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# MOLECULAR ARRANGEMENTS IN SPHINGOLIPIDS

## CONFORMATION AND HYDROGEN BONDING OF CERAMIDE AND THEIR IMPLICATION ON MEMBRANE STABILITY AND PERMEABILITY

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SUMMARY

The preferred conformation of the ceramide part of sphingolipids has been deduced from single crystal structures of a series of sphingolipid constituents: *N*-tetracosanoylphytosphingosine, glycosylphytosphingosine hydrochloride, sphingosine hydrochloride, triacetylsphingosine, DL-2-hydroxytetradecanoic acid and *N*-stearoylethanolamine. The amide group of the ceramide, which serves as a link between the hydrocarbon chains, has a basic significance for the conformation of the entire molecule. This rigid group, which comprises six atoms in a planar conformation, adopts a perpendicular orientation towards the axes of the two hydrocarbon chains. The carbonyl oxygen thereby turns into an eclipsed position with the hydrogen atom at carbon atom 2 of the sphingosine. A parallel chain stacking is achieved by a sharp perpendicular bend of the fatty acid. This bend is produced by a sequence of two $-60^\circ$ rotations about the C-C bonds at both sides of the $\alpha$-carbon atom. The orientation of the hydrogen bond donors and acceptors of the amide group and the hydroxyl groups allow lateral interaction with other lipid molecules. The proposed models are supported by infrared spectra, thin-layer chromatographic behaviour and monolayer studies of synthetic model ceramides.

The functional role of the hydrogen bonding groups in the ceramide part of sphingolipids is emphasized and their significance for the formation of lateral hydrogen bonds within the membrane layer and thereof arising effects on membrane stability and permeability are discussed.

## INTRODUCTION

Sphingolipids are amphipathic constituents of biological membranes [1]. Their hydrophilic part may contain phosphorous, as in sphingomyelins and related ceramide phosphorylethanolamines and -phosphonoethylamines [2], or carbohydrate, as in glycosphingolipids. The latter comprise a great variety of neutral and charged lipids [3, 4] ranging from simple cerebrosides and sulfatides to compounds

with very complex carbohydrate pattern such as blood group-active oligoglycosylceramides and gangliosides.

Ceramide is the characteristic lipophilic part which is common to all these sphingolipids and which anchors them in the hydrocarbon matrix of the membrane. The ceramide is composed of a long-chain amino alcohol to which a long-chain fatty acid is linked by an amide bond. In sphingolipids from different tissues the structure of these lipophilic components exhibits characteristic differences which appear to be related to the function of the particular membrane. The purpose of the present project is to elaborate from physicochemical studies of different synthetic sphingolipid components an overall model of the ceramide conformation in biological membranes which is able to explain structural variations in relation to differences in membrane function.

A great number of long-chain base species differing in chain length, number

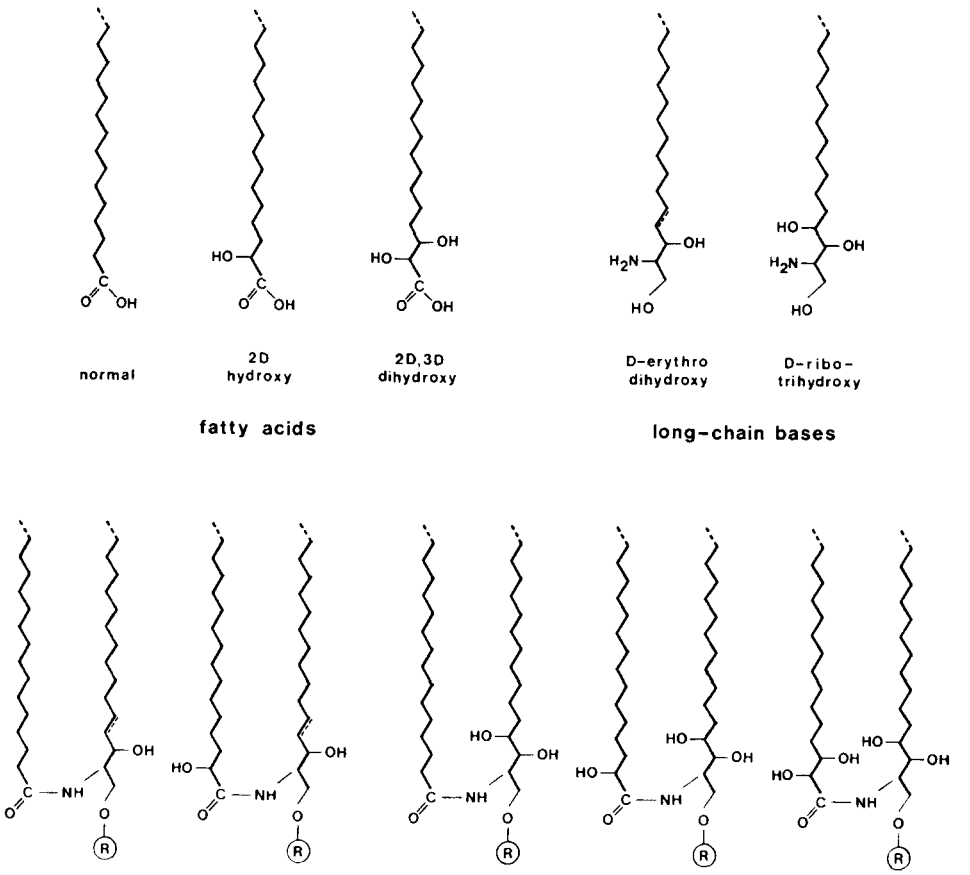



Fig. 1. Lipophilic components of sphingolipids and their most common combinations in different ceramide species, illustrating differences in number and location of hydroxyl groups.

of hydroxyl groups, double bonds and branches [5, 6] have been found in sphingolipids of animals, plants, and bacteria. According to their number of hydroxyl groups they may be classified into two major groups, dihydroxy bases and trihydroxy bases.

Similarly the fatty acids also comprise normal saturated, unsaturated or branched species which in addition may carry a hydroxyl group in D-2 position. Recently also 3-hydroxy [7] and 2,3-dihydroxy fatty acids [8, 9] have been detected in sphingolipids of *Bacteroides* species and yeast, respectively.

The combination of different fatty acids and long-chain bases gives rise to a variety of ceramide species which differ with respect to number and location of their hydroxyl groups [10]. The most common types of fatty acids, long-chain bases and possible ceramide structures are shown in Fig. 1.

The content of free hydroxyl groups and the presence of an amide group enables the ceramide to act both as a hydrogen bond donor and acceptor. This property distinguishes sphingolipids characteristically from glycerolipids (compare Fig. 2). In the latter, which constitute the major part of all membrane lipids, the ester or ether groups of the diglyceride moiety lack hydrogen donors, but can on the other hand function as effective acceptors for the formation of hydrogen bonds.

