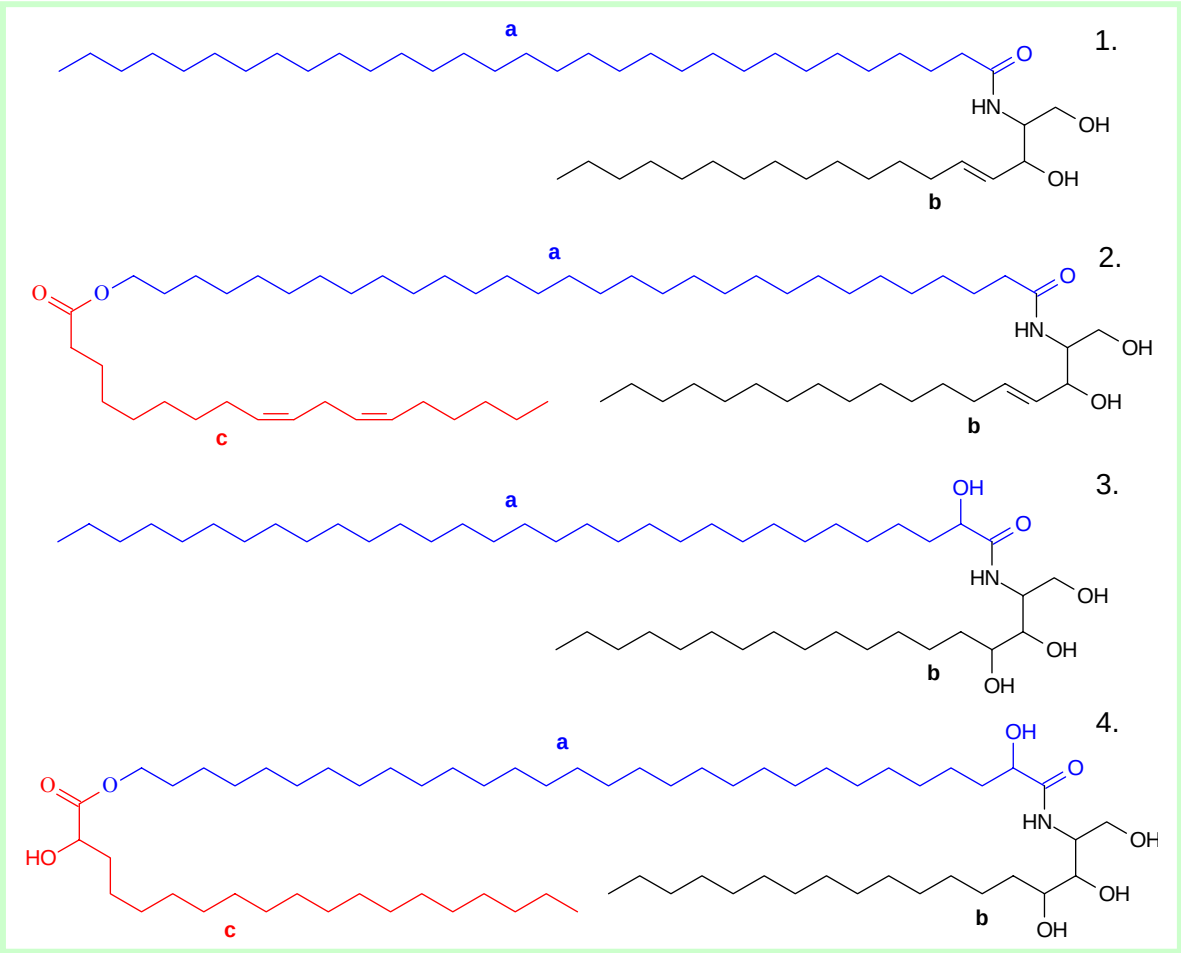
Each organism and indeed each tissue may synthesise ceramides in which there are a variety of di- and trihydroxy long-chain bases linked to fatty acids, the latter consisting mainly of longer-chain (to C_{24} or greater) saturated and monoenoic (mainly (*n*-9)) components, sometimes with a hydroxyl group in position 2. In plants, 2-hydroxy acids predominate sometimes accompanied by small amounts of 2,3-dihydroxy acids. Polyunsaturated fatty acids do not occur other than in certain testicular cells. Ceramides are usually converted rapidly to more complex sphingolipids, including sphingomyelin and the various glycosylceramides, and apart from in the skin the precursors never accumulate. Small amounts of ceramides are produced in all tissues as required for the specific biological functions described below.

