Further studies on the reconstitution of glucosylceramidase activity by Sap C and anionic phospholipids

Rosa Salvioli, Massimo Tatti, Fiorella Ciaffoni, Anna Maria Vaccaro*

Department of Metabolism and Pathological Biochemistry, Istituto Superiore Sanita, Viale Regina Elena 299, 00161 Rome, Italy

Received 16 February 2000; received in revised form 24 March 2000

Edited by Giorgio Semenza

Abstract The reconstitution of the activity of the lysosomal enzyme glucosylceramidase requires anionic phospholipids and, at least, a protein factor, saposin C (Sap C). We have previously proposed a mechanism for the glucosylceramidase activation [Vaccaro et al. (1993) FEBS Lett. 336, 159–162] which implies that Sap C promotes the association of the enzyme with anionic phospholipid-containing membranes, thus favoring the contact between the enzyme and its lipid substrate, glucosylceramide. We have further investigated the properties of Sap C using a fluorescent hydrophobic probe such as 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid (bis-ANS). The binding between bis-ANS and Sap C was pH-dependent, indicating that protonation leads to increased exposure of hydrophobic surfaces of Sap C. The interaction of Sap C with membranes, triggered by the development of hydrophobic properties at low pH values, was affected by the content of anionic phospholipids, such as phosphatidylserine or phosphatidylinositol, suggesting that anionic phospholipids have the potential to modulate the insertion of Sap C in the hydrophobic environment of lysosomal membranes. We previously showed that Sap C and anionic phospholipids are both required for the binding of glucosylceramidase to large vesicles. We have presently observed that Sap C is able to promote the association of glucosylceramidase with the lipid surface only when anionic phospholipids exceed a concentration of 5–10%. This level can be reached by summing lower amounts of individual anionic phospholipids, since they have additive effects. The present data extend and refine our model of the mechanism of glucosylceramidase activation and stress the key role of pH, Sap C and anionic phospholipids in promoting the interaction of the enzyme with membranes.

Key words: Saposin C; Glucosylceramidase; Anionic phospholipid; Membrane interaction

1. Introduction

Glucosylceramidase, the enzyme that degrades glucosylceramide in lysosomes, requires the presence of at least another protein called saposin (Sap) C to exert its action; either a deficit of glucosylceramidase or of Sap C results in the block of glucosylceramide hydrolysis [1–6].

Sap C is a member of a family of four similar glycoproteins, Sap A, B, C and D, generated from a common precursor, prosaposin, in lysosomes [4,5,7]. The four Saps are small proteins (each contains about 80 amino acids) and have six cysteine residues at similar positions. The pairings of the three disulfide bridges present in each Sap are the same: the first cysteine is linked to the last, the second to the second last and the third to the forth, without intersection of the three disulfide bridges [8–10]. The maintenance of this structure is essential for the functional properties of Saps [8,11].

Genetic diseases have evidenced the physiological role of Saps, that appear to be involved in the catabolism of sphingolipids [4,5]. The involvement of Sap C in the glucosylceramide degradation has been unequivocally demonstrated by the observation that a deficit of Sap C leads to a variant form of Gaucher disease characterized by glucosylceramide accumulation [6].

Also 'in vitro', the presence of Sap C is required for the enzymatic degradation of glucosylceramide [12]. The role of Sap C in the glucosylceramidase activation has been extensively investigated. In the past, the prevailing paradigm identified glucosylceramidase as the primary and direct target of Sap C [1–5], while our data have provided compelling evidence that phospholipid membranes rather than glucosylceramidase are the actual target of the Sap [12–14]. In fact, Sap C at low pH values tightly binds to phospholipid membranes [13] and stimulates the degradation of glucosylceramide inserted into phosphatidylserine (PS)-containing vesicles by favoring the enzyme association with the lipid surface [12].