Generally the presence of hydroxyl or amide groups in a lipophilic compound increases its polarity and thus its interaction with water. In a membrane constituent these groups should therefore in principle counteract unfavourably the hydrophobic effect that keeps the lipid in the membrane layer. Only if these groups participate in lateral hydrogen bonds within the lipid matrix will they considerably increase the

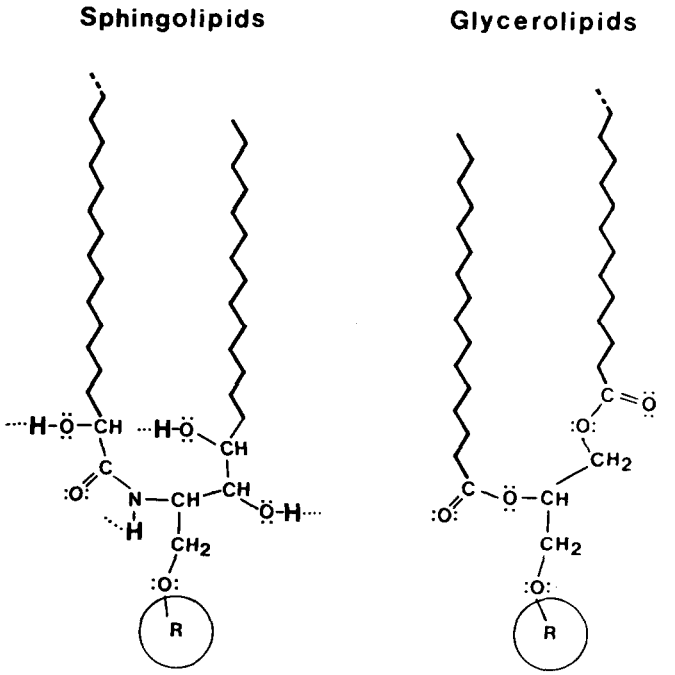



Fig. 2. Differences in hydrogen bond donors and acceptors in ceramide of sphingolipids and diglyceride of glycerolipids.

stability and impermeability of the membrane. These functional aspects of the hydrogen donor groups is highly suggested by the observation that the number of hydroxyl groups in the ceramide part is distinctly higher in sphingolipids of membranes which are exposed to a pronounced physical stress [11–14].

To elucidate the hydrogen bonding capability of different ceramide species it is necessary to investigate their conformation and the orientation of their hydrogen bond donors and acceptors. For this purpose most accurate information can be provided by single crystal structures. It has turned out, however, that it is extremely difficult to grow suitable crystals of sphingolipids and of complex membrane lipids in general.

The present paper is concerned with the preferred conformation and hydrogen bonding capability of the ceramide moiety, as derived from so far available crystal structures of sphingolipid components. The proposed conformation is supported by data from infrared and monolayer studies of synthetic model ceramides. The observed results are discussed in terms of membrane stability and permeability*

## MATERIALS AND METHODS

The resolution of 2-hydroxy fatty acids [15] and the preparation and purity of optically pure model sphingolipids as well as their thin-layer chromatographic properties are in part described elsewhere [10, 16].

The single crystal analyses of triacetylsphingosine [17] and *N*-tetracosanoylphytosphingosine [18] have been published earlier. Detailed data on the crystal structures of sphingosine hydrochloride (Nilsson, B. and Pascher, I., unpublished), glucosylphytosphingosine hydrochloride [19], DL-2-hydroxytetradecanoic acid [20] and *N*-stearoylethanolamine [21] have been submitted for publication or are in preparation.

Infrared spectra were recorded in KBr pellets on Perkin-Elmer infrared spectrophotometers model 157 and 457.

Monolayer studies were performed on a Wilhelmy surface balance. They will be subject to a separate, more detailed discussion (Löfgren, H. and Pascher, I., unpublished).

For description of conformation and torsion angles the convention according to Klyne and Prelog [22] is used.

## RESULTS AND DISCUSSION

The conformation of a molecule is determined both by intramolecular forces and by interaction with surrounding molecules.

An essential property of sphingolipids and of membrane lipids in general is their amphiphathic nature, caused by distinct polar and unpolar parts in their molecules. In different kinds of aggregation states these lipids tend to interact such that polar heads pack with polar heads and unpolar tails with unpolar tails. This usually

* The aspects on conformation and function of ceramide reported here were presented at the Eighth Scandinavian Lipid Symposium in Helsinki, June 8–12, 1975, and at the Tenth International Congress of Crystallography in Amsterdam, August 7–15, 1975.

leads to layer arrangements. In this sense the overall environment of a lipid molecule becomes rather similar, both in a crystal, a monolayer or a micell of a pure lipid, but also in the mixed lipid layer of a biomembrane. This means that intra- and

