Sphingomyelinase Activity Associated with Human Plasma Low Density Lipoprotein

POSSIBLE FUNCTIONAL IMPLICATIONS*

Received for publication, November 16, 1999, and in revised form, February 28, 2000

Juha M. Holopainen‡, Oula Penate Medina, Antti J. Metso, and Paavo K. J. Kinnunen§

From the Helsinki Biophysics and Biomembrane Group, Department of Medical Chemistry, Institute of Biomedicine, University of Helsinki, Helsinki FIN-00014, Finland

Isolated human plasma low density lipoprotein (LDL) was observed to possess sphingomyelinase activity. Accordingly, the formation of ceramide was catalyzed by LDL at 37 °C using tertiary liposomes composed of sphingomyelin (mole fraction (x) = 0.2), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (x = 0.7), 1,2-dimyristoyl-sn-glycero-3-phospho-rac-glycerol (x = 0.1), and either the fluorescent sphingomyelin analog Bodipy-sphingomyelin or [14C]sphingomyelin as substrates. However, this activity was not present in either very low density lipoprotein or the high density lipoprotein subfractions HDL2 and HDL3. Oxidation of LDL abrogated its sphingomyelinase activity. Aggregation of the liposomes upon incubation with LDL was evident from the light scattering measurements. Microinjection of LDL to the surface of giant liposomes composed of 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC), N-palmitoyl-o-sphingomyelin (C16:0-sphingomyelin), and Bodipy-sphingomyelin as a fluorescent tracer (0.75:0.20:0.05, respectively) revealed the induction of vesicular budding of vesicles, resembling endocytosis.

The occlusion of blood vessels due to atherosclerosis, resulting from the formation of cholesterol-rich lipid plaques in the arterial walls, is one of the most common causes of death in industrial countries (1, 2). High blood levels of cholesterol correlate with atherosclerosis, and the former is further related to the contents of cholesterol and saturated fat in the diet. Most of the plasma cholesterol is present in low density lipoproteins (LDL), an essential component of the plasma lipid transport system. The metabolism of LDL involves recognition of its protein moiety apolipoprotein B-100 (apoB-100) by specific receptors in the plasma membrane, followed by endocytotic uptake (for a review, see Ref. 3).

The development of atherosclerosis is initiated by interactions of LDL with the arterial wall. Although the subsequent events have remained elusive, several lines of evidence point out the importance of LDL aggregation, alterations in the endothelium, macrophage chemotaxis and foam cell formation, and muscle cell migration and alteration to the pathogenesis of atherosclerosis (2). Oxidation of LDL has been recognized to represent a major pathogenic factor in the progression of atherosclerosis (4–6). However, the mechanism(s) involved has remained elusive. This modification of LDL leads to a proteolytic fragmentation of apoB-100 as well as formation of oxidized lipids. Moreover, several cellular effects have been shown to be induced by oxidized LDL, including proliferation, endothelial dysfunction, cellular cytotoxicity, procoagulant effect, increased antigenicity, and cell adhesion and recruitment (reviewed in Ref. 6). Importantly, LDL from atherosclerotic lesions is aggregated or has an increased tendency to aggregate (7). Aggregation of LDL can be induced in vitro and in vivo by several different mechanisms including vortexing (8), accumulation of ceramide (9), and binding to negatively charged glycosaminoglycans (10, 11) and anionic phospholipids (12).

Evidence has accumulated to indicate that the sphingomyelin content of LDL and deranged sphingomyelin metabolism play roles in the development of atherosclerosis (13). In atherosclerotic lesions, the concentration of sphingomyelin is increased compared with normal arterial tissue. The hydrolytic product derived from sphingomyelin, ceramide, has also been shown to be enriched in plaques (9). Ceramide has been suggested to function as a second messenger in apoptosis, growth suppression, differentiation, and cell senescence (14) and to enhance the formation of atherosclerotic plaques (15). In cellular membranes, ceramide is formed in addition to synthesis upon the removal of the phosphocholine moiety of sphingomyelin by sphingomyelinase. At an early stage in apoptosis, activation of sphingomyelinase is observed (16), and subjecting cells to the action of externally added sphingomyelinase has been shown to cause apoptosis (17).

The exact role of apoptosis in coronary heart disease remains unknown. Yet, the association of hypertension and hypercholesterolemia with an increased rate of apoptosis suggests that both apoptosis and inflammatory changes could have roles in the pathophysiology of atherosclerosis, involving the sphingomyelin-ceramide pathway (18–20).