Both glucosylceramidase and Sap C are associated, at least in part, with lysosomal membranes [15,16]. Since the pH of the intralysosomal environment is maintained in the range 4.5–4.8 [17], acidic conditions most likely play a fundamental role in regulating the properties of the two proteins. To extend our investigation on the reconstitution of glucosylceramidase activity by Sap C and anionic phospholipids, we have further examined how the properties of Sap C are affected by variations of pH and how anionic phospholipids can modulate the interaction of Sap C and glucosylceramidase with membranes.

2. Materials and methods

2.1. Materials

Phosphatidylcholine (PC) from egg yolk and PS from bovine brain were from Avanti Polar Lipids (Alabaster, AL, USA). Phosphatidylinositol (PI) from bovine liver, cholesterol (Chol) and 4-methylumbelliferyl-β-D-glucopyranoside (MU-Glc) were from Sigma. L-α-Dipalmitoyl [dipalmitoyl-1-^{14}C]PC (110 mCi/mmol) was from NEN Research Products, DuPont de Nemours (Germany). 4,4′-Dianilino-1,1′-binaphthyl-5,5′-disulfonic acid (bis-ANS) was purchased from Molecular Probes (Eugene, OR, USA). All other chemicals were of the purest available grade.

*Corresponding author. Fax: (39)-6-49387149.
E-mail: avaccaro@iss.it

Abbreviations: Sap C, saposin C; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid; Chol, cholesterol; PS, phosphatidylserine; PC, phosphatidylcholine; PI, phosphatidylinositol; LUV, large unilamellar vesicle; SUV, small unilamellar vesicle

PII: S0014-5793(00)01417-4

2.2. Sap C preparation

Sap C was purified from spleens of patients with type 1 Gaucher's disease following a previously reported procedure [13,14]; it consisted essentially of heat treatment of a water homogenate, ion exchange chromatography on DEAE-Sephacel, gel filtration on Sephadex G-75 and reverse-phase high pressure liquid chromatography on a protein C4 column (Vydac). The purity of the final Sap preparation was verified by N-terminal sequence analysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting.

2.3. Glucosylceramidase preparation

Glucosylceramidase was purified from human placenta following the procedure described by Murray et al. [18].

2.4. Glucosylceramidase antibody

The anti-glucosylceramidase monoclonal antibody 8E4 was kindly provided by Dr. H. Aerts and Dr. S. van Weely (E.C. Slater Institute for Biochemical Research, University of Amsterdam, The Netherlands).

2.5. Vesicle preparation

Large unilamellar vesicles (LUV) were prepared by filter exclusion using a high pressure extrusion apparatus (Lipex Biomembranes, Vancouver, B.C., Canada) as previously described [19,20]. In short, the dry lipids were dispersed by vortex mixing in 2 mM L-histidine, 2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 150 mM NaCl, 1 mM EDTA, pH 7.4. The suspension was submitted to 10 cycles of freezing and thawing and then extruded 15 times through two stacked 0.1 μm pore size polycarbonate filters (Nucleopore Corp., Pleasanton, CA, USA). All vesicles were supplemented with trace amounts of labelled PC and their concentration was determined by radioactivity measurements.

2.6. Binding of bis-ANS to Sap C

The fluorescence of the bis-ANS solution (10 μM) was recorded in the absence and presence of Sap C. In the last case, the Sap (1 μM) was added to 1 ml of buffer at pre-set pH (pH 4.0–7.0) containing bis-ANS. The buffers were buffer A (10 mM acetate, 150 mM NaCl, 1 mM EDTA) adjusted to the desired pH in the range between pH 4.0 and 6.0 and buffer B (20 mM Tris, 150 mM NaCl, 1 mM EDTA) for pH 6.5 and 7.0. The fluorescence was measured at an excitation wavelength of 390 nm (2 nm slit width) and scanned from 400 to 600 nm (2 nm slit width) at 25°C using a Fluoromax spectrofluorometer equipped with a constant temperature cell holder and stirrer (Spex Industries, NJ, USA).