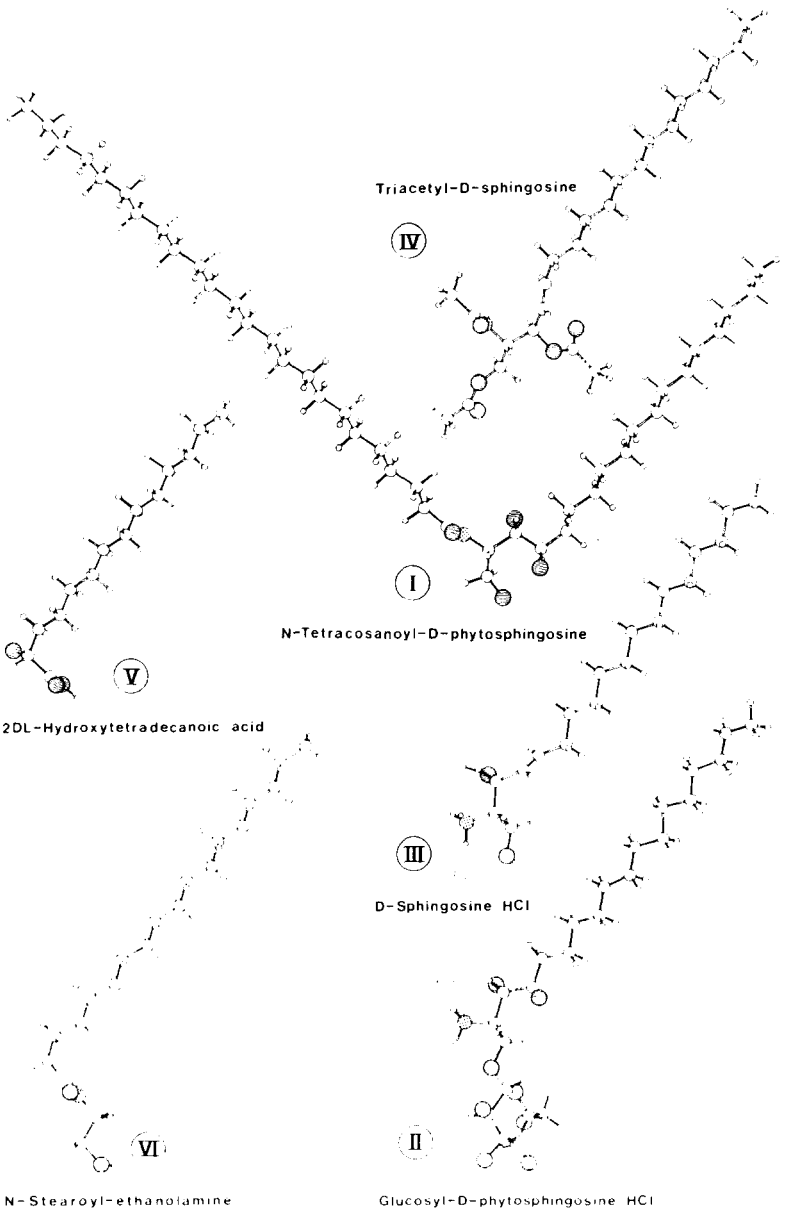



Fig. 3. Conformation of sphingolipid components as derived from single crystal analyses (refs. 17–21 and Nilsson, B. and Pascher, I., unpublished). The long-chain base derivatives (I–IV) are projected with their C-N bond in the paper plane. The amide or carboxyl group of compounds I, II, V and VI is turned almost perpendicularly to the plane of projection.

intermolecular forces which determine the conformation of these molecules will be also balanced in similar ways. If lipid molecules or essential parts of them in the crystal state recur with the same conformation in different derivatives, different crystallographic arrangements and different local environments it reflects the predominance of intramolecular forces as conformation-determining factors. Therefore, a similar overall conformation is to be expected for the lipid also in a natural membrane environment.

In Fig. 3 the conformations of a number of important sphingolipids and sphingolipid constituents are reproduced as they were obtained from single crystal analyses (refs. 17–21 and Nilsson, B. and Pascher, I., unpublished). The different long-chain base derivatives (I–IV) show interesting conformational similarities in the crystalline state. The two fatty acid components (V, VI) provide important information on chain bends. The structural features of these compounds allow general conclusions to be drawn on the conformation of the ceramide part of sphingolipids.

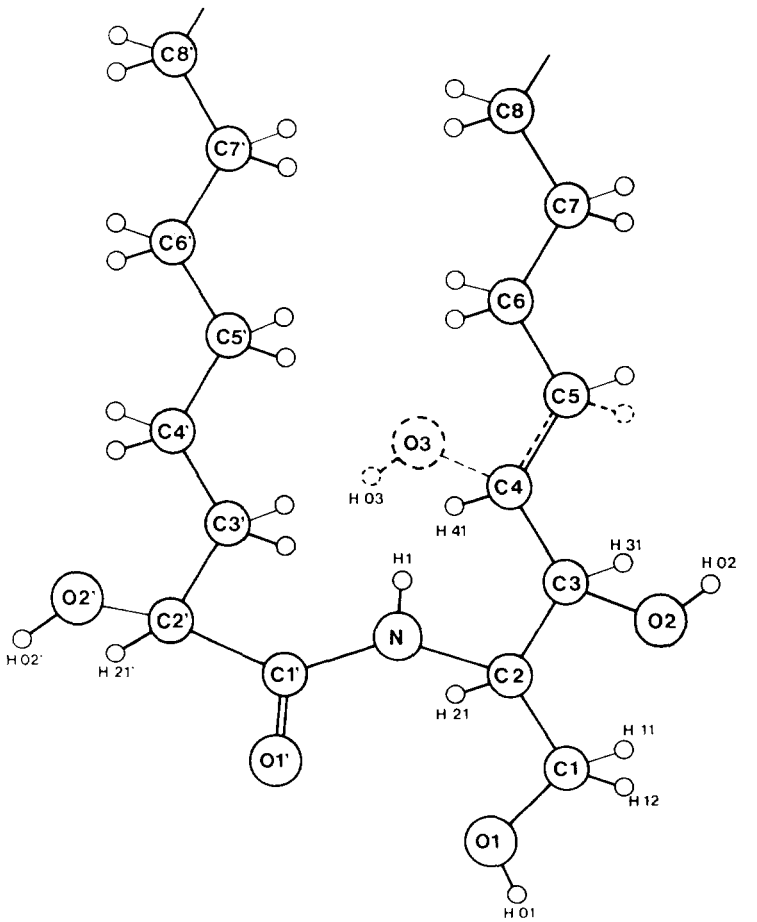



Fig. 4. Atom numbering used for the ceramide molecule.

The atom numbering of the ceramide molecule used in this discussion, is given in Fig. 4.

*The configuration of the amide group*

The amide group is known to adopt a planar resonance structure with a partial double bond character about the CO-NH bond [23]. The rotation barrier counteracting a twist about this bond has been estimated to 21 kcal [24]. As a consequence of this resonance stabilisation the amide group and the attached ligands are all rigidly oriented in a plane as shown in Fig. 5. Moreover, apparently for steric reasons, the two carbon atoms C2 and C2′ which are linked to the amide group predominantly adopt a *trans* configuration, and this is consequently also valid for the carbonyl oxygen (O1′) and the nitrogen-bound hydrogen atom (H1).

This planar conformation and *trans* configuration that is observed for practically all peptide bonds [25] has also been found in the crystal structures of the sphingolipid constituents tetracosanoylphytosphingosine (I), triacetylsphingosine (IV) and *N*-stearoylethanolamine (VI) (see Fig. 3).

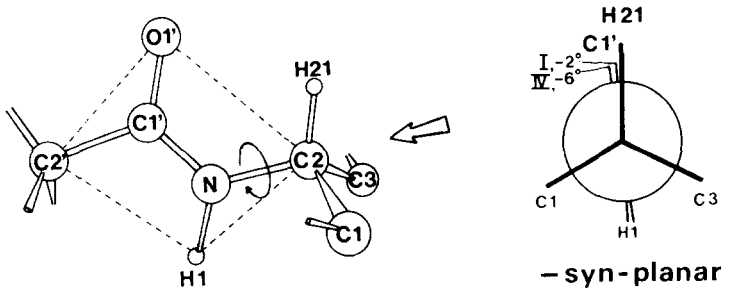



Fig. 5. Configuration of the amide group and its preferred conformation in relation to the sphingosine chain. The torsion angles between C1′ and H21 as seen along the C2-N bond are shown for *N*-tetracosanoylphytosphingosine (I) and triacetylsphingosine (IV).

*Preferred orientation of the amide group towards the sphingosine chain*

As discussed above, the amide group and its closest ligands are fixed in a planar conformation. This rather large rigid group serves as a link between the hydrocarbon chains of the long-chain base and the fatty acid. Its orientation towards these chains has therefore fundamental significance for the conformation of the entire ceramide molecule.

