FOR THE RECORD

Acid sphingomyelinase possesses a domain homologous to its activator proteins: Saposins B and D

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Abstract: An N-terminal region of the acid sphingomyelinase sequence (residues 89–165) is shown to be homologous to saposin-type sequences. By analogy with the known functions of saposins, this sphingomyelinase saposin-type domain may possess lipid-binding and/or sphingomyelinase-activator properties. This finding may prove to be important in the understanding of Niemann-Pick disease, which results from sphingomyelinase deficiency.

Keywords: acid sphingomyelinase; homology; Niemann-Pick disease; saposin

The lysosomal degradation of several sphingolipids by specific acid hydrolases, including sphingomyelinase, is stimulated by the presence of low-molecular-mass nonenzymatic factors termed sphingolipid activator proteins. Of the 5 such molecules known, 4, named saposins A, B, C, and D, are derived from a common precursor, prosaposin, by partial proteolysis (O'Brien et al., 1988). Saposins A and C each stimulate the activity of β -glucosylceramidase and β -galactosylceramidase by forming cofactor–enzyme complexes (Morimoto et al., 1989, 1990). Saposin B is a comparatively nonspecific activator that stimulates the degradation of many glycolipids and glycerolipids as well as sphingomyelin (Christomanou et al., 1986; Li et al., 1988) by solubilizing the lipid substrates for enzyme hydrolysis. Saposin D is a specific activator for sphingomyelin degradation by acid sphingomyelinase (Morimoto et al., 1988). All 4 saposins bind galactosyl- and glucosylceramide, sulfate (Soeda et al., 1993), and gangliosides (Hiraiwa et al., 1992).

The importance in determining structure–function relationships of saposins is emphasized by lysosomal storage diseases associated with their deficiencies. Deficiencies of saposin B and saposin C are known to result in activator-deficient forms of

metachromatic leukodystrophy (ML) (Kretz et al., 1990; Rafi et al., 1990; Zhang et al., 1990; Holtschmidt et al., 1991) and Gaucher disease (Schnabel et al., 1991; Wenger et al., 1991), respectively. Until now, no structural similarities had been observed between saposins and acid sphingomyelinase, whose deficiency results in a third lysosomal storage disorder, Niemann-Pick disease (types A and B) (Spence & Callahan, 1989). Here I report that an N-terminal region (residues 89–165) of acid sphingomyelinase shows significant sequence similarity with saposin-type sequences. This finding suggests a structural relationship between Niemann-Pick disease and activator-deficient forms of ML and Gaucher disease.

Previously, 5 homologues of saposins had been known, all of which are lipid-related proteins: the small subunit of human acylglycerol hydrolase (AOAH) (Hagen et al., 1991), a pore-forming peptide (PFP) of *Entamoeba histolytica* (Leippe et al., 1992), and 3 tandem repeats from pulmonary surfactant-associated protein B (SP-B) (Pilot-Matias et al., 1989; Patthy, 1991). AOAH is a leukocyte enzyme that deacylates bacterial lipopolysaccharides (Hagen et al., 1991); PFP possesses a property of spontaneous incorporation into lipid bilayers, liposomes, and cells resulting in the formation of ion channels, which may participate in the cytolytic activity of pathogenic amoebae (Lynch et al., 1982); and SP-B domain 2 (SPB-2) is an essential constituent of pulmonary surfactant, which is vital for the maintenance of lung volume during the respiratory cycle (Longo et al., 1993).

