review

Saposins: structure, function, distribution, and molecular genetics

Yasuo Kishimoto, Masao Hiraiwa, and John S. O'Brien[1]

University of California, San Diego, Center for Molecular Genetics, School of Medicine, La Jolla, CA 92093-0634

Abstract Saposins A, B, C, and D are small heat-stable glycoproteins derived from a common precursor protein, prosaposin. These mature saposins, as well as prosaposin, activate several lysosomal hydrolases involved in the metabolism of various sphingolipids. All four saposins are structurally similar to one another including placement of six cysteines, a glycosylation site, and conserved prolines in identical positions. In spite of the structural similarities, the specificity and mode of activation of sphingolipid hydrolases differs among individual saposins. Saposins appear to be lysosomal proteins, exerting their action upon lysosomal hydrolases. Prosaposin is a 70 kDa glycoprotein containing four domains, one for each saposin, placed in tandem. Prosaposin is proteolytically processed to saposins A, B, C and D, apparently within lysosomes. However, prosaposin also exists as an integral membrane protein not destined for lysosomal entry and exists uncleaved in many biological fluids such as seminal plasma, human milk, and cerebrospinal fluid, where it appears to have a different function. The physiological significance of saposins is underlined by their accumulation in tissues of lysosomal storage disease patients and the occurrence of sphingolipidosis due to mutations in the prosaposin gene. This review presents an overview of the occurrence, structure and function of these saposin proteins.—**Kishimoto, Y., M. Hiraiwa, and J. S. O'Brien.** Saposins: structure, function, distribution, and molecular genetics. *J. Lipid Res.* 1992. **33:** 1255–1267.

Supplementary key words sphingolipid activator proteins • saposin A • saposin B • saposin C • saposin D • prosaposin • lysosomes • sphingolipid hydrolases • sphingolipidosis • storage disease

I. INTRODUCTION

Saposins are a group of four small heat-stable glycoproteins that are required for the hydrolysis of certain sphingolipids by specific lysosomal hydrolases. The first saposin (now called saposin B) was described by Jatzkewitz and his colleagues in 1964 as a required heat-stable factor for hydrolysis of sulfatides by arylsulfatase A (1). The second saposin (saposin C) was discovered in 1971 by Ho and O'Brien (2) and was found to stimulate the hydrolysis of glucosylceramidase and to accumulate in tissues of Gaucher disease patients. The discovery of the two remaining saposins, A and D, resulted from cloning and sequencing of the prosaposin cDNA.

The discovery that all four saposins are part of a single precursor protein, prosaposin, began with the cloning of a cDNA encoding saposin B (3, 4) and the discovery that the human cDNA sequence was homologous with a protein in rat testis, SGP-1 (5). After determining the SGP-1 cDNA sequence Collard et al. (5) noticed that four similar polypeptide domains, including one homologous with human saposin B, were placed in tandem in SGP-1. After determining the sequence of the human cDNA encoding saposin C, it was found that the same cDNA fragment encoded both saposin B and C and contained two additional domains of similar type, consistent with the finding with SGP-1 (6). Fürst, Machleidt, and Sandhoff (7) also con-

Abbreviations: CD, circular dichroism; SAP, sphingolipid activator protein; SP, surfactant protein.

[1]To whom correspondence should be addressed.

cluded that prosaposin is the precursor of saposin B and C as well as a third protein, termed component C (identical to saposin D) which they isolated by affinity chromatography after denaturation. We independently isolated undenatured saposin D and found that it stimulates sphingomyelinase (8). Shortly thereafter we also isolated saposin A which was found to stimulate glucocerebrosidase similar to saposin C (9). The occurrence of saposin A and D in quantities similar to saposin B and C strongly suggests that all four saposins are biologically important proteins.

Based on the placement of four saposin domains in prosaposin, reading from amino-terminal to carboxyl-terminal, we proposed to name them saposins A, B, C and D (**Fig. 1**). Our nomenclature (8, 9) was proposed to avoid the confusing array of previous terms used for these proteins and to emphasize the identical genetic origin of saposins.

All saposins contain about 80 amino acids and are glycosylated (**Table 1**). Three-dimensional theoretical models of these saposins suggest they are highly compact and closely resemble one another, judging from their six identically placed cysteines, conserved prolines, and glycosylation sites as well as their similar circular dichroism (CD) spectra (10). Notwithstanding the saposins' structural similarity, their mechanisms of stimulation as well as their specificities for sphingolipid substrates or lysosomal enzymes are not identical as described in the following section.

The physiological significance of the saposins was underscored by reports of human sphingolipidosis patients with mutations in the prosaposin gene who have deficiencies of saposins or of prosaposin (11–15).

In this review, we will discuss recent progress on the structure, function, and the role of saposins in human lysosomal storage disorders. The topics covered in detail in our previous review (10) will be touched upon only briefly and readers will be occasionally referred to that reference.

II. INDIVIDUAL SAPOSINS

A. Saposin A

Saposin A was recently isolated and characterized. As described above, this saposin was identified as an N-terminal domain in the prosaposin cDNA prior to its isolation. We isolated saposin A from Gaucher spleen and confirmed its authenticity by amino acid sequence analysis (9). Two potential glycosylation sites were identified in saposin A both of which are glycosylated (see Table 1). The N-terminal amino acid was confirmed to be serine but the carboxyl terminal amino acid remains to be identified. From estimates of molecular weight after deglycosylation, saposin A contains about 84 amino acids with a molecular weight of ca. 9230. Saposin A contains six cysteine residues, all in disulfide linkage (Y. Kishimoto

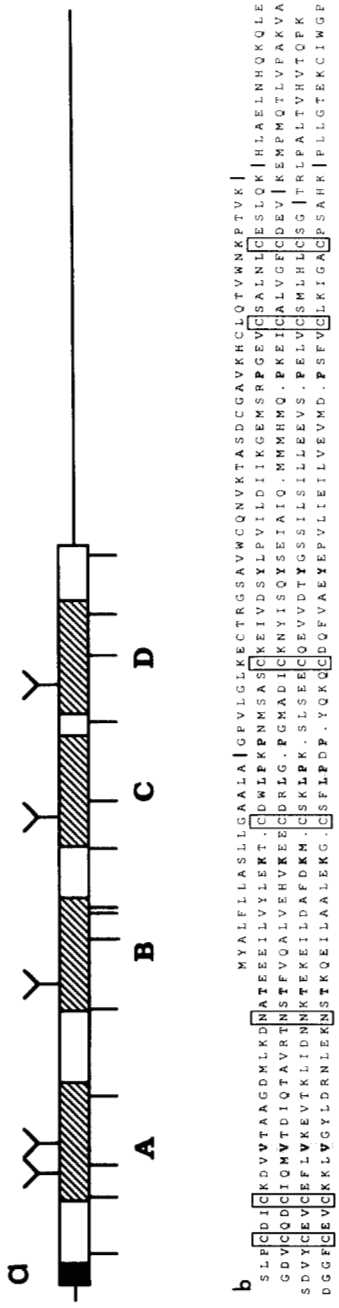

Fig. 1. Structure of saposin proteins. a) Abbreviated structure of prosaposin with saposin domains (cross-hatched) and signal peptide (solid) indicated; glycosylation sites are shown by forked symbols and exon-intron boundaries are shown by vertical lines. b) Amino acid structure of prosaposin with the four saposin domains aligned to demonstrate positions of conserved amino acids. Boxes indicate structurally significant amino acids and shaded areas indicate other conserved amino acids. Vertical bars denote proteolytic cleavage sites.

TABLE 1. Comparison of saposin physical characteristics

	Domain in Prosaposin	Amino Acid Number	Molecular Weight			Amino Acids			Isoelectric Point	Three-Dimensional Structure[a]	
			Peptide	Carbohydrate	Total	Acidic	Basic	% Hydrophobic		α-Helix	β-Sheet
A	1	84	9229	6000	15,300	15	8	55	4.6	44	8
B	2	81	9096	3000	12,100	12	8	53	4.6	26	38
C	3	80	8944	3000	12,000	16	9	55	4.6	42	1
D	4	83	9248	3000	12,300	13	10	63	4.6	53	0

[a]As indicated by CD at pH 4.5. The rest is in random coil.

and J. S. O'Brien, unpublished results) indicative of a highly organized compact structure. Circular dichroism analysis indicates a polypeptide with a high alpha helical content (44%) with the remainder being random coil (10).