The orientation of the amide group plane towards the sphingosine chain is determined by the torsion angle about the C2-N bond. Rotation about this bond, which connects the amide group to the asymmetric secondary sphingosine carbon atom (C2) is sterically restricted to a considerable extent, due to collision of the contact radii of the carbonyl oxygen (O1′) with those of the chain carbon atoms (C1) and (C3).

Potential energy calculation for the corresponding C-O bond of secondary esters [26, 27] show two distinct energy minima which favour conformations where the carbonyl group adopts an approximately staggered position with torsion angles of about $\pm 40°$ in relation to the hydrogen atom, that corresponds to H21 in Fig. 5. For

the amide group of compounds I and IV, however, the carbonyl carbon (C1′) and the hydrogen atom (H21) are found in an eclipsed (or *syn-periplanar*) position to each other. The torsion angles between these atoms, as seen along the C2-N bond are given in Fig. 5 and show only minor deviations from an exactly eclipsed orientation. By this conformation the plane of the amide group is turned into a perpendicular or almost perpendicular orientation towards the axis of the sphingosine chain, and the hydrogen atom (H21) is located in or close to the plane of the amide group.

A similar eclipsed orientation of the amide group is found for several peptides and for the parallel-pleated sheet of polypeptides [24].

If this preferred eclipsed conformation is determined by intramolecular nonbonded forces or is induced by intermolecular interactions cannot be decided unambiguously. In all these compounds the carbonyl oxygen (O1′) and the nitrogen-bound hydrogen atom (H1) of the amide group are involved in strong intermolecular hydrogen bonds.

However, the secondary ester group of phosphatidylethanolamine [28], which closely resembles an amide group, but completely lacks hydrogen bonds, deviates from a corresponding eclipsed conformation by approx. 15° only. This indicates that also for this bond the eclipsed arrangement is a favoured conformation rather than the staggered one.

*Conformation of the fatty acid hydrocarbon chain*

In a membrane layer the polar hydrophilic head groups of lipids will keep contact with the aqueous environment, while the hydrophobic paraffin chains are expelled from water and are expected to adopt a more or less parallel orientation in the lipophilic matrix of the membrane. Crystal structure of sphingolipids with two parallel hydrocarbon chains which can supply information on chain folding in vicinity of the amide group, are at present not available.

The only double chain sphingolipid analyzed so far, a ceramide (tetracosanoylphytosphingosine, compound I in Fig. 3) crystallizes in a V-shaped conformation which allows a favourable accommodation of the polar molecule part in the crystal lattice. Conclusions on the conformation and folding of the fatty acid chain near the carboxyl end, however, can be derived from the crystal structures of *N*-stearoylethanolamine (VI) and DL-2-hydroxytetradecanoic acid (V) which are shown in Fig. 3.

In both compounds the hydrocarbon chain has a sharp bend at the α-carbon atom C2′. Instead of the *anti-periplanar* zig-zag conformation which is usually adopted in straight chain fatty acids *N*-stearoylethanolamine has an (−) *anti-clinal*, (+) *syn-clinal* conformation about the N-C1′-C2′-C3′ and C1′-C2′-C3′-C4′ bonds with torsion angles of −129° and +70°, respectively (compare Fig. 6a). This sequence of torsion angles turns the plane of the amide group almost perpendicularly toward the axis of the remaining fatty acid chain.

A similar bend conformation is reported for the fatty acid attached to the β-carbon atom of glycerol in phosphatidylethanolamine [28]. The torsion angles about corresponding bonds are −133° and +74°, respectively.

In the 2-hydroxy fatty acid (V) the chain bend at carbon atom C2′ is produced by another sequence of torsion angles. A (−) *syn-clinal*, (−) *syn-clinal* conformation about the C1′-C2′ and C2′-C3′ bond with torsion angles of −63° and −58°, respec-

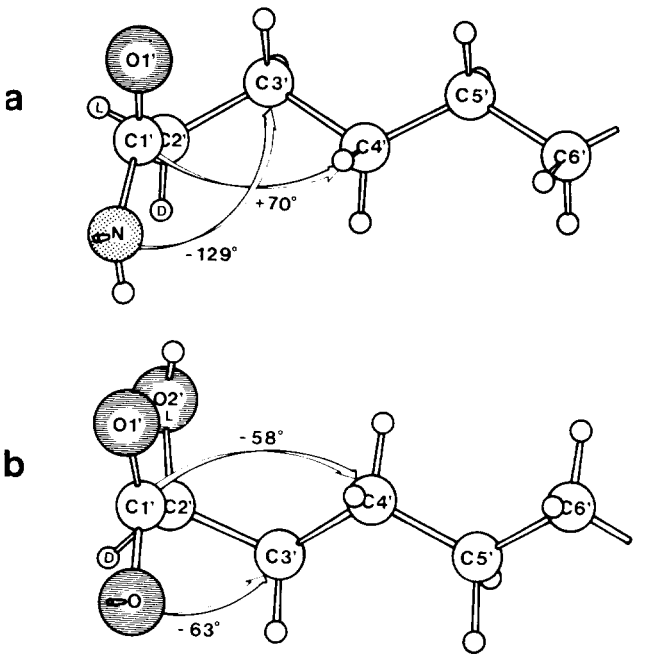



Fig. 6. Chain bends of the fatty acid in vicinity of the carboxyl end are shown for (a) *N*-stearoylethanolamine (VI) and (b) DL-2-hydroxytetradecanoic acid (V). The torsion angles about the C1′-C2′ and C2′-C2′ bond, which produce approximately perpendicular bends of the chain axis, are indicated by arrows.

tively, again produce a perpendicular (89°) orientation of the carboxyl group toward the chain axis.

The chain conformation of the two compounds is given in Fig. 6. Both produce an essentially similar perpendicular bend by either a double (−) *syn-clinal* or (+) *syn-clinal* twist at both sides of C2′. The bend of *N*-stearoylethanolamine may be regarded as a (+) *syn-clinal* (+51°), (+) *syn-clinal* (+71°) conformation if it is related to the carbonyl oxygen (O1′) rather than to the chain-connecting nitrogen atom. The only difference arising from the two sequences of torsion angles is that the undistorted zig-zag planar portion of the chain starts at carbon atom C2′ in one case with an up down (zig-zag) and in the other case with a down-up (zag-zig) pattern. This has, however, consequences for the conformation of a substituent at the α-carbon (see below).

The observed perpendicular orientation of the amide group, both towards the sphingosine chain and towards the fatty acid chain will result in a parallel arrangement of the two hydrocarbon chains. This becomes evident from Fig. 3 if the normal straight chain fatty acid in compound I is exchanged by one of the bent chain acids in compound V or VI. In such a molecule the hydrogen donor (H1) and acceptor (O1′) of the amide group adopt a perpendicular orientation with respect to the hydrocarbon chain axes of the lipid.