The acid sphingomyelinase (ASM) sequence (residues 89–165) (Schuchman et al., 1991) possesses conserved residue types at each position thought to characterize the saposin fold. These are 6 cysteine residues (at positions 1, 4, 33, 46, 72, and 80) that participate in intramolecular disulfide bridges (Johansson et al., 1991, 1992) and hydrophobic residues (at positions 7, 8, 11, 25, 28, 29, 36, 49, 50, and 53) that lie within 3 regions (1–16, 23–36, and 43–54), each of which is likely to form an amphipathic α -helix (O'Brien & Kishimoto, 1991). The average of pairwise similarity scores between the ASM sequence and other saposin-type sequences was found to be 6.3 SD from a mean of randomized sequences. This indicates that the saposin-type domain within ASM (residues 89–165, hereinafter referred to as ASM-

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Fig. 1. Multiple sequence alignment of saposin-type domains showing sequence similarity between human saposins A, B, C, and D, SP-B domains 1, 2, and 3, AOA, *E. histolytica* PFP, and human ASM. PIR accession numbers are shown following the sequence identifiers. Absolutely conserved cysteine residues are shown in white-on-black, and positions where hydrophobic residues are present in 90% or in all sequences are shaded. Sequence similarity was detected by scanning the NBRF-PIR database (V.36) with a multiple alignment of saposin, SP-B, AOA, and PFP sequences (O'Brien et al., 1988; Pilot-Matias et al., 1989; Hagen et al., 1991; Leippe et al., 1992) using a local similarity algorithm (Barton, 1993b) with Dayhoff's MDM78 matrix and a length-dependent gap penalty of 8. Subsequent multiple alignment with the ASM sequence (Schuchman et al., 1991) was achieved using the AMPS suite of programs (Barton, 1990) and displayed using ALSCRIPT (Barton, 1993a). The alignment shows an average of 21% identity between pairwise alignments of the ASM sequence and previously known saposin-type sequences, as compared with an average of 24% between all pairwise alignments. The average pairwise similarity score between the ASM sequence and the other saposin-type sequences is 6.3 SD from the mean of scores for randomized sequences of the same length and composition of the sequence pairs. The similarity score for all other pairwise comparisons is 7.9 SD.

sap) and other saposin-type domains are all likely to have diverged from a common ancestor and all share the same overall fold (Barton, 1990). ASM-sap possesses only 1 major difference from other saposin-type sequences in possessing a 2-residue insertion at its C-terminus (positions 76 and 77).

The finding of homology between ASM-sap and saposins suggests 2 possible functions for the ASM-sap domain that are not mutually exclusive. By analogy to the lipid-binding functions of saposins (Hiraiwa et al., 1992; Soeda et al., 1993), ASM-sap may mediate the known affinity of ASM for lipids (Quintern et al., 1987). Alternatively, if functionally analogous to saposins A and C (Morimoto et al., 1990), ASM-sap may participate in an intramolecular interaction with the remaining C-terminal portion of the molecule, which by default must contain its catalytic site(s), thereby stimulating ASM activity. This would suggest that addition of activators is not a prerequisite for activity, as shown experimentally (Morimoto et al., 1988).

ASM-sap possesses a function that is essential for sphingomyelinase activity; a variant of ASM containing a substitution of a critical cysteine residue (Cys-155 to Arg; position 72 of Fig. 1) within ASM-sap renders the enzyme inactive (Ida et al., 1993). Although all other mutations have been found in the presumed catalytic C-terminal portion of ASM (references in Levran et al., 1993), this finding raises the possibility that some patients with Niemann-Pick disease types A and B may be homoallelic for mutations in the region of the ASM gene that codes for ASM-sap. Moreover, others with Niemann-Pick disease types C and D also may be homoallelic for mutations in the ASM-sap region, resulting in ASM variants possessing normal catalytic activity but diminished affinity for lipids.

This finding suggests a first structural connection between Niemann-Pick disease and 2 other lysosomal storage disorders: substitution of Cys-72 (numbering as in Fig. 1) in ASM renders the enzyme inactive; substitution at the same position (Cys-72) in saposin C has been found to cause activator-deficient Gaucher disease (Schnabel et al., 1991); and mutation of Cys-46 in saposin B has caused an activator-deficient form of metachromatic leukodystrophy (Holtzman et al., 1991).

In conclusion, acid sphingomyelinase has been found to contain a region homologous to its activator proteins, saposins B and D. This finding may be important in the understanding of both the genetic bases of Gaucher, ML, and Niemann-Pick diseases and the abnormal accumulation of saposins in these lysosomal storage diseases (O'Brien & Kishimoto, 1991).

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