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The Biochemical, Immunological and Molecular Characterization of Human Sphingomyelin Synthase-1

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Doktorvater: Prof. Dr. Felix Wieland

Abstract

Sphingomyelin synthases (SMS) are a set of enzymes that catalyze the transfer of the phosphocholine moiety from phosphatidylcholine onto the primary hydroxyl group of ceramide (Cer), thus generating sphingomyelin (SM) and diacylglycerol (DAG); it can also catalyze the reverse reaction. The involvement of SMS in the synthesis of bioactive lipids such as Cer (pro-apoptotic) and DAG (pro-growth) raises an important issue about its subcellular localization, purification and characterization. In this study, we were able to purify the endogenous SMS protein using three sequential chromatography steps to yield a more than 450-fold enrichment of SMS activity and a total yield of about 15%. Furthermore, the purified fractions were employed for a biochemical characterization of the enzyme. This has provided biochemical data indicating that SMS1 is a membrane protein mainly localized to the Golgi apparatus that requires mixed detergents to maintain its activity during sequential chromatographic steps. Recently, Huitema et al. (2004) identified 2 isoforms of SMS using bioinformatics and functional cloning strategies in yeast. SMS1 was proposed to be Golgi-localized, and SMS2, plasma membrane-localized. SMS1 and SMS2 are highly conserved mammalian membrane proteins with a prediction of 6 trans-membrane domain and a luminal (exoplasmic) catalytic site. We raised antibodies against SMS1 (anti-SMS1) and could show that we had purified this particular isoform. Furthermore, by immunofluorescence confocal microscopy the
intracellular localization of SMS1 was characterized.

Cloning and expression of SMS1 yielded a high quantity of recombinant protein. The detergent conditions defined in my purification protocol allowed purification and characterization of both endogenous and recombinant enzyme.