2.7. Tyrosine fluorescence measurement

Tyrosine fluorescence of Sap C was monitored at 37°C, using a Fluoromax spectrofluorometer equipped with a constant temperature cell holder and stirrer (Spex Industries, NJ, USA). Emission spectra were obtained at an excitation wavelength of 277 nm (2.5 nm slit width) and scanned from 280 to 360 nm (2.5 nm slit width). The spectra were recorded in the absence and in the presence of LUV (60 μg total lipid) added to 1 ml of a Sap C solution (4 μM) in buffer A adjusted to pH 4.5. The spectra were corrected for the fluorescence associated with LUV themselves.

2.8. Binding of glucosylceramidase to vesicles

For binding studies, glucosylceramidase (1 μg), Sap C (10 μg) and BSA (10 μg) were incubated with LUV (100 μg total lipid) in 0.2 ml of buffer A adjusted to pH 4.5, at 37°C, for 10 min. In some experiments, Sap C was omitted. The mixtures were centrifuged with a 42.2 Ti rotor (Beckman), in polycarbonate centrifuge tubes (7×20 mm), at 100 000×*g* for 1 h. After separation of the supernatant, the pelletted vesicles were resuspended in 0.2 ml of the same buffer. Free glucosylceramidase in the supernatant and liposome-bound glucosylceramidase in the resuspended pellet were identified by SDS–PAGE and Western blotting (see below).

To further evaluate the binding of glucosylceramidase to vesicles, the enzyme activity remaining in the supernatant after centrifugation (free glucosylceramidase) was determined. The enzyme assay contained in a final volume of 0.2 ml, an aliquot of the supernatant, 0.1/0.2 M citrate/phosphate buffer, pH 5.6, 2.5 mM MU-Glc, 0.1% (v/v) Triton X-100 and 0.25% (w/v) sodium taurocholate [20]. The assay mixtures were incubated for 30 min at 37°C and the extent of reaction was estimated fluorometrically. The percentage of liposome-bound glucosylceramidase was expressed relative to the amount of free enzyme in the supernatant.

2.9. SDS–PAGE and enhanced chemiluminescence (ECL) Western blotting

SDS–PAGE was performed with 10% acrylamide separating gels and 4.5% stacking gels [21]. After electrophoresis, the proteins were electroblotted to polyvinylidene difluoride membranes (Bio-Rad) and glucosylceramidase was detected with anti-glucosylceramidase monoclonal antibody 8E4 using an ECL Western blotting kit according to the manufacturer's instructions (Amersham International plc, Buckinghamshire, UK).