Saposin A stimulates the enzymatic hydrolysis of 4-methylumbelliferyl-β-glucoside, glucocerebroside, and galactocerebroside (9). Saposin A appears to stimulate acid β-glucosidase (glucocerebrosidase) by reducing the enzyme's K_m value from 2.94 to 1.61 mM and increasing its V_{max} value from 9.5 to 50 nmol/h. Stimulation of acid β-glucosidase by saposin A is similar to that by saposin C (see below) but the degree of stimulation is about one-third lower. Our kinetic studies indicated that saposins A and C bind to β-glucosidase at the same site; the site is different from that which binds acidic lipids (16).

The stimulative activity of saposin A for acid β-glucosidase was confirmed by Fabbro and Grabowsky (17) who demonstrated that certain anti-β-glucosidase monoclonal antibodies also stimulate and that the antibody activation (as well as activation by saposin A and saposin C) mediates their activating effects by binding to distinct sites of the β-glucosidase. These results indicate that saposin A, saposin C, and antibodies cause a conformational change in the structure of the enzyme that results in increased catalysis. Our results and those of Fabbro and Grabowsky are contradictory to those of Kondoh et al. (18) who report that saposin A has no stimulatory activity on glucosylceramide β-glucosidase.

B. Saposin B

As described earlier, saposin B was the first saposin to be identified and is probably the most well studied. This protein has been called by many different names, including sphingolipid activator protein-1 (SAP-1), sulfatide activator protein, GM_1 ganglioside activator, dispersin, and nonspecific activator (see ref. 10 for individual references). The list of enzymes activated by saposin B is extensive. Earlier studies demonstrated that arylsulfatase A activity for sulfatide hydrolysis, α-galactosidase A for globotriaosylceramide hydrolysis, and acid β-galactosidase for GM_1 ganglioside hydrolysis are stimulated by this protein (19, 20).

A later report by Li et al. (21) added to the list of enzymic activities activated by saposin B including additional glycolipids as well as glycerolipids prompting the authors to call saposin B a "nonspecific activator". As discussed later in the review, several human patients with a genetic deficiency of saposin B have been reported. These patients present with a phenotype resembling metachromatic leukodystrophy with sulfatide lipidosis, indicating an important role of saposin B in the hydrolysis of sulfatides in vivo. Further analysis of accumulated lipids in tissues of such patients should provide a better understanding of saposin B specificity. Thus far, tissue analyses have been limited due to the unavailability of postmortem tissues.

Saposin B appears to be unique among the saposin family in that it stimulates enzymic activities by interacting with substrates but not with enzymes. Wenger and Inui (22), using comigration with saposin B on polyacrylamide gel electrophoresis as evidence, showed that 1 nmol of saposin B binds with 4.0 and 2.6 nmol of GM_1 ganglioside and sulfatide, respectively. Many other lipids, including GM_2 ganglioside and sphingomyelin, also bind to saposin B, although saposin B does not have stimulatory effects on the lysosomal hydrolysis of these lipids.

The kinetics of saposin B binding to lipids was further studied by Vogel, Schwarzmann, and Sandhoff (23). From saturation curves of saposin B versus substrate followed by enzymatic hydrolysis, they calculated the dissociation constants of different saposin B-lipid complexes; 3.0 μM for GM_1 ganglioside hydrolysis by β-galactosidase, 65 μM for sulfatide hydrolysis by arylsulfatase A, and 30 μM for globotriaosylceramide by α-galactosidase A. These investigators further studied complex formation using centrifugation to separate free lipids from those bound to saposin B. The molar ratios of lipids, including $GD1_a$, GM_1, GM_2, GM_3 gangliosides and sulfatide, to saposin B were found to be about 1:2, which are lower than those found by Wenger and Inui (22). Based on the assumption that saposin B exists as a dimer in aqueous solution (24, 25), the ratios of lipid to activator were approximately 1:1. The saposin B-lipid complex has not been isolated for further characterization. Vogel et al. (23) also reported that sapo-

sin B facilitates transfer of these glycolipids between donor and acceptor liposomes and that transport was dependent upon certain structural rules. Lipids having less than three hexoses were transformed at very slow rates whereas complex lipids with longer carbohydrate chains (gangliosides GM_2, GM_1, and $GD1_a$) were transferred much faster.

It is not known whether saposin B acts upon lipids within intact membranes in vivo. A recent study by Louis and Fluharty (26) demonstrates that activator-dependent hydrolysis of sulfatide by arylsulfatase A occurred using myelin membrane preparations containing sulfatide. The rate observed was about one-twentieth of that with sulfatide micelles. This study indicates that saposin-dependent lysosomal membrane recycling (autophagy) may be an early event in membrane component recycling in vivo.

While the affinity of saposin B for glycolipids has been well documented, its interaction with enzymes has not been adequately addressed. The assumption that the enzyme and saposin B do not interact is based on two observations (24). First, after gel filtration of a mixture of arylsulfatase A and saposin B, there was complete separation of the two proteins. Second, stimulation of arylsulfatase A or acid β-galactosidase by saposin B did not occur when artificial water-soluble substrates were used. In addition, Vogel et al. (23) demonstrated that sulfatide and ganglioside GM_1 derivatives with short acyl chains (C-2) did not require saposin B for hydrolysis by arylsulfatase A or β-galactosidase. The possibility still exists that saposin B may interact with the lipid binding sites of hydrolyases even though it does not interact with the catalytic sites.

C. Saposin C

Saposin C has been known for many years since its discovery in 1971 by Ho and O'Brien (2). Saposin C has been termed factor-P, sphingolipid activator protein-2 (SAP-2), coglucosidase, $A_{1\alpha}$-activator, and heat-stable factor by different investigators (10). It stimulates the hydrolysis of glucocerebroside by glucosylceramidase and galactocerebroside by galactosylceramidase. A variant of Gaucher disease that is caused by the lack of saposin C has been reported (14, 27).

Unlike saposin B, saposin C does not appear to interact with substrate lipids such as glucocerebroside but binds to the enzyme, glucocerebrosidase, which becomes activated. This conclusion is based on the following observations. Sepharose-linked saposin C acts as an affinity column for glucocerebrosidase (2). Saposin C reduces the K_m value for glucocerebrosidase for both glucosylceramide and 4-methylumbelliferyl-β-glucoside (28, 29). Saposin C does not form a complex with glucocerebroside or with 4-methylumbelliferyl-β-glucoside (29). No complex formation between glucocerebroside and saposin C has been detected as judged by polyacrylamide gel electrophoresis. Finally, a high molecular weight aggregate that contains glucocerebrosidase and saposin C has been isolated from human tissue (30).

Glucosylceramidase is also stimulated by acidic lipids, such as phosphatidylserine, phosphatidic acid, phosphatidyl inositol, sulfatide, and gangliosides (31, 32). This stimulation has caused much confusion as to the essential role of saposin C because previous investigations on the effect of saposin C were done with impure enzyme preparations which were contaminated by acidic lipids. Using purified glucosylceramidase, it was demonstrated that saposin A and C stimulate the enzymic activity by a mechanism different from that of acidic lipids, binding to the enzyme at a different site (16).

D. Saposin D

Saposin D is the least understood of the saposins. As with saposin A, occurrence of saposin D was predicted from the cDNA sequence of prosaposin (6). In 1988, Fürst et al. (7) isolated and sequenced a new protein, after its alkylation from human kidney. They found that the sequence of the new protein was identical to the predicted activator which was deduced from the published nucleotide sequence of the human prosaposin cDNA (4) after several nucleotide corrections. At the same time, we isolated from Gaucher spleen a new protein whose sequence is identical to the predicted protein from the 4th domain of prosaposin. Since the domain for the protein is located at the C-terminus of prosaposin, we called the protein saposin D (8).