*Orientation of the hydroxyl groups*

The orientation of both the amide group and the hydroxyl groups in the ceramide is of great interest, as they determine the capacity of the molecule to form intra- or intermolecular hydrogen bonds. In natural sphingolipids the amine group and the hydroxyl group(s) of the long-chain base [5] and also the hydroxyl group(s) of the fatty acid [15] have all D-configurations. These stereospecific configurations together with the requirement of parallel hydrocarbon chains and the preferred perpendicular orientation of the amide group between these chains, considerably restricts the number of possible conformations of the hydroxyl groups.

*The hydroxyl groups of the long-chain base.* As the primary hydroxyl group on C1 of the long-chain base usually is involved in the covalent linkage with the polar head group, only the remaining secondary hydroxyl group(s) are of interest as hydrogen donors. Information on the conformation of these groups are available from the crystal structures of compounds I, II and III in Fig. 3. In all three compounds the hydroxyl group O2 on C3 adopts a (−) *syn-clinal* staggered conformation in relation to the nitrogen on C2. The torsion angles between N and O2 as seen along the C2-C3 bond are shown in Fig. 7 and fall within a range of −53 to −67°.

For triacetylsphingosine (IV) in which the hydroxyl groups are acetylated, the corresponding torsion angle is 180°. This places the nitrogen and the oxygen (O2) *anti-planar* to each other.

Trihydroxy bases (phytosphingosines) contain an additional hydroxyl group on C4. In the two compounds I and II so far investigated O2 and O3 adopt the *anti-planar* staggered conformation with a torsion angle of approx. −170° (see Fig. 7).

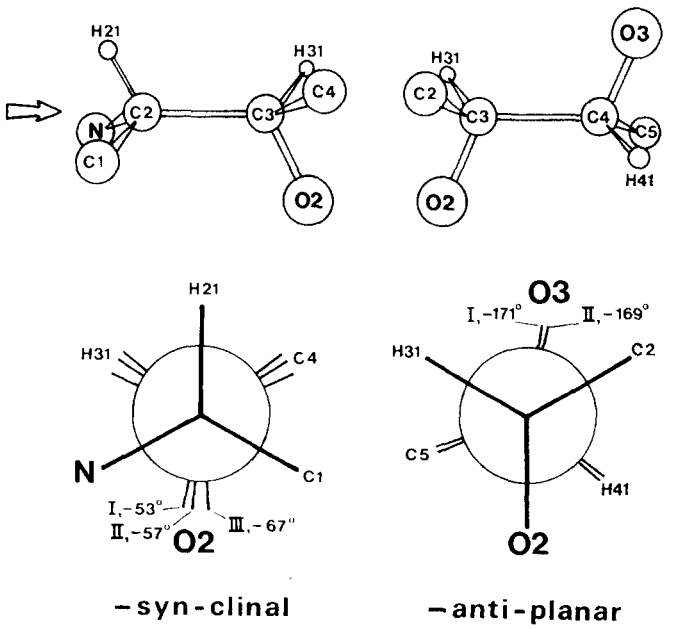



Fig. 7. Torsion angles between the nitrogen atom and the hydroxyl oxygen atoms O2 and O3 of the sphingolipid base, illustrating the conformational resemblance observed for *N*-tetracosanoylphytosphingosine (I), glycosylphytosphingosine hydrochloride (II) and sphingosine hydrochloride (III).

In all the three long-chain base compounds I–III (Fig. 3) the nitrogen and the hydroxyl groups are involved in different intermolecular hydrogen bonds with adjacent molecules or ions of the crystal lattice. In spite of the varying environments, however, the mutual orientation of the nitrogen and the hydroxyl groups is identical in these three compounds and appears to reflect a favoured conformation. The preferred 60° staggered orientation of O2 toward the ammonium or amide group has been generally observed for e.g. serine or serine containing peptides [24, 29] and reflects an electrostatic attraction between the positively polarized hydrogen (H1) of the amide group with the $\pi$ electrons of O2. The *anti-planar* conformation of the vicinal hydroxyl groups in phytosphingosine may be explained by electrostatic repulsion of the two negatively polarized oxygens.

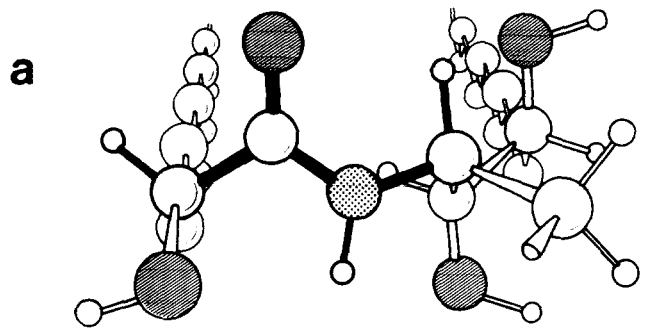
*The hydroxyl group of the fatty acid.* So far no crystal structure of a sphingolipid with a 2-hydroxy fatty acid has been solved. Again the structure of DL-2-hydroxytetradecanoic acid (Fig. 3, V) provides important information on the conformation of the fatty acid hydroxyl group.

The $\alpha$-hydroxyl group of this compound adopts an eclipsed, *syn-planar*, position to the carbonyl oxygen (compare also Fig. 6b). The torsion angle between these groups as seen along the C1′-C2′ bond is −7°. This coplanar orientation appears to be a preferred conformation due to the formation of an intramolecular hydrogen bond between the $\alpha$-hydroxyl group and the carbonyl oxygen.

If this perpendicularly bent hydroxy acid is attached to sphingosine as discussed earlier, a ceramide with the requested parallel chains is obtained, in which both the oxygen O2′ and the hydrogen HO2′ of the $\alpha$-hydroxyl group become coplanar with the whole amide group. Such a ceramide model is shown in Fig. 8b in a view straight onto the plane of the amide group.

However, if attention is paid to stereochemistry and to the preferred conformations of the long-chain base and the amide group it becomes obvious that this coplanar configuration and hydrogen bonding of the $\alpha$-hydroxyl group only can be achieved with a 2-hydroxy fatty acid which has the unnatural L-configuration. A hydroxy fatty acid with natural D-configuration attached to the long-chain base in the same manner ends up with a conformation as shown in Fig. 8a, which is not consistent with the crystal structure of the free acid. The $\alpha$-hydroxyl group continues in the zig-zag direction of the hydrocarbon chain and cannot be brought into close contact with the carbonyl oxygen without drastical changes of the conformation of the entire molecule.

