A triple-binding-domain model explains the specificity of the interaction of a sphingolipid activator protein (SAP-1) with sulphatide, G_{M1}-ganglioside and globotriaosylceramide

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The conformations of the neutral glycosphingolipid, globotriaosylceramide, and of the methyl ester of G_{M1}-ganglioside have been predicted by energy-minimization techniques and compared with those previously obtained for G_{M1}- and G_{M2}-ganglioside. A triple-binding-domain model is put forward to explain known specificities of binding between these glycosphingolipids and activator proteins. This model suggests that hydrophobic interactions, electrostatic interactions and hydrogen-bonding between sugar residues are important. The model is discussed in relation to previous studies on the effect of chemical modification of glycosphingolipids on their ligand properties.

INTRODUCTION

The role of glycosphingolipids in cell growth and differentiation and in cell transformation and the development of malignancy has recently been reviewed (Hakomori, 1984; Wiegandt, 1985). Implicit in many of these roles is the recognition by the glycosphingolipid of another macromolecule such as protein, glycoprotein or glycolipid. The carbohydrate moiety of glycosphingolipids is the main candidate for such interactive recognitions, since it is mostly found on the exterior surface of plasma membranes and it has been assumed that the lipid moiety has the function of anchoring the molecule in the membrane. Subtle alterations in the oligosaccharide component, such as addition or removal of a single monosaccharide unit, can have profound effects on the physiological function of the glycosphingolipid, and it seems likely that the conformation of the oligosaccharide is of overriding importance.

The enzymic degradation of glycosphingolipids requires the presence of some detergent-like molecule in order that the lipid can interact with the water-soluble enzyme. Lysosomes, the site of degradation *in vivo*, do not contain detergent, and the conversion of the lipid into a suitable physical form for degradation is achieved by the presence of activator proteins (see Sandhoff & Conzelmann, 1979). These appear to act by removing the lipid from membranes or lipid aggregates and, by coating the hydrophobic part of the molecule with protein, make it soluble. Although these activator proteins share a common function, specific activators have been characterized for both G_{M1}- and G_{M2}-ganglioside. The first activator protein to be described was that for sulphatide hydrolysis (Mehl & Jatzkewitz, 1964), and biochemical, immunological and pathological evidence suggest that this is identical with that required for G_{M1}-ganglioside hydrolysis (Inui *et al.*, 1983; Banks *et al.*, 1985). It has been reported recently that this same protein is also able to function in the hydrolysis of GbOse₃Cer by α -galactosidase (Li *et al.*, 1985). This sphingolipid activator protein has been named 'SAP-1'

by Inui & Wenger (1984) to differentiate it from that shown by Conzelmann & Sandhoff (1978) to be necessary for G_{M2}-ganglioside hydrolysis (SAP-2). G_{M1}- and G_{M2}-ganglioside differ only in the terminal galactose residue and, since the same activator protein is able to function with such dissimilar structures as sulphatide, GbOse₃Cer and G_{M1}-ganglioside, this galactose cannot be a major determinant in the specificity of the binding. It seems probable that there is a gross conformational difference between G_{M1}- and G_{M2}-ganglioside.

An explanation of this specificity has been suggested by Li *et al.* (1984), who studied the effect of chemical modification of the carboxylate group of the *N*-acetylneuraminy residue of G_{M1}-ganglioside on the hydrolysis of the ganglioside. In a complementary study, Mitsuyama *et al.* (1985) have examined the binding of activator protein to a variety of chemically modified sulphatides attached to affinity matrices.

Two major approaches to the study of conformation of glycosphingolipids are possible. The direct physical approach using X-ray crystallography is available in only a limited number of cases where crystalline preparations exist (Pascher & Sundell, 1977), and, even in these cases, caution is necessary in extrapolation of the results to conformation in a membrane or lipid aggregate. In other cases, indirect physical methods such as n.m.r. can be applied and, although such techniques are often difficult to interpret in terms of specific conformations, valuable information on possible hydrogen-bonding and ligand interaction can be obtained. An alternative approach is the use of computer algorithms to predict three-dimensional structures, and such methods have been shown to give excellent agreement with the data obtained from n.m.r. and X-ray crystallography for relatively simple glycolipids and oligosaccharides (Wynn *et al.*, 1986). This computational technique, which calculates the potential energy of a series of glycosphingolipid conformations and hence allows the prediction of the minimum energy state and the most probable conformer, has recently been applied to G_{M1}- and

Abbreviations used: GbOse₃Cer, globotriaosylceramide (Gal α 1-4Gal β 1-4Glc β 1-1Cer); G_{M1}-ganglioside, Gal β 1-3GalNAc β 1-4(NeuAc α 2-3)Gal β 1-4Glc β 1-1Cer (II³NeuAc-GgOse₃Cer); G_{M2}-ganglioside, GalNAc β 1-4(NeuAc α 2-3)Gal β 1-4Glc β 1-1Cer (II³NeuAc-GgOse₃Cer).