Saposin D stimulated sphingomyelinase activity when the substrate was presented in the proper concentration of Triton X-100 or in liposomes (M. Tayama, J. S. O'Brien, and Y. Kishimoto, unpublished result). Saposin D also slightly stimulated the hydrolysis of 4-methylumbelliferyl-β-glucoside by glucosylceramidase but did not stimulate hydrolysis using the natural substrate, glucocerebroside. The stimulation is highly specific, because saposin D did not stimulate hydrolysis of all other 4-methylumbelliferyl derivatives tested which included pyrophosphate, α-galactosidase, β-galactoside, α-glucoside, α-mannoside, α-fucoside, β-glucuronide, and β-N-acetylglucosaminide (8).

E. Prosaposin

The existence of prosaposin in humans was predicted from pulse chase experiments on the biosynthesis of saposin B and C in cultured human skin fibroblasts by Fujibayashi and Wenger (33). Prosaposin appeared to exist in two forms, a major intracellular form of 68 kDa and a major extracellular form of 73 kDa. In time course studies the 68 kDa form was synthesized before the 73 kDa form. Both forms were converted to a 50 kDa form after deglycosylation with N-glycosidase F. These results suggest that prosaposin is biosynthesized, glycosylated, and is both secreted extracellularly and is proteolytically pro-

cessed intracellularly to generate saposin A, B, C, and D. Griswold, Roberts, and Bishop (34) and Collard et al. (5) isolated pure prosaposin from the media of rat Sertoli cell cultures. As discussed above, rat prosaposin (named SGP-1) is homologous to human prosaposin. Using antibodies against saposin B and C, we detected prosaposin in various human tissues and body fluids (6). Brain and testes appeared to have the highest concentrations of prosaposin followed by kidney, spleen, and liver. This observation was confirmed in rat tissues by Sano et al. (35) who reported that prosaposin concentrations in rat brain increased with age. In addition to the above-mentioned tissues, they found that skeletal muscle, heart, and platelets also contain prosaposin. Hineno et al. (36) also detected prosaposin in various human secretory fluids, including cerebrospinal fluid, seminal plasma, milk, pancreatic juice, blood plasma and bile. In male rats, prosaposin (SGP-1) was also detected and quantified in various fluids (58). Concentrations (in mg/ml) were highest in fluid from efferent ducts of the rat testis (109 μg/ml) followed by seminiferous tubules (60 μg/ml), testicular lymph (7 μg/ml), and serum (vena cava) (1.5) μg/ml. We have quantified prosaposin in human cerebrospinal fluid where its concentration is relatively high (10–35 μg/ml).

Kondoh et al. (18) recently isolated prosaposin (74 kDa) from human milk. Amino acid sequencing of milk prosaposin indicated that the N-terminal amino acid is the glycine in the 17th position counting from the N-terminal end of preprosaposin. A 16-residue signal peptide is cleaved to generate prosaposin from preprosaposin and the cleavage site is identical in rat and human. The yield of prosaposin was 100 μg from 500 ml human milk. We have also isolated prosaposin from human milk and determined its concentration (7 μg/ml). In addition, we have isolated prosaposin from human seminal plasma (90 μg/ml) (37) and determined its molecular weight (66 kDa) and its N-terminal amino acid sequence; this sequence was identical to that of milk prosaposin. Deglycosylation of seminal plasma prosaposin yielded a 54 kDa protein, indicating that about 12 kDa of carbohydrate is linked to the five glycosylation sites. This observation indicates that glycosylation of prosaposin precedes its proteolysis to mature saposins as indicated by the pulse-chase experiments of Fujibayashi and Wenger (38). The higher molecular weight of milk prosaposin (74 kDa) indicates that milk prosaposin contains larger carbohydrate chains. The isoelectric point of the seminal plasma prosaposin (5.4) was found to be considerably higher than those of mature saposins (4.6) (Table 1).

Using the cDNA encoding prosaposin (6), human prosaposin was expressed in invertebrate cells using a baculovirus expression vector (E. Ginns and B. M. Martin, unpublished result). Prosaposin was isolated from the spent culture media and its N-terminal amino acid sequence and isoelectric point were found to be identical to those of human seminal fluid prosaposin, as described above (37). The molecular weight of the recombinant prosaposin was estimated by SDS-polyacrylamide gel electrophoresis at 58 kDa. Deglycosylation of recombinant prosaposin yielded a 54 kDa protein, which migrated in the same position as deglycosylated human seminal plasma prosaposin. The carbohydrate side chains of recombinant prosaposin harvested after 2 days in culture are much shorter than those of milk or seminal plasma prosaposin. ture are much shorter than those of milk or seminal plasma prosaposin.

We have determined that recombinant prosaposin has activator activity similar to that of mature saposins. Recombinant prosaposin stimulates glucosylceramidase with activity about 80% of mature human saposin C on a molar basis. Recombinant prosaposin stimulates sphingomyelinase activity to the same extent as saposin D and stimulates GM_1 ganglioside β-galactosidase activity to a greater extent than saposin B. Human milk prosaposin has been reported to have only slight activity in stimulation of glucosylceramidase (18). Trypsin digestion of the milk prosaposin preparation yielded some stimulatory activity. Human milk prosaposin, which has larger carbohydrate chains than recombinant prosaposin, may have lower stimulatory activity due to masking of stimulatory regions by the oligosaccharide side chain.

F. Distribution of saposins

Saposins appear to be present in almost every tissue examined, including liver, spleen, brain, placenta, and saliva of different animal species. They can be categorized as "housekeeping proteins" necessary for lysosomal hydrolysis (31).

Lysosomal localization of saposin B was first clearly shown in normal human liver and colon by Tamaru et al. (39) using immunohistochemical technique. Recent immunohistochemical studies by Taniike et al. (40) demonstrated that in normal human brain saposin B reactivity occurred primarily in gray matter with low reactivity in white matter. In gray matter, saposin B reactivity was detected in glia but was much more evident in neurons. Saposin B was found almost exclusively in lysosomes and in multilamellar bodies in neurons. Lysosomal localization in cultured human skin fibroblasts was also confirmed for saposin B (41) and for saposin C (42).

III. BIOGENESIS OF SAPOSINS

A. Synthesis of prosaposin and its processing

As discussed above, Fujibayashi and Wenger (38) studied the biosynthesis of saposin B in cultured human skin fibroblasts by pulse-chase analysis, identifying prosaposin as 68 kDa and 73 kDa proteins. Using the same approach, they demonstrated that saposin C is generated

by a precursor of similar size (33). Some intermediates (35–52 kDa) during conversion of prosaposin to mature saposins were found in both experiments.

Rijnboutt et al. (43), using cultured human hepatoma cells, HepG2, and antisera against saposin C detected a 68 kDa precursor protein that was converted to a 73 kDa form within 30 min of chase. A small amount of the 73 kDa form was secreted into the culture medium. A 50 kDa intermediate form was present after 1 h of chase which could represent the 48 kDa trisaposin species (lacking the A domain) we have characterized as the first proteolysis product of prosaposin (see below). Finally, mature saposin was detectable as a protein of 12 kDa after 1.5 h of chase. The 68 kDa precursor, the 50 kDa intermediate, and mature saposin C were released after treatment with saponin, which induces release of secreted proteins but not of integral membrane proteins. However, the 73 kDa intermediate was almost completely retained in the cells with about half being released by mannose-6-phosphate. These results suggest that a portion of prosaposin is an integral membrane protein bound by a mannose-6-phosphate-independent mechanism and another portion is targeted to lysosomes via mannose-6-phosphate receptor-mediated uptake; a small portion is secreted from the cell. Using human skin fibroblasts and anti-saposin B antiserum, Schnabel et al. (44) also reported that 70 kDa prosaposin synthesized in fibroblasts was secreted into the culture media and another portion was processed to mature saposin B.

The functional significance of the "integral membrane form" of prosaposin is not understood. Membrane association of prosaposin may occur independent of N-linked oligosaccharide chains as with cathepsin D and glucocerebroside (43) and may be mediated by hydrophobic polypeptide regions which are abundant in each saposin domain. We recently demonstrated that prosaposin binds glycolipids with high affinity, especially gangliosides, which may indicate that membrane association of the "integral membrane form" of prosaposin could be mediated by its co-association with gangliosides or other glycosphingolipids.

