ACID SPHINGOMYELINASE FROM HUMAN URINE: PURIFICATION AND CHARACTERISATION

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ABSTRACT

Acid sphingomyelinase (sphingomyelin phosphodiesterase, EC 3.1.4.12) was purified from human urine in the presence of 0.1 % Nonidet P-40. The activity could be enriched up to 30 000 fold by sequential chromatography on Octyl-Sepharose, Concanavalin-A Sepharose, Blue Sepharose and DEAE Cellulose. The last purification step yielded an enzyme preparation with a specific activity of about 2.5-10 mmol sphingomyelin cleaved/h per mg protein and with a yield of about 3-16 %. Purified sphingomyelinase appeared to be homogeneous in sodium dodecyl sulphate polyacrylamide gel electrophoresis with a mass of about 70 kDa. In the presence of 0.08 % sodium taurodeoxycholate the preparation showed phosphodiesterase activity towards several phospholipids. Sphingomyelin, phosphatidylcholine, phosphatidylglycerol and at a slow rate phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine were hydrolysed. The phospholipase C activity towards phosphatidylcholine and phosphatidylglycerol and acid sphingomyelinase activity co-purified during the entire purification procedure, indicating that acid sphingomyelinase has phospholipase C activity towards these lipids.

Addition of 100 μM tripalmitoylglycerol to the assay system (which contains 100 μM sphingomyelin) instead of detergent, stimulated the reaction about 20-fold, thus offering a sensitive system for the assay of acid sphingomyelinase in a system free of detergents. Sphingomyelin degradation was strongly inhibited by phosphatidylinositol 4', 5'-bis phosphate, adenosine 3', 5'-diphosphate and adenine-9-β-D arabinofuranoside 5'-
monophosphate (50% inhibition at inhibitor concentrations of 1-5 \( \mu \text{M} \) and a substrate concentration of 100 \( \mu \text{M} \) sphingomyelin).

INTRODUCTION

Acid sphingomyelinase catalyses the hydrolysis of the membrane lipid sphingomyelin to ceramide and phosphorylcholine (1). Different purification procedures with human placenta and human brain as starting material yield enzyme preparations with quite different specific activities containing different polypeptide mixtures (2-5). So the molecular mass of human acid sphingomyelinase and the specific activity of the purified enzyme is still uncertain. Deficiencies of this enzyme are the underlying causes for Niemann-Pick disease types A and B (6-7). In another group of Niemann-Pick patients (types C, D, and E) the level of acid sphingomyelinase activity appears close to normal (6) but sphingomyelin accumulates in some organs. Until now there has been no explanation for the biochemical defect underlying these forms of the disease. Fibroblasts of patients with Niemann-Pick disease type A and B also deficient in acid phospholipase C acting on 1,2-diacylglycerophosphocholine (8-9) and 1,2 diacylglycerophosphoglycerol (10). If these phosphatidylcholine and phosphatidylylglycerol degrading activities and acid sphingomyelinase activity are localized on the same protein, a pure enzyme preparation should be able to hydrolyse the mentioned substrates. Freeman et al. (11) demonstrated, that enriched placental sphingomyelinase, which was prepared by a method of Jones et al (3), could degrade sphingomyelin and phosphatidylcholine. This purification procedure yields a final preparation which cleaves about 60 \( \mu \text{mol} \) sphingomyelin/h per mg protein and contains proteins with different molecular masses (determined with sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)). A different pattern of proteins is found in the final preparations of Pentchev et al. (2) and Sakuragawa (4). They described enzyme preparations with 270 and 380 \( \mu \text{mol}/h \) per mg protein, respectively. These data indicate that the purification procedure of Jones et al. may yield a final enzyme preparation which is contaminated with other proteins. However, to clarify if phosphatidylcholine, phosphatidylylglycerol and sphingomyelin are hydrolyzed by the same enzyme, acid sphingomyelinase should be purified to homogeneity.

Acid sphingomyelinase is a membrane-associated protein. Detergents are required for maximal extraction of the enzyme. Besides membrane-associated sphingomyelinase soluble forms have been described. In cell culture, human skin fibroblasts secrete a soluble form of the enzyme into the medium (12). A soluble form of sphingomyelinase is also present in urine (13). In previous reports we described the enrichment of acid sphingomyelinase from human urine (14-15).

This paper describes a method for the purification of acid sphingomyelinase from human urine to homogeneity, its substrate specificity and some very potent inhibitors of the enzyme. Furthermore we present a sensitive procedure to