2. Skin Ceramides

Exceptionally, the stratum corneum of the skin in which the outer-most layer consists of dead cells contains relatively high levels of ceramides, including O-acylceramides. These are present mainly in the extracellular domains (interstices) and are accompanied by nearly equimolar amounts of cholesterol, and free fatty acids. This ratio is believed to be essential for the normal organization of the tissue into the membrane structures that are responsible for functioning of the epidermal barrier. Ceramides exist both in the free form and linked by ester bonds to structural proteins. The lipid organization in the membranes of skin is different from that of other biological membranes in that two lamellar phases are present, which form crystalline lateral phases mainly, with repeat distances of approximately 6 and 13 nm. Small sub-domains of lipids in a liquid phase may also exist.

Some of these skin ceramides have distinctive structures not seen in other tissues. They can contain the normal range of longer-chain fatty acids (some with hydroxyl groups in position 2), linked both to dihydroxy bases with *trans*-double bonds in position 4 or to trihydroxy bases (*e.g*. formulae 1 and 3 in the above figure). In addition, there are *O*-acyl ceramides in which the long-

chain fatty acid component (typically C_{30}) (a) has a terminal hydroxyl group, which may be in the free form or esterified with either linoleic acid or a 2-hydroxy acid (c); the sphingoid base can be either a di- or trihydroxy base (the latter is not a common feature in sphingolipids of animal origin) (b) (*e.g.* formulae 2 and 4 in the figure above). In addition, the *omega*-hydroxy-ceramides may occur covalently bound to proteins in certain skin cells. Such lipids have been studied in particular detail in the skin of the pig, but they have also been found in humans and rats (see our web pages on **waxes**). In addition, several molecular forms of glucosylceramide, based on similar ceramide structures, have been characterized in skin, and these are also essential for its proper function.



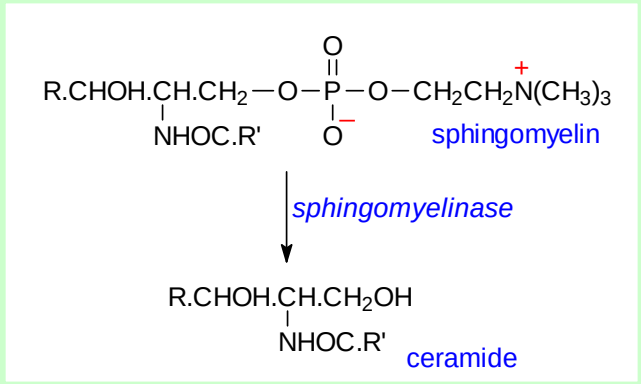
Depending on the particular layer of the skin (epidermis, stratum corneum, *etc*.), the lipid composition can vary. These lipids have an obvious role in the barrier properties of the skin, limiting loss of water and solutes and at the same time preventing ingress of harmful substances. As the aliphatic chains in the ceramides and the fatty acids are mainly non-branched long-chain saturated compounds with a high melting point and a small polar head group, the lipid chains are mostly in a solid crystalline or gel state, which exhibits low lateral diffusional properties with low permeability at physiological temperatures. There is a report that the stratum corneum layer of the skin has a water permeability only one thousandth that of other biomembranes. Natural and synthetic ceramides are now commonly added to cosmetics and other skin care preparations.

The distinctive ceramides in the skin are derived from sphingomyelin and glucosylceramide synthesised in specific organelles termed 'lamellar bodies' in the epidermal cells. These organelles must fuse with the apical plasma membrane of the outermost cell layer of the epidermis in order that their lipid and contents can be secreted. It is only then that the final step of hydrolysis of the lipid precursors with generation of ceramides occurs (see next section). This mechanism ensures that ceramides, with their potentially harmful effects, never accumulate within nucleated cells. In diseased skin, there is often an altered lipid composition and organization and impaired barrier

properties. Thus, diminished levels of ceramide in the epidermis, reflecting altered sphingolipid metabolism, have been implicated in such skin disorders as psoriasis and atopic dermatitis.

Our web page on **waxes** describes the non-polar lipids secreted onto skin by the sebaceous glands.