We have isolated products of prosaposin proteolysis to obtain insight into the preferential cleavage sites during proteolytic processing of that portion of prosaposin that is destined for lysosomes (37). Two proteins detected by immunoblotting using anti-saposin antibodies of 48 kDa and 29 kDa were isolated from human seminal plasma which is a rich source of prosaposin. The N-terminal amino acid sequence of the 48 kDa protein was identical to that beginning with the threonine at position 165 of prosaposin between the saposin A and B domains. From its molecular weight, reactivities with antibodies against individual saposins, and the sequencing data, we concluded that the 48 kDa protein is a trisaposin containing domains for saposin B, C, and D. The 29 kDa protein interacts only with antibodies against saposin C and D and is a disaposin containing domains for saposin C and D.

We have also isolated two intermediate proteins with 39 kDa and 26 kDa molecular weights from the spent culture medium of recombinant prosaposin synthesis. The 39 kDa protein had an N-terminal sequence identical to that of prosaposin beginning with the tyrosine at position 179. The 26 kDa protein had an N-terminal sequence identical to that following the leucine at position 298 of prosaposin. These observations, as well as their reactivities with antibodies against individual saposins, indicate that the 39 kDa protein is a trisaposin containing domains B, C, and D, and the 26 kDa protein is a disaposin containing domains C and D. Identification of these intermediates demonstrates that, in both human seminal plasma and invertebrate cells, proteolysis of prosaposin apparently occurs sequentially from the N-terminal end with the saposin A domain being cleaved first, followed by cleavage of the saposin B domain. The size of the initial product in seminal plasma is similar to that reported during pulse-chase experiments of prosaposin processing in cultured mammalian cells (43), although the identity of the two proteins needs to be established. Two additional interesting observations are *1)* that the cleavage site is different in human seminal plasma and insect cells and *2)* that the first processing step generates a saposin A derivative whose N-terminal amino acids are different (about 20 residues longer) than that of mature saposin A. If these products are reflective of those produced intracellularly, further proteolysis is apparently necessary to generate mature saposins after they are initially produced.

B. Human gene for prosaposin

Inui et al. (45) and Fujibayashi et al. (46) localized the gene encoding saposin B and C to human chromosome 10. By in situ hybridization the prosaposin gene was later localized to the long arm of chromosome 10 (q21–22) (47). Holtschmidt et al. (48) recently reported that the prosaposin gene encompasses 17 kb of genomic sequence with 14 exons. Not yet analyzed is the region encoding the 13 amino acids of the signal peptide and the 5'-untranslated sequence. From the regularity of the positions of introns, they suggested that the prosaposin gene arose by sequential duplication of an ancestral gene as proposed previously (5, 6). Noteworthy, however, is the interruption of the exons encoding the saposin domains by introns: two in saposin A, three in saposin B, one in saposin C, and two in saposin D. If tandem duplication occurring in the ancestral gene gave rise to the prosaposin gene containing four repeats, subsequent evolution has not preserved the domains as uninterrupted coding regions.

IV. STRUCTURE AND FUNCTION OF SAPOSINS

A. Structure of mature saposins

Each saposin contains about 80 amino acid residues and has six equally placed cysteines, two prolines, and a glycosylation site (two in saposin A, one each in saposins B, C, and D). These residues are also completely conserved in saposins from other animal species (10) and can be assumed to be essential for biological function. From their extreme heat-stability, abundance of disulfide linkages, and resistance to most proteases, mature saposins are assumed to be extremely compact and rigidly disulfide-linked molecules. CD spectra of each saposin indicate that they are highly structured and rich in α-helical regions (10). The α-helical content of each saposin is maximal at pH 4.5, the optimal pH for most lysosomal hydrolases, suggesting that the α-helical structure is important in stimulation. Although the secondary structures have been examined with CD spectroscopy, as described above, tertiary structures of saposins have not yet been elucidated. Resistance of the saposins to proteases has made assignment of disulfide linkages difficult.

Wynn (49) first proposed an amphipathic helical lipid binding center for saposin B from its partial amino acid sequence (3). Based on helical wheel modeling of the amino acid sequence deduced from the cDNA sequence for prosaposin, we proposed a triple helix structure, stabilized by disulfide linkages, for saposin B and C (6) and saposin A (9). According to the Chou-Fasman secondary structural prediction (50), analysis of the amino acid sequence of saposin B suggests that most of its first 24 amino acids from the N-end are in β-sheet configuration, whereas this region is helical in the other saposins (10). A higher β-sheet content of saposin B (34% versus 8, 1, and 0% in saposin A, C, and D, respectively) than others was confirmed by CD analysis (10). Gel filtration data suggest that saposins B and C, and possibly the other saposins, exist as homodimers in aqueous solution but the forces giving rise to the formation of dimers are not understood.

Saposins are rich in carbohydrates; about 20% in saposins B, C, and D and about 40% in saposin A, isolated from Gaucher spleen. Saposin B, C, and D contain a single N-linked chain whereas saposin A contains two N-linked chains. The carbohydrate structure of saposin B from normal human liver was studied in detail by Yamashita et al. (51) who found that oligosaccharide chains are a mixture of secondary degradation products from hexasaccharides to a single N-acetylglucosamine. All of them were derived from complex type sugar chains. They also studied the carbohydrate chain of saposin B isolated from the liver of a GM_1 gangliosidosis patient and found that the carbohydrates were a mixture of sialylated and non-sialylated mono- to tetra-antennary complex-type sugar chains. We have also studied the carbohydrate chains obtained from saposins A, C, and D from Gaucher spleen and found a mixture of mono- to tetra-antennary complex-type sugar chains, with and without fucose attached to the first N-acetylglucosamine residue (K. Ito, N. Takahashi, J. S. O'Brien, and Y. Kishimoto, unpublished result). In the rat prosaposin contains sulfated oligosaccharide chains which may play a role in secretion (34).

B. Function of mature saposins

The similar sequences of each saposin would indicate a common structure–function rationale. However, the functional properties of saposin B, which interacts with lipid substrates, were thought to differ from those of other saposins which interact with enzymes. If the hydrophobic pocket formed by the three helical regions present in each saposin is a lipid binding site, then all saposins should form complexes with lipids. We have recently demonstrated that all four saposins form glycolipid complexes with especially high affinity for gangliosides. The binding of saposins A, B, C, and D and prosaposin to gangliosides was saturable, pH-dependent (maximal at pH 4), and occurred within minutes at room temperature. Demonstration of the complex by physical means was made by isoelectric focussing revealing a shift in isoelectric point of the [^{3}H]ganglioside–[^{14}C]saposin complex after co-association, and by molecular sieve and ion-exchange chromatography. Apparent K_Ds were calculated for binding to 13 gangliosides with varying complexities of their oligosaccharide chains. In general, saposins A, B, C, and D bound to gangliosides with similar affinities whereas prosaposin was more tightly bound.

Several rules for binding emerged from considerations of the structures of the gangliosides studied. Binding increased with an increase in length of the oligosaccharide chains and increased with increasing number of sialic acid residues. Gangliosides of the a series were bound more tightly than those of b series. Acetylation of sialic acid residues greatly reduced binding.

We recently demonstrated that even though all saposins and prosaposin bind ganglioside GM_1, there are major differences in their ability to stimulate its hydrolysis by acid β-galactosidase. On a molar basis, prosaposin has the greatest activity followed by saposin B and saposin D; saposin A and C are minimally active. Calculation of K_m values for activation reveals identical values for prosaposin and saposin B and a higher value for saposin D. Thus, although all five saposin proteins bind ganglioside GM_1, binding differs from the stimulatory property, a finding reported previously for saposin B by Wenger and Inui (22) and Vogel et al. (20). Although all four saposins bind gangliosides, we have recently demonstrated, using ganglioside $GD1_a$ and GM_1 as substrates, that acid sialidase activity is not stimulated by saposins (J. S. O'Brien, M. Hiraiwa, and Y. Kishimoto, unpublished results).

Saposins A, C, and D appear to stimulate enzyme activities by interacting with enzymes but the mechanism of

interaction is not known. With pure glucosylceramidase and saposin C, we demonstrated that a 5000 molar excess of saposin C is necessary to obtain maximum activity of the enzyme (16). This observation indicates that binding between saposin C and glucosylceramidase is weak. A recent study on complex formation between these two proteins by gel filtration confirms that interprotein binding is weak (S. Soeda, M. Hiraiwa, Y. Kishimoto, and J. S. O'Brien, unpublished results). In spite of weak binding, the activity of glucosylceramidase, in the absence of acidic lipids, appears totally dependent upon saposins A and C; the enzyme is nearly inactive by itself. It is possible that the lysosomal concentration of saposins is sufficiently high at the site of the enzyme to be maximally effective.