3. Results

3.1. pH modulates the hydrophobic properties of Sap C

We have previously obtained indications that the superficial

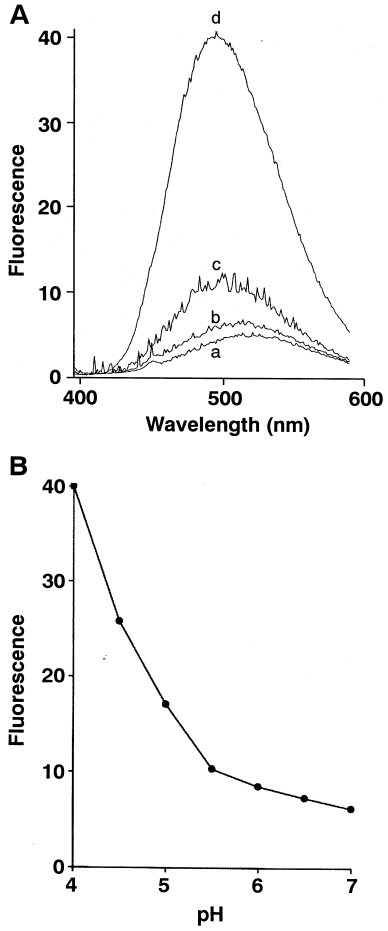

Fig. 1. pH dependence of the bis-ANS fluorescence in the presence of Sap C. (A) Fluorescence emission spectra of bis-ANS (10 μM) in the absence (a) and presence of 1 μM Sap C at pH 7.0 (b) or pH 4.0 (d). To test the reversibility of bis-ANS binding to Sap C, the pH of the sample recorded at pH 4.0 (d) was readjusted at pH 7.0 by injecting appropriate amounts of 1 M NaOH and the emission spectrum collected again after 5 min (c). Without Sap C, the same spectrum of bis-ANS was obtained either at pH 7.0 or pH 4.0. (B) pH dependence of fluorescence intensities for bis-ANS in the presence of Sap C (λ_{ex} = 390 nm, λ_{em} = 495 nm). Fluorescence was measured 5 min after addition of 1 μM Sap C to buffers of the indicated pHs containing 10 μM bis-ANS. The points represent means of at least four different experiments. The deviation for all samples was less than ± 5% of the corresponding mean value.

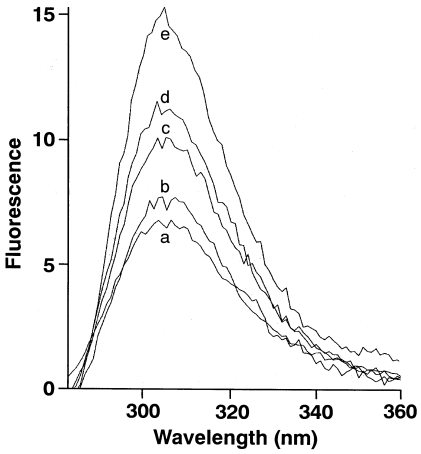

Fig. 2. Intrinsic fluorescence spectra of Sap C at pH 4.5 in the absence (a) and presence of LUVs (b, c, d, e). Sap C in the presence of LUV composed of Chol:PC:PI (25:65:10) (b) or Chol:PC:PS (25:65:10) (c) or Chol:PC:PI (25:55:20) (d) or Chol:PC:PS (25:55:20) (e). The addition of LUV composed of Chol:PC (25:75) did not change the fluorescence spectrum of Sap C (a).

hydrophobicity of Sap C increases at low pH values [14]. To further analyze the pH dependence of Sap C hydrophobicity, the Sap interaction with bis-ANS, a hydrophobic fluorescent probe, has been examined as a function of pH (Fig. 1A,B). Bis-ANS is known to have a high affinity for non-polar cavities in proteins and a quantum yield which is very much higher in such hydrophobic environments than in aqueous solutions. An increase in bis-ANS fluorescence along with a concomitant blue shift are generally assumed as evidence for its binding to apolar sites of proteins [22,23]. Upon addition of Sap C, the fluorescence intensity of bis-ANS was poorly enhanced at pH 7, while a marked increase and a blue shift of the emission maximum (about 20 nm) were observed at pH 4.0. The effect was partly reversible as evidenced by the decrease of the bis-ANS fluorescence after switching from pH 4.0 to 7.0 (Fig. 1A). The pH profile for fluorescence indicated that the major changes begun at pH lower than 5.5 (Fig. 1B). The enhancement observed under acidic conditions most likely reflects increased binding of bis-ANS as a result of the exposure of hydrophobic sites on the surface of Sap C. These results support our previous hypothesis [14] that the hydrophobic properties of Sap C are regulated by the pH of the environment.

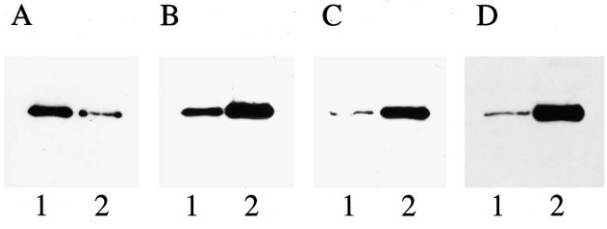

Fig. 3. Glucosylceramidase binding to LUV of different composition in the presence of Sap C. A mixture of glucosylceramidase and Sap C was incubated at pH 4.5 with LUV of the following composition: A, Chol:PC:PS (25:70:5); B, Chol:PC:PI (25:55:20); C, Chol:PC:PS (25:55:20); D, Chol:PC:PI:PS (25:55:10:10). Free and liposome-bound glucosylceramidase were separated by centrifugation and analyzed by SDS–PAGE and Western blotting as reported under Section 2. Lanes 1, free glucosylceramidase; lanes 2, membrane-bound glucosylceramidase. Each experiment was repeated at least three times with similar results.

