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Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine

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Abstract The tricyclic antidepressant desipramine causes a decrease in cellular acid sphingomyelinase (A-SMase, EC 3.1.4.12) activity when added to culture medium of human fibroblasts. This effect can be prevented by incubation of the cells with the protease inhibitor leupeptin, which suggests that desipramine induces proteolytic degradation of the lysosomal enzyme. By using surface plasmon resonance (SPR, Biacore) we were able to monitor the interactions of A-SMase and substrate-containing lipid bilayers immobilized on the surface of a Pioneer™ L1 sensor chip. SPR binding curves show that the enzyme hardly dissociates from the lipid surface at acidic pH values. On the other hand, a drop in binding signals (resonance units, RU) of approximately 50% occurred after injection of 20 mM desipramine. Our findings indicate that desipramine interferes with the binding of A-SMase to the lipid bilayers and thereby displaces the enzyme from its membrane-bound substrate. The application of control substances suggests a key role for the cationic moiety of desipramine. We hypothesize that the displacement of the glycoprotein A-SMase from the inner membranes of late endosomes and lysosomes by desipramine renders it susceptible to proteolytic cleavage by lysosomal proteases.

Key words: Acid sphingomyelinase; Desipramine; Drug-induced lipidosis; Surface plasmon resonance

1. Introduction

Acid sphingomyelinase (A-SMase, EC 3.1.4.12) is a lysosomal hydrolase that catalyzes the degradation of sphingomyelin (SM) to phosphorylcholine and ceramide. Inherited deficiencies of A-SMase activity lead to different forms of Niemann–Pick disease, which are characterized by lysosomal SM accumulation [1].

Membrane constituents of eukaryotic cells like SM, a phospholipid mainly located in the exoleaflet of the plasma membrane, are internalized by endocytotic processes and degraded within the acidic organelles. During transport through the endosomal compartments, plasma membrane components form intraendosomal and later on intralysosomal structures and vesicles [2,3]. Inside the lysosomes, they become substrates for various exohydrolases that remove sugar residues from glycosphingolipids or, in the case of A-SMase, phosphorylcholine from SM.

Acidic compartments of the cell contain the phospholipid bis(monoacylglycero)phosphate (BMP) [4–6] which was detected at highest levels in internal vesicles and membranes of lysosomes [7]. BMP was shown to remain negatively charged even at pH 4.2 [8]. Since A-SMase has an isoelectric point of around 6.8, it possesses positively charged regions in the acidic lysosomal environment. These regions most likely contribute to the interaction of the enzyme with the anionic membrane-bound BMP. Linke et al. [9] performed surface plasmon resonance (SPR) experiments using liposomes with varying lipid composition and demonstrated that the binding of A-SMase to liposomes containing BMP is enhanced by almost 50% compared to vesicles free of BMP. On the other hand, the presence or absence of SM in the vesicles had no significant effect [9].

In 1981, Albouz et al. [10] reported that the tricyclic antidepressant desipramine causes a decrease of A-SMase activity in cultured murine neuroblastoma cells and human fibroblasts. Lysosomal inclusions similar to those known from patients with lipid storage diseases were observed in various organs of rats upon treatment with amphiphilic cationic drugs, including lung and liver as well as the central nervous system. Retinal alterations, corneal opacities, hepatosplenomegaly, abnormalities of liver function and neuro-myopathies accompanied by inclusions especially abundant in Schwann cells were observed in humans [11–14].

Added at a concentration of 25 μM to the cell culture medium, desipramine gave rise to a reduction of A-SMase activity down to 20% of controls within 2 h [15]. A direct inhibition of the enzyme by cationic amphiphilic drugs and a diminished biosynthesis of A-SMase were ruled out to be responsible for this effect, as well as an association of SMase activity with the desipramine-induced downregulation of β-adrenoceptors [16,17]. Hurwitz et al. [15] showed that treatment with desipramine leads to intracellular degradation of the mature enzyme which can be prevented by preincubation of the cells with the protease inhibitor leupeptin. To provide new insight into the interactions of A-SMase and lipid bilayers in the presence of desipramine we here present the results of our studies using SPR (Biacore).

2. Materials and methods

2.1. Materials

SM, phosphatidylcholine and cholesterol, 1,7-diaminoheptane, 1,2,3-heptanetriol, hexanoic acid and desipramine were obtained

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from Sigma (Taufkirchen, Germany) and BMP (dioleoyl) from Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

2.2. Expression and purification of human A-SMase (hA-SMase)

The authors thank Stephanie Lansmann (Bonn, Germany) for providing purified hA-SMase. hA-SMase was expressed using the baculovirus expression vector system [18] and purified from insect Sf21 cells to homogeneity as described by Lansmann et al. [19]. The recombinant enzyme had a specific activity of 0.8 mmol/h/mg purified in the presence of Zn^{2+} (0.1 mM) and measured in a micellar assay system.