Datta, Snider, and Radin (52) demonstrated that uptake of glucosylceramidase by neuroblastoma cells is stimulated by saposin C. They showed that glucosylceramide that accumulated in the neuroblastoma cells rapidly disappeared when a ternary mixture of glucosylceramidase, saposin C, and phosphatidylserine was added to the culture media. Their finding also demonstrates binding between saposin C and glucosylceramidase that facilitates uptake.

Carbohydrate side chains are not important in the stimulatory activity of saposins. Sano and Radin (53) removed the carbohydrate chain of pig saposin C and found that deglycosylated saposin C had full activity in glucosylceramidase activation. Similarly, we have also shown that deglycosylation of saposin A (9) from Gaucher spleen did not reduce stimulation of glucosylceramidase.

Analysis of homologous proteins may facilitate an understanding of structure and function. As first noted by Sano et al. (54) and discussed in detail by us (10) and Pathy (55), there is an extensive similarity in the amino acid sequence of saposins and that of the major pulmonary surfactant protein SP-B. These include a similar chain length and placement of six cysteine residues and a number of proline residues, which are important in determining the tertiary structure of proteins. Many other amino acids are also identically positioned, although the glycosylation site present in saposins is missing in SP-B. Homology between SP-B and the four saposins is as great as that among the saposins themselves (10). SP-B is one of three hydrophobic proteins found in pulmonary surfactant together with phospholipids and cholesterol. SP-B is capable of restoring the surfactant property when mixed with phospholipids by lowering surface tension of the lipid monolayer (56). Despite their similarity, biological functions of the saposins and SP-B appear to be significantly different. SP-B provided by Dr. Hawgood did not stimulate acid glucosidase activity and saposin B possessed only a weak effect in lowering surface tension (S. Hawgood, J. S. O'Brien, and Y. Kishimoto, unpublished results). SP-B is more hydrophobic than the saposins and the proportion of charged residues is also different. The number of charged molecules in saposin A, for example, is 21 (13 negatively charged and 8 positively charged) and that in SP-B is 12 (4 negative and 8 positive); chain lengths are similar in the two proteins. A recent study of SP-B by Cochrane and Revak (57) demonstrated that chains of hydrophobic amino acids dotted with positively charged amino acids appear to be essential for surfactant activity. These differences may contribute to the observed differences in the activities of these proteins.

V. STRUCTURE AND FUNCTION OF PROSAPOSIN

Whether the saposin domains in the prosaposin molecule possess functional activity similar to mature saposins has recently been investigated. Kondoh et al. (18) reported that human milk prosaposin does not stimulate activity for glucosylceramidase, indicating that the saposin C domain in milk prosaposin is either sterically hindered or improperly folded. Our group has found that recombinant prosaposin and rat testicular prosaposin (SGP-1) stimulate glucosylceramidase almost to the same extent as saposin C (37). Recombinant prosaposin also stimulates GM_1 ganglioside, β-galactosidase, and sphingomyelinase but does not stimulate the hydrolysis of cerebroside sulfate by arylsulfatase A (A. Fluharty, personal communication).

Little is known of the function of the prosaposin that appears to be an integral membrane component (not bound to the mannose-6-phosphate receptor) discussed above. Studies by Griswold et al. (34) on rat prosaposin (SGP-1) secreted by Sertoli cells demonstrate that SGP-1 is one of a set of proteins that are synthesized and secreted when spermatozoa become mature and that bind to the mature spermatozoa. Collard et al. (5) and Sylvester et al. (58) propose two roles for prosaposin in spermatogenesis: one for the release of spermatozoa and another for membrane modification by glycolipid transport.

Prosaposin has the highest affinity for ganglioside GQ1b among other gangliosides examined. Ganglioside GQ1b has the unique ability to enhance neuronal cell proliferation and to increase the number and total length of neurites (neurite sprouting) in neuroblastoma cell lines (59). Ganglioside binding proteins with affinities high enough to act as receptors have been searched for in the brain. One such receptor was reported in rat brain membranes using ganglioside GT1b covalently attached to bovine serum albumin (a neoganglioprotein) as a ligand (60). Binding to this receptor was highest for gangliosides of the b series which differs from the affinity of prosaposin for the a series gangliosides. A subsequent study by the same group (61) localized the receptor to central nervous system myelin membranes.

The apparent K_m values we observed for binding of gangliosides to prosaposin (1–2 μM) were uncorrected for

concentrations of bound versus unbound protein due to procedural difficulties. We anticipate that, after correction, actual K_Ds for binding will be much higher than the apparent K_Ds, in the receptor binding nanomolar range.

A recent report in which prosaposin transcripts were analyzed during mouse brain development describes the highest levels of prosaposin mRNA in the brain stem and dorsal ganglia in the 12-day embryo at the time neuroblast differentiation is rapidly proceeding (62). One possibility is that prosaposin represents a biologically important ganglioside transport protein involved in membrane modulation during neuronal differentiation and neuritogenesis.

From the structural similarity between saposin B and influenza neuraminidase, Potier et al. (63) proposed that human lysosomal sialidase is derived from prosaposin. They isolated a 60 kDa protein, which is recognized by an anti-prosaposin antibody, from human placenta and showed the protein to have the characteristics of a neuraminidase. We have also isolated a protein from placenta which appears to be identical to the one reported by Potier et al. (63) from placenta and have demonstrated that it is not a sialidase but an immunoglobulin G heavy chain protein (69). This study and a recent report of cells from a patient with prosaposin deficiency due to a mutated initiation codon (44) demonstrate that prosaposin is not a precursor of sialidase.

VI. SAPOSINS AND DISEASES

A. Accumulation of saposins in tissues of lysosomal storage disease patients

Saposins are found in considerable excess in tissues of some lysosomal storage disease patients. Inui and Wenger (64) determined concentrations of saposin B by using immunoelectrophoresis and found more than a 10-fold accumulation in the liver of patients with type 1 GM_1 gangliosidosis and type A Niemann-Pick disease and in brains of patients with type 1 GM_1 gangliosidosis, Tay-Sachs disease, and Sandhoff disease. Moderate accumulations were also found in diseases including metachromatic leukodystrophy and Krabbe disease. Our more recent study with high performance liquid chromatography (for saposins A, C, and D) and an activation assay (for saposin B) revealed up to an 80-fold accumulation of saposins A, C, and D in the spleen of patients with Gaucher disease and type 1 Niemann-Pick disease, in liver of fucosidosis and type 1 Niemann-Pick disease, and in brain of patients with Tay-Sachs disease and Sandhoff disease (65). These observations indicate that saposins accumulate in tissues where sphingolipids accumulated.

The accumulation of saposins may be explained by two hypotheses. First, synthesis of saposins may be stimulated by the accumulation of either defective enzyme or lipids as a compensatory mechanism. Second, saposins may be co-deposited with the accumulated lipids. Datta, Snider, and Radin (52) found 61–70% increased levels of saposin C in mouse brain and liver in response to increasing the level of glucosylceramide by injecting either glucosylceramidase inhibitor (conduritol B epoxide) or glucosylceramide emulsion. Taniike et al. (41) loaded sulfatides into cultured skin fibroblasts from a metachromatic leukodystrophy patient (caused by deficient cerebroside sulfatase) and found that simultaneous accumulation of sulfatide and saposin B occurred in 59% of lysosomes examined. These observations indicate that the storage of glycosphingolipids is accompanied by the accumulation of saposins.