The two models shown in Fig. 8 are primarily based on the crystal conformation of tetracosanoylphytosphingosine (I) and of DL-2-hydroxytetradecanoic acid (V). A substitution of the long-chain base with a fatty acid with the bent conformation as observed for *N*-stearoylethanolamine (VI) would cause a change in the orientation of the $\alpha$-hydroxyl group. As is apparent from Fig. 6a, a 2-hydroxyl group with L-configuration would continue in the zig-zag direction of the hydrocarbon chain while a D-hydroxyl group would come into close contact with the amide nitrogen. As the electron pair of the nitrogen is engaged in a resonance with the carbonyl group, it will not serve as a hydrogen acceptor. On the other hand, the nitrogen could in this case participate as a hydrogen donor in a bond towards the $\alpha$-hydroxyl oxygen. This would in its turn have a directing effect on the hydrogen bond in which the $\alpha$-hydroxyl group can serve as a donor. The elucidation of this possibility as well as questions



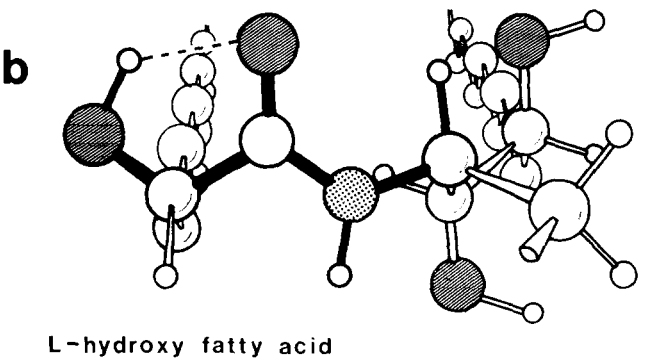




Fig. 8. Deduced conformation of ceramide. The models summarize the preferred conformational features observed in different ceramide components. The two molecules differ with respect to the configuration of the fatty acid hydroxyl group. They are seen with the amide group projected towards the viewer and with the hydrocarbon chains pointing away. All atoms connected with solid bonds are localized in or close to the plane of the amide group.

which concern the conformation of the hydrocarbon chains of the different long-chain base species has to be subject to further investigations. The rigid *trans* double bond of sphingosine (III) does not admit an adequate chain packing if the hydroxyl group O2 is kept in a (—) *syn-clinal* conformation towards the nitrogen. Therefore, rather an *anti-planar* orientation as found in the triacetylderivative (IV) has to be adopted in condensed layers (Löfgren, H. and Pascher, I., unpublished). There are, however, a series of observations which clearly support the overall conformation of the ceramide proposed in Fig. 8 especially with respect to the amide group – fatty acid orientation. If the given models are correct considerable differences in physical properties are to be expected for the two diastereomeric compounds,

While in the natural D-compound the fatty acid hydroxyl group points away from the molecule and will be capable of intermolecular interactions, the hydroxyl group of the L-compound will adopt a close contact with the carbonyl oxygen and interact with it in an intramolecular hydrogen bond. This formation of an intra-

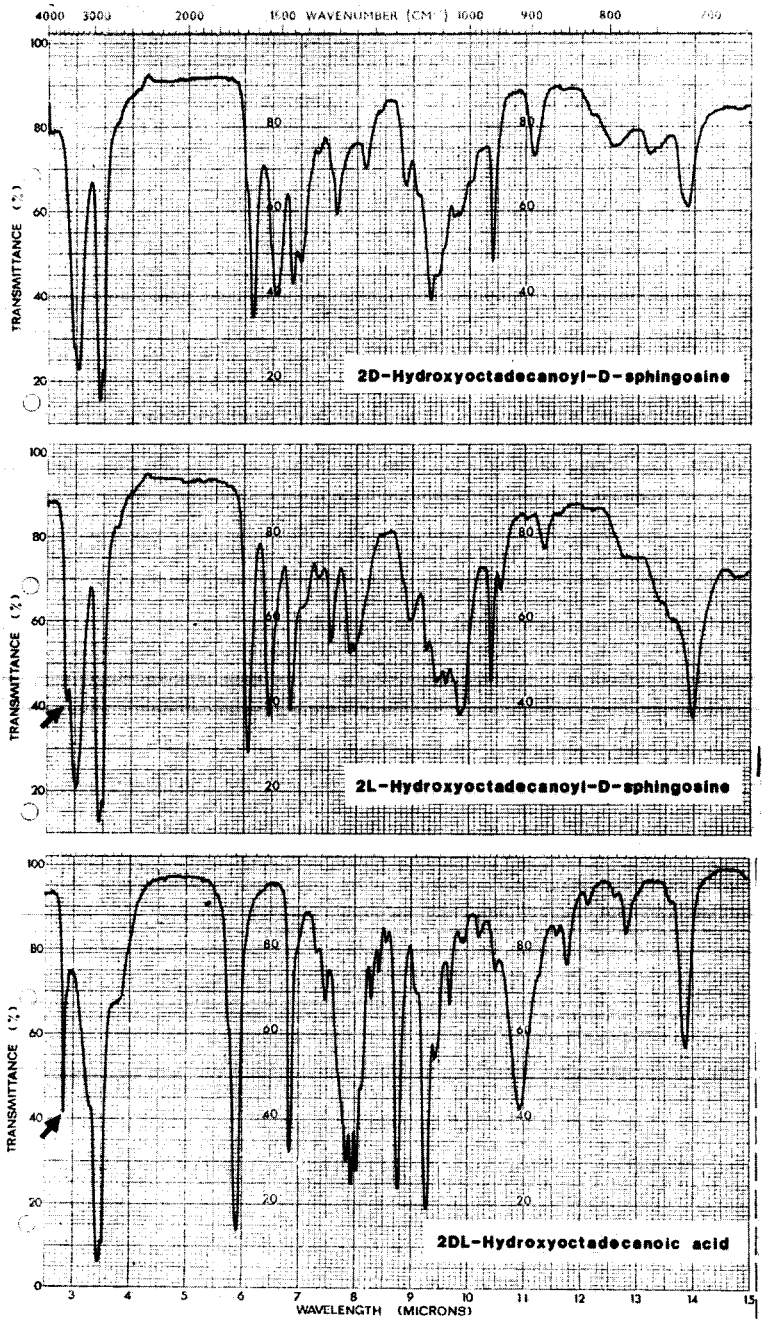



Fig. 9. Infrared spectra of diastereomeric ceramide species containing D-sphingosine (D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene) in combination with D-and L-2-hydroxyoctadecanoic acid, respectively. For comparison the spectrum of DL-2-hydroxyoctadecanoic acid is included. The arrows show the sharp O-H stretching absorption at 3515 $cm^{-1}$ which is characteristic for an intramolecular hydrogen bond.

molecular hydrogen bond should be recognizable by spectroscopic methods. Moreover, the internal compensation of polar forces would lead to a decrease in polarity which should be detectable by other physical methods.

*Experimental support of the postulated ceramide conformation*

A series of ceramides was synthesized from long-chain bases with different numbers of hydroxyl groups and natural all D-configuration by combination with either D- or L-2-hydroxy fatty acids. The observed behaviour of these diastereomers is in perfect agreement with properties expected for the predicted ceramide models.