Fig. 1. Stereo representation of the minimum-energy conformer of GbOse₃Cer

G_{M2}-ganglioside (Wynn, 1986). Here the predicted conformations of GbOse₃Cer and the methyl ester of G_{M1}-ganglioside are reported and a model of the interaction between activator protein SAP-1 and glycosphingolipids is discussed.

METHODS

The conformations of GbOse₃Cer and the methyl ester of G_{M1}-ganglioside were predicted by energy-minimization techniques as previously described (Wynn, 1986; Wynn *et al.*, 1986).

RESULTS AND DISCUSSION

The predicted conformation of GbOse₃Cer is shown as a stereodiagram in Fig. 1. Although there are a series of local energy minima of similar energy to this global energy minimum, these other minima show only minor variations in the torsion angles of the hydroxy groups of the sugar residues. Conformers showing other orientations about the glycosidic linkages and/or the side chains of the sugars have far higher energies. The regular anti-planar zig-zag conformation of the hydrocarbon chains, which is necessary for the parallel stacking of these chains, is clearly visible in this global-minimum-energy conformer together with the 'shovel' shape obtained when the glucose moiety is attached to the ceramide. These features have been noted previously for galactosyl- and glucosyl-ceramide (Pascher & Sundell, 1977; Wynn *et al.*, 1986), the 'shovel' shape being explicable by the hydrogen atom of the amide group being involved as a donor in two intramolecular hydrogen bonds, one with the oxygen atom of the fatty acid hydroxy group and the other with the oxygen of the glycosidic link.

Another salient feature of the GbOse₃Cer conformation is the location of the hydroxy groups of the oligosaccharide. As viewed in Fig. 1, the C-2 and C-3 hydroxy groups of the glucose residue, the C-2 and C-3 hydroxy groups of the β -galactose residue, the C-2, C-3 and C-6 hydroxy groups of the α -galactose residue, together with the secondary hydroxy group of the ceramide, lie almost in a plane on the upper (proximal) surface of the molecule, whereas hydroxy groups are uncommon on the lower (distal) surface. This preferred orientation of hydroxy groups in the minimum-energy

conformer of GbOse₃Cer is emphasized in Fig. 2(a), where the stereo representation of the oligosaccharide moiety is shown.

Comparison with the oligosaccharide moiety of G_{M1}-ganglioside reveals that the minimum-energy conformer of this ganglioside also contains a plane of hydroxy groups shown in Fig. 2(b) on the upper (proximal) face of the molecule, whereas the charged carboxy group of the sialic acid residue is on the lower (distal) face. These hydroxy groups are on the C-6 atom of glucose, the C-6 atom of the chain galactose, the C-4 and C-7 atoms of the sialic acid, the C-4 and C-6 atoms of the *N*-acetylgalactosamine and the C-4 and C-6 atoms of the terminal galactose. Although the plane is somewhat distorted, there is no other comparable plane in the molecule that contains more than three, or possibly four, hydroxy groups. In contrast, the oligosaccharide of the minimum energy conformer of G_{M2}-ganglioside shown in Fig. 2(c) has no such planar concentration of hydroxy groups, the groups being evenly distributed throughout this rather open structure.

These results on the orientation of the hydroxy groups in various glycosphingolipids, taken in conjunction with those previously reported (Wynn, 1986) on the relative orientation of the charged group in G_{M1}- and G_{M2}-ganglioside and sulphatide lead me to propose a triple-binding-domain model to explain the specificity of interaction of the sphingolipid activator protein, SAP-1, with a variety of glycosphingolipids. In this model there are three essential domains in the glycosphingolipid molecule which can be recognized by the activator protein and which form the basis of their specific interaction. Firstly, the hydrocarbon chains of the fatty acid and sphingosine moieties form a hydrophobic domain which can interact with a complementary hydrophobic domain in the protein molecule. Secondly, there is an electrostatic interaction between the charged sialic acid or sulphate group and a positively charged group or groups on the protein. Thirdly, there is hydrophilic interaction, presumably mediated by hydrogen-bond formation, between a plane of hydroxy groups in the glycosphingolipid molecule and a similar and complementary plane in the activator protein. Since the activator protein is a lysosomal glycoprotein, it is possible that this complementary plane may be provided by the core oligosaccharide present in this molecule. It seems likely that, if at least two of these