3.2. *Anionic phospholipids modulate the fluorescent properties of Sap C*

At low pH values, Sap C tightly binds to phospholipid membranes [14]. We have presently characterized the spectroscopic properties of Sap C in order to use them as a probe to monitor possible conformational changes during lipid binding. Sap C contains two tyrosines, two phenylalanines and no tryptophan residues. As a result, the intrinsic fluorescence spectrum of Sap C is dominated by tyrosines, albeit with a low quantum yield, and shows a characteristic maximum at 306 nm (Fig. 2). The addition of vesicles containing PS or PI, two anionic phospholipids normally present in lysosomes, greatly enhanced the intrinsic fluorescence of Sap C at pH 4.5, indicating a decreased polarity of the tyrosines environment. As shown in Fig. 2, the increase was related to the amount of anionic phospholipids in the membrane. The effect of PS was significantly more pronounced than that of a matched amount of PI. Unlike PS- or PI-containing vesicles, the addition of LUV composed of only PC and Chol did not change the fluorescence spectrum of Sap C. At pH 7.0, the presence of vesicles with or without PS and PI had no effect on the fluorescence properties of the Sap (data not shown). It thus appears that the amount and the nature of anionic phospholipids regulate the mode of interaction of Sap C with membranes and/or its conformation.

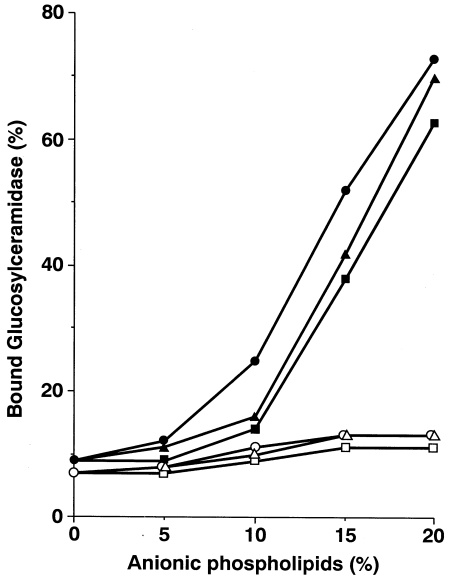

Fig. 4. Dependence of glucosylceramidase binding on the level of anionic phospholipids and on the presence of Sap C. Glucosylceramidase was incubated at pH 4.5 with LUV in the presence (▲,●,■) or in the absence of Sap C (△,○,□). Vesicles contained 25% Chol and the indicated amount of anionic phospholipids. To keep the total phospholipid content constant (75%), the percentage of PC was accordingly varied. The anionic phospholipids were PI (■,□), or PS (△,▲), or an equimolar mixture of PS and PI (○,●). Liposome-bound glucosylceramidase was evaluated by measuring the enzyme activity remaining free in the supernatant after centrifugation as reported under Section 2. The points represent means of at least four different experiments. The deviation for all samples was less than ±5% of the corresponding mean value.

3.3. Anionic phospholipids and Sap C modulate the interaction of glucosylceramidase with membranes

Anionic phospholipids not only affect the interaction of Sap C with membranes, but are also an almost absolute requirement for the reconstitution of glucosylceramidase activity [3,24,25]. We have early shown that PS, together with Sap C, promotes the binding of the enzyme to LUV at low pH values [13]. To further investigate the interactions between glucosylceramidase, Sap C and membranes at pH 4.5, a pH value similar to that of the lysosomal environment, the two proteins have been incubated with LUV of different composition. After centrifugation, the free and vesicle-bound glucosylceramidases have been qualitatively recognized by Western blot analysis. As shown in Fig. 3, the enzyme poorly interacts with vesicles that contain a low amount of PS (5%), but a higher concentration (20%) of either PS or PI or a mixture of PS and PI promotes the association of most of glucosylceramidase with the lipid surface.