2.3. Preparation of liposomes

Preparation of large unilamellar liposomes (LUVs) has been described previously [9,20]. Briefly, a lipid mixture consisting of 10 mol% SM, 50 mol% phosphatidylcholine, 20 mol% cholesterol and 20 mol% BMP (for neutral liposomes: 10 mol% SM, 70 mol% phosphatidylcholine, 20 mol% cholesterol), dissolved in organic solvents, was dried under a stream of nitrogen and then rehydrated in phosphate-buffered saline buffer, pH 7.4 (loading buffer). The mixture was freeze-thawed six times in liquid nitrogen and in an incubator at 37°C, respectively, and passed 17 times through a polycarbonate membrane with a pore diameter of 100 nm.

2.4. SPR (Biacore)

SPR measurements were performed using a Biacore 3000 system at 25°C. LUVs (total lipid concentration 0.1 mM) were immobilized on the surface of a Pioneer™ L1 sensor chip (Biacore) in loading buffer. The running buffer used was 50 mM sodium acetate buffer, pH 4.5 or a 50 mM sodium acetate solution, pH 7.4 in control experiments. A-SMase (0.2 μM, 70 μl) was injected in running buffer at a flow rate of 20 μl/min. 1,7-Diaminoheptane, 1,2,3-heptanetriol, hexanoic acid and desipramine (60 μl, dissolved in water) were injected for 180 s (flow rate 20 μl/min) in the concentrations indicated in the figures.

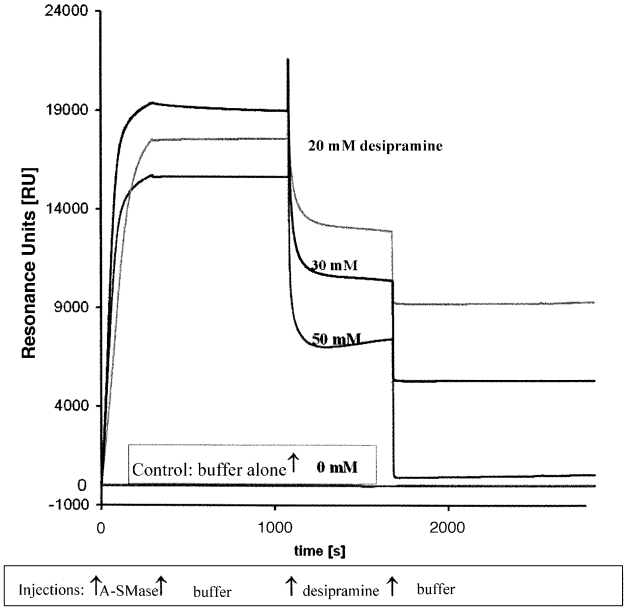

Fig. 1. Interaction of A-SMase with immobilized anionic liposomes in the presence of desipramine at pH 4.5. LUVs (average diameter 100 nm, total lipid concentration 0.1 mM) were immobilized on a Pioneer™ L1 sensor chip. The vesicles were composed of 50 mol% phosphatidylcholine, 20 mol% cholesterol, 20 mol% BMP and 10 mol% SM. Response signals measured subsequent to the binding of the liposomes were defined as zero. A-SMase (0.2 μM), desipramine or running buffer (50 mM sodium acetate buffer, pH 4.5) were injected as indicated.

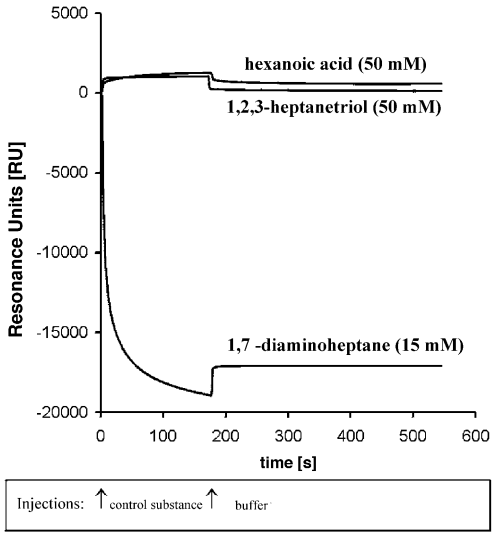

Fig. 2. Influence of control substances on the binding of A-SMase to immobilized anionic liposomes at pH 4.5. After immobilization of LUVs and A-SMase as described under Fig. 1, hexanoic acid, 1,2,3-heptanetriol and 1,7-diaminoheptane, respectively, were injected at the concentrations indicated. Response signals measured subsequent to the binding of A-SMase to the liposomal surface were defined as zero.