Fig. 2. Stereo representation of the minimum-energy conformer of the oligosaccharide moieties of various oligosaccharides

(a) GbOse₃Cer; (b) G_{M1}-ganglioside; (c) G_{M2}-ganglioside. The linkage to the ceramide is indicated by ★.

structural features are present in the glycosphingolipid, strong binding to the protein will take place. Thus G_{M1}-ganglioside contains all three and binds very strongly, whereas sulphatide only has the charge and the hydrophobic domain, and GbOse₃Cer has the hydrophobic and planar hydroxy-group domain. G_{M2}-ganglioside, which only possesses the hydrophobic domain, since the charge is in the wrong orientation, binds weakly, if at all. Conzelmann *et al.* (1982), in a study of the complexing of glycolipids to the activator protein for G_{M2}-ganglioside degradation (SAP-2), were able to show binding ranging from very strong to very weak. By definition, all glycosphingolipids possess the

hydrophobic binding domain and will show weak interaction with activator protein.

The gene for SAP-1 has recently been cloned and the amino acid sequence of the first 67 residues of this protein determined by base-sequence analysis (Dewji *et al.*, 1986). A glycosylation site is present 21 residues from the *N*-terminus. A preliminary analysis of the precursor of this protein shows that the next two residues are lysine and arginine (N. Dewji, personal communication), and this would be a suitable site for proteolytic cleavage during maturation, leaving the *C*-terminus of the mature protein as a lysine residue. Bovine serum albumin, which binds fatty acids strongly, has a tertiary structure where

Fig. 3. Stereo representation of the minimum-energy conformer of the methyl ester of G_{M1} -ganglioside

six helices form a cylindrical structure with a hydrophobic core that is capable of accepting the lipid hydrocarbon tail (Brown & Shockley, 1982). Preliminary studies in this laboratory on the prediction of secondary and tertiary structures show that the SAP-1 monomer contains two or possibly three helical regions and that there is significant homology between the protein and serum albumin. In the dimer, which is the physiological state of the activator protein, these helical regions could associate to form the cylindrical hydrophobic domain, as in serum albumin, for interaction with the hydrophobic domain of the glycosphingolipids. Interaction between the oligosaccharide of the protein and the plane of hydroxy groups of the lipid and between the lysine and the negative charge on the lipid would complete the binding.

Fig. 3 shows the global energy minimum of the methyl ester of G_{M1} -ganglioside. Although there are significant alterations to the torsion angles of the hydroxy groups and side chains, the orientation of the sugar rings is very similar to that reported for the parent ganglioside (Wynn, 1986). Thus, on the triple-domain theory, one would predict that the activator protein could still interact with this ester via the hydrophobic and hydrophilic domains. This is in accord with the results of Li *et al.* (1984), who showed that the ester was a substrate for the activator-enhanced β -galactosidase and concluded that the carboxy function of sialic acid in G_{M1} -ganglioside is not vital for the enzyme/activator action. The affinity of modified sulphatides for activator protein as shown by Mitsuyama *et al.* (1985) led them to suggest also that the charge was not vital for interaction and that the activator must be able to recognize the galactose moiety as well as one of the hydrocarbon chains. This would again be entirely consistent with the triple-domain theory.

The role of the solvent has been deliberately excluded from these calculations. Gangliosides are normally present in membranes and are therefore in a partially non-polar environment, whereas the oligosaccharide moiety will have some interaction with the aqueous solvent. The complex nature of this environment preclude detailed calculations of its contribution at the present time, and it is recognized that caution is needed in the interpretation of these predictions. However, the

agreement between predicted conformers and X-ray-crystallographic data (Wynn *et al.*, 1986) suggests that computational methods may prove useful in the study of ganglioside conformation, particularly when taken in conjunction with physical data.

I gratefully acknowledge the kindness of Dr. B. Robson and other members of the Epsitron Protein Engineering Group in allowing me to use the SIMPLEX energy-minimization program and also for their helpful advice and criticism.

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