Saposin accumulation may be nonspecific, that is, unrelated to the catabolic defect. For example, saposin B and D accumulate in the tissues of Gaucher disease patients and they do not appear to be involved in hydrolysis of glucosylceramide. Similarly, accumulation of saposins in brain in Tay-Sachs disease and Sandhoff disease is massive but saposins do not appear to be involved in the hydrolysis of GM_2 ganglioside, the major storage product. As discussed, all four saposins bind strongly to gangliosides including ganglioside GM_2 (M. Hiraiwa S. Soeda, Y. Kishimoto, and J. S. O'Brien, unpublished results). We have recently found that saposins also bind to cerebrosides. These observations may explain why all four saposins accumulate in tissues that store glycosphingolipids. Whether fucosylated glycolipids bind to saposins should also be examined since saposins are stored in massive quantities in the viscera in fucosidosis. Recent observations on the distribution of saposins in brain of GM_1 gangliosidosis and spleen of Gaucher disease patients (66) demonstrate that the majority of saposins are localized in the light subcellular fractions in both tissues, while GM_1 ganglioside and glucosylceramide are found in the heavy particulate fractions. If saposins and glycosphingolipids co-associate, they appear to dissociate during fractionation.

B. Mutations of the prosaposin gene

Discovery of a mutation in prosaposin resulting in saposin deficiencies demonstrated the essential role of saposins in sphingolipid hydrolysis in human tissues. Patients with saposin deficiencies have normal or near normal in vitro activities of lysosomal hydrolases. Two mutations in saposin B have been elucidated. One of these is a point mutation in the open reading frame (a single C-T transition) (11, 12) resulting in a substitution of threonine to isoleucine and the loss of the single glycosylation site in saposin B. The lack of the carbohydrate chain was proposed as responsible for the instability of saposin B, perhaps by exposing a cleavage site for tryptic-like enzymes. However, we have recently found that removal of the carbohydrate chain from saposin B does not result in increased susceptibility to trypsin. This observation may indicate that the tertiary structure of saposin B may be

changed by the mutation to render it proteolytically sensitive.

In a second patient with saposin B deficiency, a C-A transversion was present in the middle of a 4 kb intron in the prosaposin gene (13, 67), generating a new splice site resulting in the insertion of a 33 bp intronic fragment into the saposin B domain. In this case the inserted amino acids were very hydrophilic and saposin B was deficient, presumably due to instability. Another saposin B-deficient patient was recently reported (68) but the etiology of the defect has not yet been elucidated.

A deficiency in saposin C was first demonstrated in a variant form of Gaucher disease (27). A recent report by Schnabel, Schröder, and Sandhoff (14) demonstrated that a G^{1154}-T transversion (counting from the A of the ATG initiation codon) occurred in the prosaposin cDNA resulting in the substitution of Phe for Cys^{385} in saposin C. This substitution may result in failure to form a normal tertiary structure and make the mutant saposin C unstable. However, this defect was found in only one allele in the patient's reverse transcribed prosaposin mRNA; amplification of genomic DNA gave both the mutant and the normal sequence. Since the patient's cells were deficient in immunoreactive saposin C (27), the authors suggest that a second unidentified mutation occurred in the other allele which might lead to mRNA deficiency with the patients being compound heterozygotes. This explanation is difficult to accept since the remaining coding sequence of prosaposin was normal which should give rise to normal levels of saposin A. Since saposin A is nearly as active as saposin C in vitro, it should compensate for saposin C deficiency and prevent storage of glucosylceramide. Quantification of saposin A in such patients should be carried out as it is our present belief that isolated saposin C deficiency cannot give rise to Gaucher disease.

Another type of mutation resulting in total prosaposin deficiency was recently reported in two siblings of fourth cousin parents (15). Biochemical analysis demonstrated the accumulation of different sphingolipids including glucosylceramide, galactosylceramide, and ceramide in the patient's tissues. In these siblings sequence analysis of mRNA demonstrated an A-T transversion in the initiation codon (44) resulting in absent preprosaposin synthesis. Clinically the index case presented with a severe neuropathic phenotype with clonic tonic seizures at birth, hypotonia, failure to thrive, and death by 16 weeks after serial convulsive attacks which were refractory to anticonvulsants. The early onset and severity of the central nervous system involvement underscores the proposal that prosaposin is an important protein in nervous system development. Unfortunately, postmortem examination of the brain was not carried out.

VII. CONCLUSION

Although saposins B and C have been known for many years, recent progress in immunochemical, biochemical, and, most importantly, molecular biological techniques has contributed to rapid progression of saposin research in the past several years. The initial confusion as to the identity of individual saposins has been sorted out. The discovery that all four saposins, A, B, C, and D, are encoded in a single precursor, prosaposin, and released by proteolytic processing significantly contributed to the discovery and definition of saposin proteins and precipitated the new nomenclature proposed by us. Discovery of metabolic defects due to mutations in the prosaposin gene, as well as the availability of pure saposins and corresponding lysosomal hydrolytic enzymes, gave insight into the physiological significance of saposin proteins.

The presence of two different pools of prosaposin, one intracellular and the other extracellular, suggests that prosaposin has multiple functions, one of which is to generate saposins that activate lysosomal hydrolysis of sphingolipids. This function has been well documented enzymatically and by subcellular localization of saposins. In spite of this clear indication, the mechanism of activation is incompletely understood. One puzzle is the difference in the mode of activation by saposin B from other saposins. Our recent finding that all saposins bind strongly with gangliosides and other sphingolipids demonstrates that lipid binding may be a common feature of all saposins. Possibly, a ternary mixture of enzyme-saposin-sphingolipid may be the obligatory intermediate of the hydrolytic reaction.

Our working hypothesis on a second function of prosaposin is that it may serve as an transport protein for gangliosides. Immunocytochemical studies indicate high concentrations of saposins within neurons and high prosaposin mRNA concentrations in the developing nervous system. Studies need to be carried out to determine whether prosaposin-ganglioside complexes are important in ganglioside transport, neuronal differentiation, neuritogenesis, and intracellular movement of gangliosides such as axoplasmic flow.

Recombinant prosaposin is now being produced in insect cells. The increased availability of prosaposin as well as mature saposins will offer an opportunity to study the interactions between enzyme-saposin complexes as well as lipid-saposin complexes in greater detail and to study the cellular biological functions of prosaposin. This approach should not only deepen our understanding of lysosomal hydrolysis of sphingolipids, but also of protein-protein and lipid-protein interactions in general, mediated by these unique proteins.

This work was supported in part by NIH grants NS08682 and HD18983, and the Gould Family Foundation (JSO), and NS13559 (YK). We thank Mrs. Susan O'Brien for word processing of the manuscript.

Manuscript received 28 April 1992.