To better evaluate the dependence of glucosylceramidase binding on the level and nature of anionic phospholipids, the enzyme activity remaining in the supernatant after centrifugation of the vesicles (free glucosylceramidase) was measured. As shown in Fig. 4, up to a level of about 10% of PS and PI, the enzyme poorly associated with LUV both in the presence and in the absence of Sap C. It is worth noting that at the pH of the experiment, namely pH 4.5, Sap C is tightly bound to the vesicles also in the absence of anionic phospholipids [14]. As the concentration of PS and PI increases, most of glucosylceramidase partitions on the lipid surface in the presence but not in the absence of Sap C. No significant difference in the capacity of PS and PI to favor the enzyme association was observed, their effect being additive (Fig. 4).

4. Discussion

In the present study, we have further refined the model of the glucosylceramide degradation by glucosylceramidase and Sap C, developed since our first observations that glucosylceramidase hydrolyzes its natural substrate by associating to anionic phospholipid and glucosylceramide-containing vesicles at low pH values [26] and Sap C favors the enzyme binding by interacting with [13] and perturbing [27] the target membrane.

Our early studies suggested that the trigger for the Sap C interaction with membranes was an increase of the Sap C hydrophobicity that occurs at low pH values similar to that of the lysosomal environment [14]. The ability of Sap C to change its hydrophobic properties has presently been confirmed using bis-ANS as a hydrophobic probe. The pH-dependent enhancement of the bis-ANS fluorescence in the presence of Sap C parallels the previously observed pH dependence of the partitioning of Sap C between the detergent and aqueous phases of a Triton X-114 solution [14]. Our past and present results conclusively demonstrate that organized hydrophobic surfaces of Sap C are exposed as the Sap passes through a transition which is centered at pH 5.5–5.0. It can be envisaged that Sap C, which is generated in lysosomes from its precursor, prosaposin, undergoes a dramatic increase of hydrophobicity when in contact with the acidic medium of these organelles, small local variations in pH resulting in marked changes of the Sap C properties.

For several proteins, for instance proteins of enveloped viruses such as Semliki Forest virus and influenza virus, the interaction with membranes is specifically triggered by acidic pH [28,29]. In early studies, we showed that also Sap C uses its hydrophobic properties to interact with membranes. Actually, at pH values lower than 5.0, Sap C extensively binds to LUV composed of PC and Chol slightly altering the bilayer integrity; the Sap C-induced destabilization increases sharply when the membrane is supplemented with an anionic phospholipid such as PS [14]. On the other hand, we have presently found that membranes containing anionic phospholipids such as PS and PI dramatically affect the fluorescent properties of the Sap, the effect being related to the level and the nature of the anionic phospholipids. The enhancement of the Sap C fluorescence induced by PS and PI is indicative of conformational alterations or/and of a deeper insertion of the Sap in the membrane. These results show that, even when Sap C is completely bound, there are differences in the mode of interaction of the Sap with the lipid surface that depends on the content of anionic phospholipids.