3. Biosynthesis and Metabolism



The biosynthesis of ceramides *de novo* is discussed in our web pages dealing with **sphingoid bases**, as important structural features of the latter are introduced only when they are incorporated into ceramides. In brief, sphinganine is coupled to a long-chain fatty acid to form dihydroceramide by means of one of several ceramide synthases, before the double bond is introduced into position 4 of the sphingoid base. However, a second CoA-independent pathway may exist in plants. Ceramides are also produced during the catabolism of the complex sphingolipids, for example by the action of one or other of the sphingomyelinases or of phospholipase C on **sphingomyelin** (see our web pages on this lipid) in animal tissues as part of the 'sphingomyelin cycle'. Many agonists including chemotherapeutic agents, tumor necrosis factor-alpha, 1,25-dihydroxy-vitamin D_3, endotoxin, gamma-interferon, interleukins, nerve growth factor, ionizing radiation and heat stimulate hydrolysis of sphingomyelin to produce ceramide. In addition, reversal of the sphingomyelin synthesis reaction may generate ceramide.

Glycosphingolipids can also be hydrolysed by glycosidases to ceramides in tissues, but the process tends to be less important in quantitative terms (other than in skin). The key enzymes of sphingolipid metabolism were first characterized from the yeast *Saccharomyces cerevisiae*, and these were found to be sufficiently similar to the corresponding enzymes in mammals to facilitate their study in the latter.

Much remains to be learned of how the distinctive fatty acid compositions of ceramides and thence of complex sphingolipids are attained (see the **introductory webpage**). As discussed in our webpage on **long-chain bases**, there are specific ceramide synthases that utilize specific fatty acids for ceramide biosynthesis, but it does not appear to be known how these are compartmentalized or regulated within cells.

Most of the ceramide required for the production of complex lipids is synthesised in the endoplasmic reticulum, with subsequent metabolism occurring in the Golgi apparatus. A key cytoplasmic protein, ceramide transporter or 'CERT', mediates the transport of ceramide between these organelles in a non-vesicular manner. It has a phosphatidylinositol-4-monophosphate-binding domain, which targets the Golgi apparatus, and a 'START' domain capable of catalysing inter-membrane transfer of ceramide. There is also a short peptide motif that recognizes a specific protein in the endoplasmic reticulum. The CERT protein extracts ceramides only from membrane bilayers with some specificity for those containing C_{14} to C_{20} fatty acids, and delivers it for the synthesis of sphingomyelin but not for glycosylceramide. The pool of ceramide utilized for synthesis of the latter is delivered to the Golgi by a vesicular transport mechanism.

In animals, catabolism of ceramides by ceramidases, of which three are known yields sphingoid bases and free fatty acids. These are classified according to their pH optima, i.e. acid, neutral and alkaline, and they are located in the lysosomes, the endoplasmic reticulum and Golgi apparatus,

and mitochondria, respectively. However, this reaction can also operate in reverse to generate ceramides in some circumstances. 'Acid' ceramidase is of particular importance, and aberrations in its synthesis or activity is involved in several human disease states. Ceramidases are also present in yeasts and plants.

4. Biological Functions

Ceramides, like other lipid second messengers in signal transduction, are produced rapidly and transiently in response to specific stimuli in order to target specific proteins. While they can be produced by synthesis *de novo*, activation of one of the sphingomyelinases under physiological stress or other agents is a more rapids means of generation in animal tissues at least. Indeed, ceramides appear to be formed under all conditions of cellular stress by a multiplicity of activators in eukaryotic organisms. However, it should be noted that ceramides are formed in different compartments or membranes of the cell with different compositions by a variety of different mechanisms at different times and potentially with distinct functions. In discussing the biological functions of ceramides, it is necessary to consider all of these factors.

It is now recognized that ceramides exert a wide range of biological functions in relation to cellular signalling. They are especially important in triggering apoptosis, and they have also been implicated in the activation of various protein kinase cascades. The mechanism of these interactions is the subject of intensive study at present, but in relation to the latter, two intracellular targets for ceramide action of special important have been discovered – a specific protein phosphatase (ceramide-activated protein phosphatase) and a family of protein kinases (ceramide-activated protein kinases). For example, the phosphatase may be involved in the regulation of glycogen synthesis, insulin resistance and response to apoptotic stimuli.