REFERENCES

1. Mehl, E., and H. Jatzkewitz. 1964. Eine cerebrosidsulfatase aus schweineniere. *Hoppe-Seyler's Z. Physiol. Chem.* **339:** 260–276.
2. Ho, M-W., and J. S. O'Brien. 1971. Gaucher's disease: deficiency of "acid" β-glucosidase and reconstitution of enzyme activity in vitro. *Proc. Natl. Acad. Sci. USA.* **68:** 2810–2813.
3. Dewji, N. N., D. Wenger, S. Fujibayashi, M. Donoviel, F. Esch, F. Hill, and J. S. O'Brien. 1986. Molecular cloning of the sphingolipid activator protein-1 (SAP-1), the sulfatide sulfatase activator. *Biochem. Biophys. Res. Commun.* **134:** 989–994.
4. Dewji, N. N., D. A. Wenger, and J. S. O'Brien. 1987. Nucleotide sequence of cloned cDNA for human sphingolipid activator protein 1 precursor. *Proc. Natl. Acad. Sci. USA.* **84:** 8652–8656.
5. Collard, M. W., S. R. Sylvester, J. K. Tsuruta, and M. D. Griswold. 1988. Biosynthesis and molecular cloning of sulfated glycoprotein-1 secreted by rat Sertoli cells: sequence similarity with the 70 kDa precursor to sulfatide GM1 activator. *Biochemistry.* **27:** 4557–4564.
6. O'Brien, J. S., K. A. Kretz, N. N. Dewji, D. A. Wenger, F. Esch, and A. L. Fluharty. 1988. Coding of two sphingolipid activator proteins (SAP-1 and SAP-2) by same genetic locus. *Science.* **241:** 1098–1101.
7. Fürst, W., W. Machleidt, and K. Sandhoff. 1988. The precursor of sulfatide activator protein is processed to three different proteins. *Biol. Chem. Hoppe-Seylers.* **369:** 317–328.
8. Morimoto, S., B. Martin, Y. Kishimoto, and J. S. O'Brien. 1988. Saposin D: a sphingomyelinase activator. *Biochem. Biophys. Res. Commun.* **156:** 403–410.
9. Morimoto, S., B. M. Martin, Y. Yamamoto, K. A. Kretz, J. S. O'Brien, and Y. Kishimoto. 1989. Saposin A: a second cerebrosidase activator protein. *Proc. Natl. Acad. Sci. USA.* **86:** 3389–3393.
10. O'Brien, J. S., and Y. Kishimoto. 1991. Saposin Proteins: structure, function and role in human lysosomal storage disorders. *FASEB J.* **5:** 301–308.
11. Kretz, K. A., G. S. Carson, S. Morimoto, Y. Kishimoto, A. L. Fluharty, and J. S. O'Brien. 1990. Characterization of a mutation in a family with saposin B deficiency: a glycosylation site defect. *Proc. Natl. Acad. Sci. USA.* **87:** 2541–2544.
12. Rafi, M. A., X-L. Zhang, G. DeGala, and D. A. Wenger. 1990. Detection of a point mutation in sphingolipid activator protein-1 mRNA in patients with a variant from of metachromatic leukodystrophy. *Biochem. Biophys. Res. Commun.* **166:** 1017–1023.
13. Zhang, X., M. A. Rafi, G. DeGala, and D. A. Wenger. 1991. The mechanism for a 33-nucleotide insertion in mRNA causing sphingolipid activator protein (SAP-1) deficient metachromatic leukodystrophy. *Hum. Genet.* **87:** 211–215.
14. Schnabel, D., M. Schröder, and K. Sandhoff. 1991. Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease. *FEBS Lett.* **284:** 57–59.
15. Harzer, K., B. C. Paton, A. Poulos, B. Kustermann-Kuhn, W. Roggendorf, T. Grisar, and M. Popp. 1989. Sphingolipid activator protein deficiency in a 16-week-old atypical Gaucher disease patient and his fetal sibling; biochemical signs of combined sphingolipidoses. *Eur. J. Pediat.* **149:** 31–39.
16. Morimoto, S., Y. Kishimoto, J. Tomich, S. Weiler, T. Ohashi, J. A. Barranger, K. A. Kretz, and J. S. O'Brien. 1990. Interaction of saposins, acidic lipids and glucosylceramidase. *J. Biol. Chem.* **265:** 1933–1937.
17. Fabbro, D., and G. A. Grabowski. 1991. Human acid β-glucosidase: use of inhibitory and activating monoclonal antibodies to investigate the enzyme's catalytic mechanism and saposin A and C binding sites. *J. Biol. Chem.* **266:** 15021–15027.
18. Kondoh, K., T. Hineno, A. Sano, and Y. Kakimoto. 1991. Isolation and characterization of prosaposin from human milk. *Biochem. Biophys. Res. Commun.* **181:** 286–292.
19. Inui, K., M. Emmett, and D. A. Wenger. 1983. Immunological evidence for deficiency in an activator protein for sulfatide sulfatase in a variant form of metachromatic leukodystrophy. *Proc. Natl. Acad. Sci. USA.* **80:** 3074–3077.
20. Vogel, A., W. Fürst, M. A. Abo-Hashish, M. Lee-Vaupel, E. Conzelmann, and K. Sandhoff. 1987. Identity of the activator proteins for the enzymatic hydrolysis of sulfatide, ganglioside G_{M1}, and globotriaosylceramide. *Arch. Biochem. Biophys.* **259:** 627–638.
21. Li, S-C., S. Sonnino, G. Tettamanti, and Y-T. Li. 1988. Characterization of a nonspecific activator protein for the enzymatic hydrolysis of glycolipids. *J. Biol. Chem.* **263:** 6588–6591.
22. Wenger, D. A., and K. Inui. 1984. Studies on the sphingolipid activator protein for the enzymatic hydrolysis of GM1 ganglioside and sulfatide. *In* Molecular Basis of Lysosomal Storage Disorders. R. O. Brady and J. Barranger, editors. Academic Press, New York. 1–18.
23. Vogel, A., G. Schwarzmann, and K. Sandhoff. 1991. Glycosphingolipid specificity of the human sulfatide activator protein. *Eur. J. Biochem.* **200:** 591–597.
24. Fischer, G., and H. Jatzkewitz. 1975. The activator of cerebroside sulfatase: purification from human liver and identification as a protein. *Hoppe-Seylers Z. Physiol. Chem.* **356:** 605–613.
25. Li, S-C., and Y-T. Li. 1976. An activator stimulating the enzymic hydrolysis of sphingolipids. *J. Biol. Chem.* **251:** 1159–1163.
26. Louis, A. I., and A. L. Fluharty. 1991. Activator-dependent hydrolysis of myeline cerebroside by arylsulfatase A. *Dev. Neurosci.* **13:** 41–46.
27. Christomanou, H., A. Aignesberger, and R. P. Linke. 1986. Immunochemical characterization of two activator proteins stimulating enzymic sphingomyelin degradation in vitro. Absence of one of them in a human Gaucher disease variant. *Biol. Chem. Hoppe-Seylers.* **367:** 879–890.
28. Peters, S. P., P. Coyle, C. J. Coffee, R. H. Glew, M. S. Kuhlenschmidt, L. Rosenfeld, and Y. C. Lee. 1977. Purification and characterization of a heat-stable glucocerebrosidase activating factor from control and Gaucher spleen. *J. Biol. Chem.* **252:** 563–573.
29. Berent, S. L., and N. S. Radin. 1981. Mechanism of activation of glucocerebrosidase by Co-β-glucosidase (glucosidase activator protein). *Biochim. Biophys. Acta.* **664:** 572–582.
30. Aerts, J. M. F. G., W. E. Donker-Koopman, C. van Laar, S. Brul, G. Murray, D. A. Wenger, J. A. Barranger, J. M. Tager, and A. W. Schram. 1987. Relationship between the two immunologically distinguishable forms of glucocerebrosidase in tissue extracts. *Eur. J. Biochem.* **163:** 583–589.