*Infrared spectroscopy.* Evidence for the engagement of the L-2-hydroxyl group of the fatty acid in an intramolecular hydrogen bond in the solid state is obtained by infrared spectroscopy. In Fig. 9 the spectra of a pair of diastereomeric ceramides are compared with the spectrum of the DL-2-hydroxyoctadecanoic acid. The natural ceramide with D-2-hydroxy fatty acid exhibits a broad absorption band at 3400–3300 $cm^{-1}$. This peak comprises the stretching vibrations of the different O-H groups and the N-H group and the relatively low frequency shows that all groups are involved in fairly strong polymeric hydrogen bonds [30]. In the spectrum of the L-isomer there is an additional sharp peak in this region with a frequency of 3515 $cm^{-1}$ indicating a hydroxyl group which is free or participates in a very weak single bridged hydrogen bond [30]. A hydroxyl stretching vibration with the same high frequency is found for one of the crystal forms of DL-2-hydroxy fatty acid. The single crystal analysis of DL-2-hydroxytetradecanoic acid revealed that this very characteristic O-H stretching frequency at 3515 $cm^{-1}$ is caused by the $\alpha$-hydroxyl group which is involved in a weak intramolecular hydrogen bond with the adjacent carbonyl oxygen. Based on the results from the crystal structure analysis and on the infrared spectra of this 2-hydroxy fatty acid, safe conclusions on the solid state conformation of the fatty acid L-hydroxyl group in the ceramide molecule can be drawn which confirm the postulated models. The preferred fatty acid conformation observed in crystalline ceramides is apparently also adopted under the following two non-crystalline conditions.

*Thin-layer chromatography.* Diastereometic pairs of ceramides which only differ with respect to their configuration of the fatty acid hydroxyl group exhibit on thin-layer chromatography considerable differences in their mobility [10]. As shown in Fig. 10, the stereoisomers which contain the L-hydroxy fatty acid always travel faster than the corresponding D-compounds. This increase in mobility reflects a decrease in polarity, which is consistent with the postulated internal neutralisation of polar forces in the unnatural L-hydroxy derivatives.

*Surface balance.* Important information on conformation and hydrogen bonding capacity of different ceramides and other sphingolipids is obtained from studies of their monolayer behaviour (Löfgren, H. and Pascher, I., unpublished).

These studies provide the most interesting results concerning properties and function of the hydroxyl groups in the lipophilic components of these membrane lipids. Fig. 11 shows the pressure isotherms of two diastereomeric ceramide species with D- and L-hydroxy fatty acid. On compression the surface pressure of the L-compound rises slowly from an area of about 80 $Å^2$/molecule until the layer reaches the collapse point at 39 $Å^2$/molecule.

For the natural D-compound no surface pressure is observed down to a molecular area of 45 $Å^2$. Beyond this value the pressure increases rapidly and the film

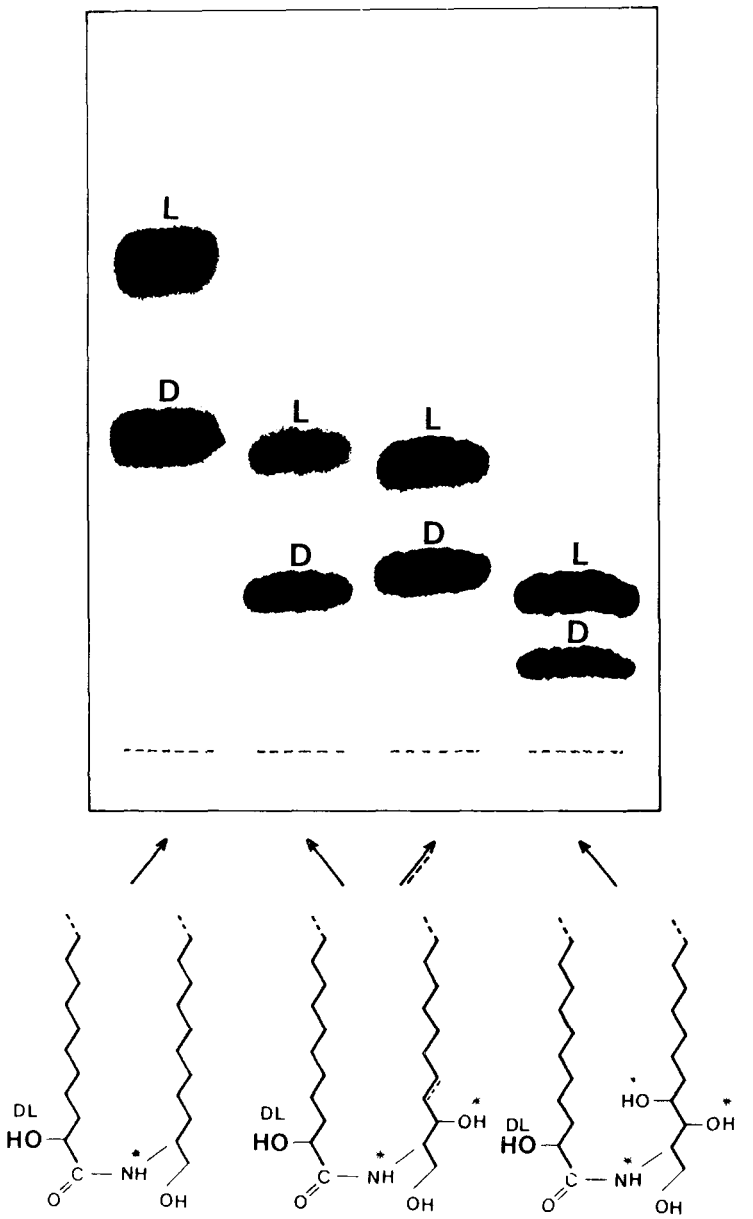



Fig. 10. Thin-layer chromatographic mobility of diastereomeric pairs of ceramides, which differ with respect to the configuration of the 2-hydroxyl group on the fatty acid [10]. The bases in the different ceramides pairs are: lane 1: 1-hydroxy-D-2-aminooctadecane (sphingine); lane 2: D-*erythro*-1,3-dihydroxy-2-aminooctadecane (dihydrosphingosine); lane 3: D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene (sphingosine); lane 4: D-*ribo*-1,3,4-trihydroxy-2-aminooctadecane (phytosphingosine). Solvent: chloroform/methanol (95 : 5, v : v).