The role of ceramides in the regulation of apoptosis, and cell differentiation, transformation and proliferation has received special attention. Apoptosis, the process by which a cell actively commits suicide, is essential in many aspects of normal development and is required for maintaining tissue homeostasis. Failure to properly regulate apoptosis can have catastrophic consequences, and many disease states including cancer, diabetes, neuropathies, Alzheimer's disease, Parkinson's disease, and atherosclerosis, are thought to arise from deregulation of apoptosis. For example, ceramide has been implicated in the actions of tumor necrosis factor-α and in the cytotoxic responses to amyloid Aβ peptide, which are involved in Alzheimer's disease and neuro-degeneration. In addition, ceramides appear to be involved in many aspects of the biology of aging and of male and female fertility. The mechanism by which ceramide mediates anti-proliferative pathways or inhibits pro-survival effects not yet well defined, but it may involve regulation of the specific protein phosphatases or kinases. Ceramides are also intimately involved in the induction of autophagy, the 'maintenance' process by which cellular proteins, and excess or damaged organelles are removed from cells.

The biological function of ceramides in animal tissues may require the presence of the 4,5-double bond in the long-chain base, although the *trans* conformation may not be essential in that synthetic ceramide containing a *cis*-4,5-double bond is an equally potent inducer of apoptosis at least. On the other hand, dihydroceramides may have separate functions of their own.

As animals and plants have multiple isoforms of ceramide synthase that are specific for the chain-length of the base and fatty acid, it has been suggested that ceramides containing different fatty acids have distinct roles in cellular physiology. In particular, C_{16} ceramide appears to be especially important in apoptosis in non-neuronal tissues, while C_{18} ceramide has growth-arresting properties and may be involved in apoptosis in some carcinomas treated with chemotherapy agents. In addition, a transferase has been identified that transfers the acetyl group from **platelet activating factor** to sphingosine with a high specificity. The product, *N*-acetylsphingosine - the simplest of all

ceramide molecules, has signalling functions that are distinct from those of the parent lipids or of other ceramides.

In contrast, the ceramide metabolite, **sphingosine-1-phosphate** (see the relevant webpage), has opposing effects on apoptosis and the balance between the two lipids is obviously of great importance. Drug therapies that influence the relative concentrations of these lipids are generating considerable interest, especially in relation to cancer treatment. Pathways mediated by ceramide and sphingosine-1-phosphate have been identified in both the development and progression of cancer, with the former acting to suppress tumors by inducing anti-proliferative and apoptotic responses in cancer cells, and the latter functioning to promote tumor growth. A further ceramide metabolite, **ceramide-1-phosphate**, has anti-apoptosis effects also, as well as being involved in inflammatory responses by activating a specific phospholipase A_2 (see a separate webpage). Again, the balance between the precursor and product is of great biological importance. For practical reasons, the metabolism and functions of these two sphingolipids and of ceramides and sphingoid bases are discussed separately here, but an integrated view is necessary for a full understanding.

Although ceramides and diacylglycerols have structural similarities, their occurrence, location and behaviour in membranes are different. Ceramides cross synthetic lipid bilayers relatively quickly *in vitro*, but it is not clear whether they can flip across more complex biological membranes equally readily, especially in raft microdomains. Restricted flipping could have important effects upon the signalling role of ceramides in that those generated by different enzymes on each side of a membrane could have distinct functions. Ceramides appear to increase the permeability of membranes.

While ceramides are minor components of membranes in general, their physical properties ensure that they are concentrated preferentially into lateral microdomains ('**rafts**' or 'caveolae') with other sphingolipids, especially **sphingomyelin** (see also the web pages on this lipid) and cholesterol. Indeed they can displace cholesterol from rafts, modifying their properties. In addition, ceramides may be generated within rafts by the action of sphingomyelinases, causing small rafts to merge into larger units and modifying the membrane structure in a manner that is believed to permit oligomerization of specific proteins. Through the medium of rafts, they are then able to function in signal transduction. Specific receptor molecules and signalling proteins cluster within such domains, thereby excluding potential inhibitory signals, while initiating and greatly amplifying primary signals. Ceramide-enriched rafts may also provide an entry route into cells for viral and bacterial pathogens.

Comparatively little information is available on the role of ceramides in cell signalling in plants, but there are suggestions that sphingolipid catabolic products may be linked to programmed cell death, signal transduction, membrane stability, host-pathogen interactions and stress responses. Ceramides aggregate in rafts in plant membranes also, together with other sphingolipids and sterols.

5. Analysis

The analysis of ceramides presents no particular problems. They can be isolated by adsorption chromatography (TLC and HPLC), and further analysed by HPLC or GC after conversion to less polar derivatives. Nowadays, modern mass spectrometric methods are increasingly being used for the purpose. One widely used method for analysis of molecular species of sphingomyelin involves hydrolysis with phospholipase C to ceramides to simplify the technical problems.

Recommended Reading

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