The importance of anionic phospholipids as activators of glucosylceramidase is well documented [3,24,25]. In the lysosomal compartment, PS, PI and lysobisphosphatidic acid have been reported to be the major anionic phospholipids and the most effective lipid activators of glucosylceramidase [30,31]. In the past, we showed that the presence of anionic phospholipids and the size of the liposomes used as model of biological membranes are critical parameters in the interaction of glucosylceramidase with lipid surfaces, namely the enzyme spontaneously binds to vesicles, provided that they are small (small unilamellar vesicle (SUV)) and contain anionic phospholipids; as the diameter of the liposomes increases, the glucosylceramidase binding decreases probably as a consequence of the tighter packing of the lipids in the outer surface of LUV, compared with the loose packing in SUV [20]. The capacity of anionic phospholipid-containing LUV to bind glucosylceramidase is restored after the addition of Sap C, that facilitates the insertion of the enzyme by perturbing the lipid organization [12–14]. It was presently observed that Sap C is able to promote an extensive glucosylceramidase association only when the level of anionic phospholipids in membranes exceeds a threshold of about 10%. At lower concentrations, most of the glucosylceramidase remains free in solution (see Figs. 3 and 4), while Sap C partitions on the lipid surface [14]. The critical level can be reached by summing lower amounts of individual anionic phospholipids since they have additive effects. These results indicate that anionic phospholipids, that altogether represent more than 10% of the phospholipid content of lysosomal membranes [31,32], have the potential of serving as the 'in situ' glucosylceramidase binding lipids. According with this view, changes in their content and/or the formation of membrane domains containing these lipids can have a key role in regulating the topology and activity of glucosylceramidase on the lysosomal membranes.

Our past and present results indicate that the Sap C activation of glucosylceramidase is a complex multistep process which is triggered by the low lysosomal pH and comprises a pH-dependent change of Sap C hydrophobicity, the Sap C association with lipid surfaces and the Sap C-mediated binding of the enzyme to membranes containing an appropriate amount of anionic phospholipids. Conversely, it was claimed for a long time and widely accepted that the Sap C activation of glucosylceramidase was due to a direct interaction between glucosylceramidase and Sap C [1–5]. Some authors, who de-

fended in the past this mechanism [5,33–34], have lately presented data in full agreement with our model [35,36]. In fact, our previous findings on the reconstitution of glucosylceramidase on binding to acidic phospholipid-containing vesicles [12–14,37] have received further support by fluorimetric studies [35] showing that glucosylceramidase penetrates into the outer leaflet of membranes composed of negatively charged phospholipids undergoing a conformational change. Moreover, surface plasmon resonance experiments [36] have recently offered additional evidence to our early studies showing that Sap C spontaneously binds to phospholipid liposomes, especially in the presence of anionic phospholipids [12–14,27].

We thus conclude that, under conditions mimicking the lysosomal milieu, the glucosylceramide degradation in lysosomes depends on the Sap C-induced binding of glucosylceramidase to membranes and can be modulated by variations in pH and in anionic phospholipid levels.

Acknowledgements: This work was partly supported by ISS Research Project 'Prevention of risk factors of maternal and child health'. The authors thank Mr. E. Raia for technical assistance.