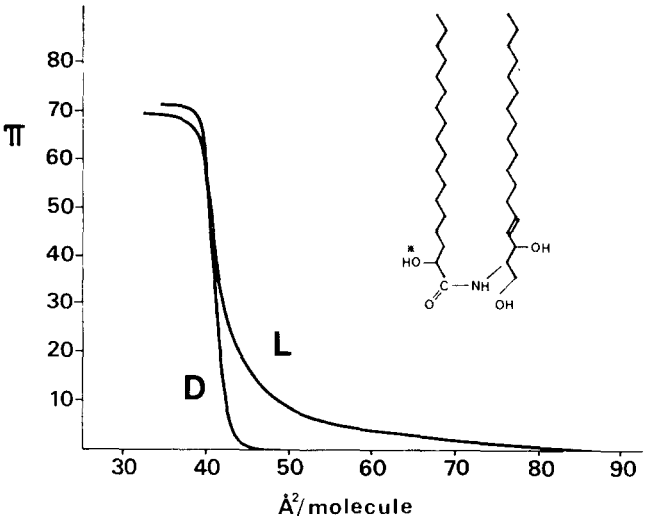



Fig. 11. Surface pressure vs. area isotherms (22 °C) for diastereomeric ceramides containing D-sphingosine in combination with D- or L-2-hydroxyoctadecanoic acid. The infrared spectra and thin-layer chromatogram of these species are given in Fig. 9, and Fig. 10 (lane 3), respectively.

collapses at 39 $Å^2$/molecule. These results indicate that the D-compound on the water surface spontaneously transforms to a condensed film with close molecular packing, while on the other hand considerable pressure has to be applied to condense the L-compound. The observed self-condensation of the natural ceramide is satisfactorily explained only by the formation of hydrogen bonds between the lipid molecules, due to the stereospecific orientation of their hydrogen donor and acceptor groups. This behaviour has to be considered as another strong support of the discussed ceramide conformation. Simultaneously it gives new interesting aspects on the structure-function relation of the ceramide part of sphingolipids.

*Lateral hydrogen bonds and their implication on membrane stability*

The major forces that keep lipids in place in the membrane arises from the hydrophobic effect of their hydrocarbon chains. The change in free energy for the transfer of a hydrocarbon from a lipophilic phase into an aqueous environment or vice versa has been estimated to approx. 1 kcal per methylene group [31]. Consequently, a total of about 30–40 kcal is necessary to remove a lipid with two hydrocarbon chains from a membrane layer. Lateral attractions between hydrocarbon chains within the layer, however, are generally due to van der Waals forces, and thus rather weak, especially when lipids are in the liquid crystalline state. From cross-sectional areas and resulting interchain distances of lipids in monolayers the magnitude of van der Waals forces can be estimated to about 2–3 kcal per hydrocarbon chain [32, 33]. The formation of a lateral hydrogen bond within the lipophilic matrix would contribute with a bond energy of 3–10 kcal to the lipid-lipid interaction and thus considerably increase stability and impermeability of the membrane.

Biochemical studies at this department have revealed significant differences in the hydroxyl group content of the ceramide structures of sphingolipids from differ-

ent tissues, which may be related to the function of the particular membrane. In mammals, sphingolipids with more hydroxylated ceramides (trihydroxy base and hydroxy fatty acid) are primarily found in kidney [11] and intestine [12, 13], and similar organ-specific ceramide compositions have also been reported for sphingolipids of invertebrates, e.g. for the ceramide-*N*-methylaminoethylphosphonate of the viscera of shellfish [34]. For dog small intestine it could be shown that glycosphingolipid of glandular cells, exposed to the intestinal lumen contained the more hydroxylated ceramides, while sphingolipids of the lamina propria contained dihydroxy base and normal fatty acid [14]. Both the kidney tubuli and the intestine membranes are exposed to a pronounced physical stress, due to perpetual changes of their environment. Especially the digestion fluid with high concentrations of lipophilic components, both in monomeric form or as lipophilic phases, such as bile salt micelles, exert a high demand on membrane stability. A lipid exchange, demonstrated experimentally on both model systems and natural membranes may give rise to a deteriorating effect on the membrane function and is considered to be one of the reasons for pathological changes in the membranes of aorta intima [35] or erythrocytes [36] which are observed in association with increased lipid concentration in blood.

One way of creating a higher stability of surface membranes may be to increase lateral hydrogen bonding by addition of hydroxyl groups to sphingolipids. As hydroxyl groups can act both as hydrogen bond donors and acceptors each group will normally give rise to the formation of two bonds. If these bonds were directed to water molecules the introduction of each additional hydroxyl group in the sphingolipid would increase the lipid-water contact, either by moving the molecule selectively further out of the membrane layer or by increasing its molecular area at the membrane interface. These effects are both very unlikely as they would actually decrease membrane stability by reducing or preventing an efficient packing of the hydrocarbon chains. The formation of a boundary of lateral hydrogen bonds at the layer interface would on the other hand promote a close packing in the hydrocarbon chain matrix and thus create a barrier effect both for unpolar and polar molecules. Comparative monolayer studies on sphingolipids with varying numbers of hydroxyl groups and glycerolipids with normal fatty acids which lack hydrogen donors in their diglyceride moiety, in fact indicate the significance of hydrogen bonding of this type for the condensation state and stability of the lipid layer (ref. 37 and Löfgren, H. and Pascher, I., unpublished).

Sphingolipids occur predominantly in the plasma membrane of the cells and are considered to be located in the outer half of the bilayer [38]. In membranes such as myelin [39] or in the microvillus membrane of small intestine [40] sphingolipids constitute about 25 % of the total lipids and thus should make up half of the lipids of the outer layer. As the hydrogen donors of ceramide can interact with free electron pairs of ester and ether groups in glycerolipids, the entire lipid layer of the membrane may be interconnected by a network of hydrogen bonds at the boundary between hydrocarbon matrix and polar head groups. The effectivity of this network, and thus of membrane stability and permeability, may be regulated to fit specific requirements of different cell types by variations in ceramide structure and lipid composition.

The suggested functional significance of hydrogen bonding groups in membrane lipids is also indicated by recent studies on the effect of supplementations of branched chain fatty acids to *Tetrahymena pyriformis* [41]. These studies have shown

that, parallel with an increased incorporation of methyl-branched fatty acids there is a considerable increase of hydroxy fatty acids in the membrane lipids of this ciliate. As branched chain hydrocarbon chains affect the cross-sectional area and thus the lateral interaction of lipids, this disturbance appears to be balanced by the simultaneous increase in hydrogen bonding through hydroxyl groups. Methyl-branched 2-hydroxy fatty acids have also been found in phosphatides of certain *Streptomyces* species [42, 43]. There again the methyl branches in the lipid rise the fluidity in the lipophilic matrix, while hydroxyl groups may be necessary to maintain appropriate condensation of the lipid layer.

The fundamental significance of hydrogen bonds for the conformation and association of macromolecular structures, such as proteins or nucleic acids, has been subject to detailed investigations and is well known today. Concerning their influence on lipid-lipid and lipid-protein interaction very limited information is at present available. The predominating volume of research on this subject has been performed on phosphatidylcholine and cholesterol systems, lipids with no or limited capacity to form hydrogen bonds.

It is therefore of great interest to investigate in detail the influence of different sphingolipids on membrane properties to provide explicit knowledge about their function.

By successive modification of the different polar groups of the lipophilic components it will be possible to elucidate both the mutual intermolecular orientation of the different hydrogen donor and acceptor groups and the amount of their free energy contribution to lipid-lipid interactions in membrane layers.

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