31. Radin, N. S. 1984. The cohydrolases for cerebroside β-glucosidase. *In* The Molecular Basis of Lysosomal Storage Disorders. R. O. Brady and J. A. Barranger, editors. Academic Press, New York. 93–112.
32. Glew, R. H., A. Basu, K. L. LaMarco, and E. M. Prence. 1988. Mammalian glucocerebrosidase: implications for Gaucher's disease. *Lab. Invest.* **58:** 5–25.
33. Fujibayashi, S., and D. A. Wenger. 1986. Synthesis and processing of sphingolipid activator protein-2 (SAP-2) in cultured human fibroblasts. *J. Biol. Chem.* **261:** 15339–15343.
34. Griswold, M. D., K. Roberts, and P. Bishop. 1986. Purification and characterization of a sulfated glycoprotein secreted by Sertoli cells. *Biochemistry.* **25:** 7265–7270.
35. Sano, A., T. Hineno, T. Mizuno, K. Kondoh, S. Ueno, Y. Kakimoto, and K. Inui. 1989. Sphingolipid hydrolase activator proteins and their precursors. *Biochem. Biophys. Res. Commun.* **165:** 1191–1197.
36. Hineno, T., A. Sano, K. Kondoh, S. Ueno, Y. Kakimoto, and K. Yoshida. 1991. Secretion of sphingolipid hydrolase activator precursor, prosaposin. *Biochem. Biophys. Res. Commun.* **176:** 668–674.
37. Hiraiwa, M., J. S. O'Brien, Y. Kishimoto, B. M. Martin, E. L. Ginns, and A. L. Fluharty. 1992. Isolation and characterization of prosaposin, the precursor of saposins (sphingolipid activator proteins). *FASEB J.* **6:** A969.
38. Fujibayashi, S., and D. A. Wenger. 1986. Biosynthesis of the sulfatide/GM1 activator protein (SAP-1) in control and mutant cultured skin fibroblasts. *Biochim. Biophys. Acta.* **875:** 554–562.
39. Tamaru, T., S. Fujibayashi, W. R. Brown, and D. A. Wenger. 1986. Immunocytochemical localization of sphingolipid activator protein-1, the sulfatide/GM1 ganglioside activator, to lysosomes in human liver and colon. *Histochemistry.* **86:** 195–200.
40. Taniike, M., K. Inui, K. Shinoda, S. Okada, H. Yabuuchi, and Y. Shiotani. 1991. Localization of sphingolipid activator protein-1 (SAP-1) in the brain of a normal human and a patient with metachromatic leukodystrophy. *Acta Histochem. Cytochem.* **24:** 215–222.
41. Taniike, N., K. Inui, K. Shinoda, S. Okada, Y. Shiotani, and H. Yabuuchi. 1988. Correlation of subcellular localization of disease specific inclusions and sphingolipid activator protein-1 (SAP-1) in sulfatide sulfatase deficient fibroblasts. *Acta Histochem. Cytochem.* **21:** 565–573.
42. Paton, B. C., J. L. Hughes, K. Harzer, and A. Poulos. 1989. Immunocytochemical localization of sphingolipid activator protein 2 (SAP-2) in normal and SAP-deficient fibroblasts. *Eur. J. Cell Biol.* **51:** 157–164.
43. Rijnboutt, S., H. M. F. G. Aert, H. J. Geuze, J. M. Tager, and G. J. Strous. 1991. Mannose 6-phosphate-independent membrane: association of cathepsin D, glucocerebrosidase, and the sphingolipid activating protein in HepG2 cells. *J. Biol. Chem.* **266:** 4862–4868.
44. Schnabel, D., M. Schröder, W. Fürst, A. Klein, R. Hurwitz, T. Zenk, J. Weber, K. Harzer, B. C. Paton, A. Poulos, K. Suzuki, and K. Sandhoff. 1992. Simultaneous deficiency of sphingolipid activator proteins 1 and 2 is caused by a mutation in the initiation codon of their common gene. *J. Biol. Chem.* **267:** 3312–3315.
45. Inui, K., F. T. Kao, S. Fujibayashi, C. Jones, H. G. Morse, M. L. Law, and D. A. Wenger. 1985. The gene coding for a sphingolipid activator SAP-2, is on chromosome 10. *Hum. Genet.* **69:** 197–200.
46. Fujibayashi, S., F-T. Kao, C. Jones, H. Morse, M. Law, and D. A. Wenger. 1985. Assignment of the gene for human sphingolipid activator protein-2 (SAP-2) to chromosome 10. *Am. J. Hum. Genet.* **37:** 741–748.
47. Kao, F. T., M. L. Law, J. Hartz, C. Jones, X-L. Zhang, N. N. Dewji, J. S. O'Brien, and D. A. Wenger. 1988. Regional localization of the gene coding for sphingolipid activator protein (SAP-1) on human chromosome 10. *Somat. Cell Molec. Genet.* **13:** 685–688.
48. Holtschmidt, H., K. Sandhoff, W. Fürst, H. Y. Kwon, D. Schnabel, and K. Suzuki. 1991. The organization of the gene for the human cerebroside sulfate activator protein. *FEBS Lett.* **280:** 267–270.
49. Wynn, C. H. 1986. A triple-binding-domain model explains the specificity of the interaction of a sphingolipid activator protein (SAP-1) with sulphatide, GM1-ganglioside and globotriaosylceramide. *Biochem. J.* **240:** 921–924.
50. Chou, P. Y., and G. D. Fasman. 1978. Empirical predictions of protein conformation. *Annu. Rev. Biochem.* **47:** 251–276.
51. Yamashita, K., K. Inui, K. Totani, N. Kochibe, M. Furukawa, and S. Okada. 1990. Characteristics of asparagine-linked sugar chains of sphingolipid activator protein purified from normal human liver and GM1 gangliosidosis (Type 1) liver. *Biochemistry.* **29:** 3030–3039.
52. Datta, S. C., R. M. Snider, and N. S. Radin. 1986. Uptake by neuroblastoma cells of glycosylceramide, glucosylcereamide glucosidase, its stimulator protein and phosphatidylserine. *Biochim. Biophys. Acta.* **877:** 387–398.
53. Sano, A., and N. S. Radin. 1988. The carbohydrate moiety of the activator protein for glucosylceramide β-glucosidase. *Biochem. Biophys. Res. Commun.* **154:** 1197–1203.
54. Sano, A., N. S. Radin, L. L. Johnson, and G. E. Tarr. 1989. The activator protein for glucosylceramide β-glucosidase from guinea pig liver: improved isolation method and complete amino acid sequence. *J. Biol. Chem.* **263:** 19597–19601.
55. Pathy, L. 1991. Homology of the precursor of pulmonary surfactant-associated protein SP-B with prosaposin and sulfated glycoprotein 1. *J. Biol. Chem.* **266:** 6035–6037.
56. Hawgood, S. 1991. Surfactant: composition, structure and metabolism. *In* The Lung. R. G. Crystal and J. B. West, editors. Raven Press Ltd., New York. 247–261.
57. Cochrane, C. G., and S. D. Revak. 1991. Pulmonary surfactant protein B (SP-B): structure-function relationships. *Science.* **254:** 566–568.
58. Sylvester, S. R., C. Morales, R. Oko, and M. D. Griswold. 1989. Sulfated glycoprotein-1 (saposin precursor) in the reproductive tract of the male rat. *Biol. Reprod.* **41:** 941–948.
59. Tsuji, S., M. Arita, and Y. Nagai. 1983. GQ1b, a bioactive ganglioside that exhibits novel nerve growth factor (NGF)-like activities in the two neuroblastoma cell lines. *J. Biochem.* **94:** 303–306.
60. Tiemeyer, M., Y. Yasuda, and R. L. Schnarr. 1989. Ganglioside-specific binding protein on rat brain membrane. *J. Biol. Chem.* **264:** 1671–1681.
61. Tiemeyer, M., P. Swank-Hill, and R. L. Schnarr. 1990. A membrane receptor for gangliosides is associated with central nervous system myelin. *J. Biol. Chem.* **265:** 11990–11999.
62. Levy, H., A. Orr-Urteger, P. Lonai, and M. Horowitz. 1991. Structural and functional analysis of the prosaposine gene. *In* 8th European Study Group on Lysosomal Diseases. #49.
63. Potier, M., S. Lamontagne, L. Michaud, and J. Tranchemontagne. 1990. Human neuraminidase is a 60-kDa processing product of prosaposin. *Biochem. Biophys. Res. Commun.* **173:** 449–456.
64. Inui, K., and D. A. Wenger. 1983. Concentrations of an activator protein for sphingolipid hydrolysis in liver and brain samples from patients with lysosomal storage diseases. *J.*

Clin. Invest. **72:** 1622–1628.

65. Morimoto, S., Y. Yamamoto, J. S. O'Brien, and Y. Kishimoto. 1990. Distribution of saposin proteins (sphingolipid activator proteins in lysosomal storage and other diseases. *Proc. Natl. Acad. Sci. USA* **87:** 3493–3497.
66. Tayama, M., J. S. O'Brien, and Y. Kishimoto. 1992. Distribution of saposins (sphingolipid activator proteins) in tissues of lysosomal storage disease patients. *J. Molec. Neurosci.* In press.
67. Zhang, X., M. A. Rafi, G. DeGala, and D. A. Wenger. 1990. Insertion in the mRNA of a metachromatic leukodystrophy patient with sphingolipid activator-1 deficiency. *Proc. Natl. Acad. Sci. USA.* **87:** 1426–1430.
68. Scholte, W., K. Harzer, H. Christomanou, B. C. Paton, B. Kustermann-Kuhn, B. Schmid, J. Seeger, U. Bendt, I. Shuster, and U. Langenbeck. 1991. Sphingolipid activator protein 1 deficiency in metachromatic leukodystrophy with normal arylsulfatase A activity. A clinical, morphological, biochemical and immunological study. *Eur. J. Pediat.* **150:** 584–591.
69. Hiraiwa, M., Y. Uda, S. Tsuji, T. Miyatake, B. M. Martin, M. Tayama, J. S. O'Brien, and Y. Kishimoto. 1991. Human placental sialidase complex: characterization of the 60 kDa protein that cross-reacts with anti-saposin antibodies. *Biochem. Biophys. Res. Commun.* **177:** 1211–1216.