References

[1] Ho, M.W. and O'Brien, J.S. (1971) Proc. Natl. Acad. Sci. USA 68, 2810–2813.
[2] Ho, M.W. and Light, N.D. (1973) Biochem. J. 136, 821–823.
[3] Glew, R.H., Basu, A., La Marco, K. and Prence, E. (1988) Lab. Invest. 58, 5–25.
[4] O'Brien, J. and Kishimoto, Y. (1991) FASEB J. 5, 301–308.
[5] Sandhoff, K., Harzer, K. and Furst, W. (1995) in: The Metabolic and Molecular Bases of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., Eds.), pp. 2427–2441, McGraw-Hill, Book Co., New York.
[6] Christomanou, H., Chabas, A. and Pampols, T. (1989) Klin. Wochenschr. 67, 999–1003.
[7] O'Brien, J.S., Kretz, K.A., Dewji, N., Wenger, D.A., Esch, F. and Fluharty, A.L. (1988) Science 241, 1098–1101.
[8] Vaccaro, A.M., Salvioli, R., Barca, A., Tatti, M., Ciaffoni, F., Maras, B., Siciliano, R., Zappacosta, F., Amoresano, A. and Pucci, P. (1995) J. Biol. Chem. 270, 9953–9960.
[9] Tatti, M., Salvioli, R., Ciaffoni, F., Pucci, P., Andolfo, A., Amoresano, A. and Vaccaro, A.M. (1999) Eur. J. Biochem. 263, 486–494.
[10] Vaccaro, A.M., Salvioli, R., Tatti, M. and Ciaffoni, F. (1999) Neurochem. Res. 24, 307–314.
[11] Munford, R.S., Sheppard, P.O. and O'Hara, P.J. (1995) J. Lipid Res. 36, 1653–1663.
[12] Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R. and Barca, A. (1997) J. Biol. Chem. 272, 16862–16867.
[13] Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R., Maras, B. and Barca, A. (1993) FEBS Lett. 336, 159–162.
[14] Vaccaro, A.M., Ciaffoni, F., Tatti, M., Salvioli, R., Barca, A., Tognozzi, D. and Scerch, C. (1995) J. Biol. Chem. 270, 30576–30580.
[15] Van Dongen, J.M., Willemsen, R., Ginns, E.I., Sips, H.J., Tager, J.M., Barranger, J.A. and Reuser, A.J.J. (1985) Eur. J. Cell Biol. 39, 179–189.
[16] Paton, B.C., Hughes, J.L., Harzer, K. and Poulos, A. (1990) Eur. J. Cell Biol. 51, 157–164.
[17] Mellman, I., Fuchs, R. and Helenius, H. (1986) Annu. Rev. Biochem. 55, 663–700.
[18] Murray, G.J., Youle, R.J., Gandy, S.E., Zirzow, G.C. and Barranger, J.A. (1985) Anal. Biochem. 147, 301–310.
[19] Mayer, L.D., Hope, M.J. and Cullis, P.R. (1986) Biochim. Biophys. Acta 858, 161–168.
[20] Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R., Barca, A. and Roncaioli, P. (1993) Biochim. Biophys. Acta 1149, 55–62.
[21] Laemmli, U.K. (1970) Nature 227, 680–685.
[22] Rosen, C.G. and Weber, G. (1969) Biochemistry 8, 3915–3920.
[23] Brand, L. and Gohlke, J.R. (1972) Annu. Rev. Biochem. 41, 843–868.
[24] Basu, A. and Glew, R.H. (1984) Biochem. J. 224, 515–524.
[25] Basu, A., Glew, R.H., Daniels, L.B. and Clark, L.S. (1984) J. Biol. Chem. 259, 1714–1719.
[26] Vaccaro, A.M., Tatti, M., Salvioli, R., Ciaffoni, F. and Gallozzi, E. (1990) Biochim. Biophys. Acta 1033, 73–79.
[27] Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R., Serafino, A. and Barca, A. (1994) FEBS Lett. 349, 181–186.
[28] Doms, R.W., Helenius, A. and White, J. (1985) J. Biol. Chem. 260, 2973–2981.
[29] Bron, R., Wahlberg, J.M., Garoff, H. and Wilschut, J. (1993) EMBO J. 12, 693–701.
[30] Prence, E., Garrett, K.O., Panitch, H., Basu, A., Glew, R.H., Wherrett, J.R. and Huterer, S. (1986) Clin. Chim. Acta 156, 179–190.
[31] Basu, A., Glew, R.H., Wherrett, J.R. and Huterer, S. (1986) Arch. Biochem. Biophys. 245, 464–469.
[32] Gennis, R.B. (1989) Biomembranes: Molecular Structure and Function, Springer-Verlag, New York.
[33] Fabbro, D. and Grabowski, G.A. (1991) J. Biol. Chem. 266, 15021–15027.
[34] Qi, X., Leonova, T. and Grabowski, G.A. (1994) J. Biol. Chem. 269, 16746–16753.
[35] Qi, X. and Grabowski, G.A. (1998) Biochemistry 37, 11544–11554.
[36] Wilkening, G., Linke, T. and Sandhoff, K. (1998) J. Biol. Chem. 273, 30271–30276.
[37] Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R. and Roncaioli, P. (1992) Biochim. Biophys. Acta 1119, 239–246.