3. Results and discussion

In order to monitor the binding of A-SMase to substrate-containing membranes, we immobilized liposomes composed of 10 mol% SM, 20 mol% BMP, 20 mol% cholesterol and 50 mol% phosphatidylcholine on the surface of a Pioneer™ L1 sensor chip. The enzyme was injected in sodium acetate buffer, pH 4.5. As observed before [9], the enzyme binds and hardly dissociates from the lipid surface when the flow is switched to sodium acetate buffer devoid of A-SMase.

Cationic amphiphilic drugs such as desipramine strongly accumulate inside the acidic compartments of living cells since they become trapped as membrane-impermeable forms subsequent to their protonation in the acidic environment. This effect should especially be considered with patients who take desipramine for a long period of time. It is known that the lysosomal concentration of cationic amphiphilic drugs can reach millimolar levels. Hostetler et al. [21] measured 6.3 mM chloroquine in lysosomes isolated from the liver of rats treated with 100 mg/kg/day of the drug at 12 h and 74 mM at 72 h.

To simulate that situation, we injected 20 mM desipramine in our Biacore system, which caused a drop in binding signals (resonance units, RU) of approximately 50%. At a concentration of 50 mM desipramine, the enzyme was completely released from its membrane-bound substrate without removing the lipid vesicles (Fig. 1). These findings suggest that the cationic amphiphile desipramine interferes with the binding of A-SMase to the anionic liposomes at acidic pH values and thereby displaces the enzyme from its substrate.

As a control, we replaced desipramine with a different cationic, a neutral and an anionic compound. 1,7-Diaminoheptane, a substance that is devoid of the tricyclic aromatic core of desipramine but carries two positively charged groups, effectively displaces A-SMase from the lipid surface at pH 4.5 (Fig. 2). Injection of the neutral 1,2,3-heptanetriol and the

partially anionic hexanoic acid did not lead to a release of the enzyme from the liposomes at acidic pH values (Fig. 2). Since 1,2,3-heptanetriol and hexanoic acid are amphiphilic molecules like desipramine, these data prove that the amphiphilic character of a substance is not sufficient to affect the binding of A-SMase to the lipid vesicles.

Our results support the assumption that the cationic groups of the amphiphile desipramine contribute to the decreased binding of the enzyme to its membrane-bound lipid substrate, presumably by competing with the interactions of the at acidic pH values cationic A-SMase with the anionic BMP in the membranes.

50 mM desipramine neither interfered specifically with the binding of A-SMase to BMP-containing liposomes at pH 7.4 nor when neutral liposomes without BMP were used at pH 4.5. In both cases, however, desipramine acted like an unspecific detergent resulting in effects similar to those of the neutral detergent CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), for instance. Response signals returned to the level measured for the Pioneer™ L1 sensor chip alone which indicates that lipids and protein together were completely washed off the sensor chip (data not shown). Thus, in accordance with our hypothesis, the cationic form of desipramine as well as anionic lipid bilayers interacting with a cationic A-SMase are crucial to cause the release of the enzyme from the liposomes.

A-SMase is a glycoprotein with six potential *N*-glycosylation sites, five of which were shown to be used by expression of mutated hA-SMase cDNA constructs in COS cells [22]; all glycosylation sites of recombinant hA-SMase purified from insect cells are used [23].

These complex oligosaccharide chains possibly protect the membrane-associated enzyme from proteolytic cleavage in the lysosome. However, once released from the substrate-bearing membranes, A-SMase may expose different sites to the lysosomal proteases and thus become susceptible for proteolytic degradation. As demonstrated before, treatment of cultured fibroblasts with desipramine leads to intracellular degradation of mature A-SMase which can be prevented by incubation of the cells with the protease inhibitor leupeptin [15]. Our results suggest that desipramine possibly induces intracellular proteolysis of A-SMase by displacing the enzyme from its membrane-bound lipid substrate and thereby making it sensitive to proteolysis. The resulting loss of lysosomal A-SMase activity may well trigger the lysosomal lipid accumulation described before [10–14].

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