A non-glycosylated and functionally deficient mutant (N215H) of the sphingolipid activator protein B (SAP-B) in a novel case of metachromatic leukodystrophy (MLD)

D. WROBE¹, M. HENSELER², S. HUETTLER¹, S. I. PASCUAL PASCUAL³, A. CHABAS⁴ and K. SANDHOFF¹*

¹ Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn, Bonn; ² Pharmazeutisches Institut, Biozentrum der Universität Frankfurt, Frankfurt am Main, Germany; ³ Hospital Infantil ‘La Paz’, Madrid; ⁴ Institut Bioquímica Clínica Barcelona, Barcelona, Spain

* Correspondence: Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk Str. 1, D-53121 Bonn, Germany.
E-mail: sandhoff@uni-bonn.de

MS received 20.04.99 Accepted 10.08.99

Summary: The lysosomal degradation of sphingolipids with short oligosaccharide chains depends on small glycosylated non-enzymatic sphingolipid activator proteins (SAPs, saposins). Four of the five known SAPs, SAP-A, -B, -C and -D, are derived by proteolytic processing from a common precursor protein (SAP-precursor) that is encoded by a gene on chromosome 10 consisting of 15 exons and 14 introns. SAP-B is a non-specific glycolipid binding protein that stimulates in vitro the hydrolysis of about 20 glycolipids by different enzymes. In vivo SAP-B stimulates in particular the degradation of sulfatides by arylsulphatase A. So far, four different point mutations have been identified on the SAP-B domain of the SAP-precursor gene. The mutations result in a loss of mature SAP-B, causing the lysosomal accumulation of sulfatides and other sphingolipids, resulting in variant forms of metachromatic leukodystrophy (MLD). Here we report on a patient with SAP-B deficiency that is caused by a new homoallelic point mutation that has been identified by mRNA and DNA analysis. A 643A > C transversion results in the exchange of asparagine 215 to histidine and eliminates the single glycosylation site of SAP-B. Metabolic labelling experiments showed that the mutation had no effect on the intracellular transport of the encoded precursor to the acidic compartments and its maturation in the patient’s cells. All four SAPs (SAP-A to SAP-D) were detectable by immunochemical methods. SAP-B in the patient’s cells was found to be slightly less stable than the protein in normal cells and corresponded in size to the deglycosylated form of the wild-type SAP-B.
Feeding studies with non-glycosylated SAP-precursor, generating non-glycosylated SAP-B, showed that the loss of the carbohydrate chain reduced the intracellular activity of the protein significantly. The additional structural change of the patient’s SAP-B, caused by the change of amino acid 215 from asparagine to histidine, presumably resulted in an almost completely inactive protein.

The lysosomal degradation of sphingolipids is accomplished by the stepwise action of specific acid exohydrolases. Some of these enzymes need the assistance of small non-enzymatic glycoprotein cofactors, so-called sphingolipid activator proteins (SAPs), to attack their lipid substrates (for review see Fürst and Sandhoff 1992; Sandhoff et al 1995; Sandhoff and Kolter 1996). Four of the five known activator proteins (saposins A to D, or SAP-A to SAP-D) are derived from a common precursor by proteolytic processing. High concentrations of SAP-precursor are found in brain, in semen and in other human secretory fluids, whereas the individual SAPs are mainly present in liver, kidney and spleen (Hineno et al 1991; Sano et al 1989). Biosynthetic studies on SAP-B and -C (Fujibayashi and Wenger 1986a,b) and on processing of SAP-precursor (Vielhaber et al 1996) revealed a 65 kDa precursor protein in the cells that is generated by cotranslational glycosylation of the 53 kDa polypeptide at all five potential glycosylation sites. The oligosaccharide moieties are modified in the Golgi apparatus to yield a precursor protein of 73 kDa containing complex-type oligosaccharide chains. A fraction of this precursor protein is transported via endosomes into the lysosomes and is converted by proteolytic cleavage into the 8–13 kDa mature SAPs A to D. Another fraction of the 73 kDa protein is secreted into the culture medium. The investigation of sphingolipidoses that are caused by defects of the SAPs (for review see Sandhoff et al 1995) has helped to elucidate the role of sphingolipid activator proteins in vivo. The crucial role of SAP-B and SAP-C is demonstrated by the fatal consequences of their deficiencies in patients with atypical forms of metachromatic leukodystrophy (MLD; McKusick 249900) and Gaucher disease (McKusick 230800), respectively. SAP-C activates the degradation of glucosylceramide by β-glucocerebrosidase (EC 3.2.1.45) (Christomanou et al 1986) and the degradation of galactosylceramide by β-galactocerebrosidase (EC 3.2.1.46) (Harzer et al 1997). Recently, SAP-D was shown to be essential for the degradation of ceramide (Klein et al 1994). So far, SAP-A has been characterized in vitro only. Like SAP-C, SAP-A stimulates the degradation of glucosylceramide and galactosylceramide by the respective glycosidases, but to a lesser extent (Fabbro and Grabowski 1991; Morimoto et al 1989, 1990). SAP-B is important for the in vivo degradation of sulphatides, globotriaosylceramide, digalactosylceramide and ganglioside GM3 by specific lysosomal enzymes (Sandhoff et al 1995; Vogel et al 1991). The clinical findings in SAP-B deficiencies are similar to those in juvenile MLD (arylsulphatase A (EC 3.1.6.1) deficiency) but not identical. An abnormal accumulation of sulphatides occurs in several organs, particularly in the white matter of the brain and in the myelin of peripheral nerves. Several mutations causing a SAP-B deficiency have been described in patients with MLD (Henseler et al 1996a; Holtschmidt et al 1991b; Kretz et al 1990; Rafi et al 1990;