The present findings demonstrate sphingomyelinase activity to be associated with human plasma LDL. We hypothesize that the increased ceramide content of cells involved in the formation of atherosclerotic plaques is at least partly the result of this enzyme activity. Physiologically, the sphingomyelinase associated with human plasma LDL could provide a non-recep-
tort-mediated mechanism for endocytic uptake of LDL cholesterol by cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—Hepes, EDTA, SOPC, and POPC were from Sigma, and Bodipy-ceramide and Bodipy-sphingomyelin were from Molecular Probes, Inc. (Eugene, OR). C16:0-sphingomyelin was purchased from Northern Lipids Inc. (Vancouver, Canada), DMPC was from Coatsome (Amagasaki, Hyogo, Japan), and [N-methyl-14C]sphingomyelin (specific activity 2.07 GBq/mmol) was from Amersham Pharma Biotech. CaCl2 dihydrate and MgCl2 hexahydrate were from Merck. The purity of the above lipids was checked by thin layer chromatography and revealed no impurities (21). The concentrations of Bodipy-sphingomyelin and Bodipy-ceramide were determined spectrophotometrically using 91,000 cm−1 at 505 nm as their molar extinction coefficient. Concentrations of the other lipids were determined gravimetrically using a high precision electrobalance (Cahn, Cerritos, CA). Protein analysis grade centrifugal ultrafiltration from pooled or single donor plasma from healthy subjects (22). For removal of KBr, LDL was dialyzed against 5 ml of 1.125 g/ml, and HDL3 (1.019–1.063 g/ml), HDL2 (1.063–1.125 g/ml), and HDL1 (1.215–1.25 g/ml) were isolated on TLC and thereafter visualized by UV illumination. In order to determine the time course of hydrolysis of sphingomyelin in LDL, we came to the conclusion that it would be worthwhile to explore the possibility that sphingomyelinase activity was associated with LDL. In a qualitative assay, we used sphingomyelin contained in liposomes (POPC/DMPG/C16:0-sphingomyelin, mole fraction 0.7:0.1:0.15, respectively) and also incorporating the fluorescent sphingomyelin analog Bodipy-sphingomyelin (x = 0.05) as a tracer. Isolated human plasma LDL (0.125 mg/ml) was added, and after a 24-h incubation at 37 °C lipids were isolated on TLC and visualized by UV illumination (Fig. 1). The formation of ceramide is evident in the presence of LDL but not in the control lacking this lipid probe. Other lipoproteins, viz., human plasma VLDL, HDL4, and HDL3, were also devoid of sphingomyelinase activity (data not shown).

**RESULTS**

In the course of our investigations on the properties and metabolic processing of LDL, we came to the conclusion that it would be worthwhile to explore the possibility that sphingomyelinase activity was associated with LDL. In a qualitative assay, we used sphingomyelin contained in liposomes (POPC/DMPG/C16:0-sphingomyelin, mole fraction 0.7:0.1:0.15, respectively) and also incorporating the fluorescent sphingomyelin analog Bodipy-sphingomyelin (x = 0.05) as a tracer. Isolated human plasma LDL (0.125 mg/ml) was added, and after a 24-h incubation at 37 °C lipids were isolated on TLC and visualized by UV illumination (Fig. 1). The formation of ceramide is evident in the presence of LDL but not in the control lacking this lipid probe. Other lipoproteins, viz., human plasma VLDL, HDL4, and HDL3, were also devoid of sphingomyelinase activity (data not shown).

The time course of enzymatic hydrolysis of sphingomyelin in POPC/DMPG/C16:0-sphingomyelin (0.7:0.1:0.2, mole fraction) LUVs was determined by following the loss of [14C]sphingomyelin radioactivity by TLC and radioimaging (Fig. 2). Initial degradation of sphingomyelin was relatively slow, and no hydrolysis was evident after the first 30 min. After 90 min, approximately 33% of the radioactive sphingomyelin was degraded, and upon further incubation for 3 h, the extent of hydrolysis increased to approximately 65%. Of the 9 nmoles of sphingomyelin in the LUV substrate, >95% was converted into ceramide within 24 h. Importantly, the extent of hydrolysis exceeded 50% of the initial sphingomyelin in the bilayer. This indicates rearrangements in the transbilayer lipid distribution in the course of the enzyme reaction, in keeping with our previous observations (30). Reasons for the lag are unclear at present yet could be related to lipid packing. Accordingly, the activity of sphingomyelinase toward sphingomyelin monolayers remained constant up to a surface pressure of 18 millinewtons/meter, whereas increasing the pressure attenuated the hydrolysis and prolonged the lag time (31). The sphingomyelinase activity of different LDL samples exhibited considerable variation. Accordingly, six different LDL preparations either pooled or from a single donor were used, and the maximum total lipid concentration. Approximately 1 µmol of this solution was applied onto the surface of a Pt-electrode (26), which was then dried with a stream of nitrogen and evacuation in vacuum for 1 h. A glass chamber with the attached electrodes and with a quartz window bottom was placed on the stage of a Zeiss LM-35 inverted fluorescence microscope. A 12-V AC voltage (f = 4 Hz) was applied prior to adding 1.3 ml of 0.05 M Hepes, pH 7.4. During the first minute of hydration, the voltage was raised to 1 V. After 24 h, the AC field was turned off, and giant liposomes were observed through a Nikon ELWD (×20) objective. The excitation and emission wavelengths were selected with filters (Melles Griot) transmitting in the range of 420–480 nm and >500 nm, respectively. Fluorescence images were taken with a Peltier-cooled digital camera (C4742-96, Hamamatsu, Japan) connected to a computer. The local similarity program SIM (29).
hydrolysis in 24 h varied from 5 to 100% of the total sphingomyelin present. The reason(s) for this variation remains unknown. The activities from single donors did not correlate with age, sex, or body mass index (data not shown), and further investigations are needed to address this issue. One possibility could be a varying degree of initial oxidation between the LDL preparations (see below).

The conjugated lipid dienes generated during lipid peroxidation of LDL exhibit an absorption maximum around 234 nm and can be directly measured from aqueous LDL solution (25). In order to determine if the sphingomyelinase activity associated with LDL was connected to its oxidation, we measured the mobility of Bodipy-ceramide. The TLC plate was developed with 1,2-dichloroethane/methanol/water (90:20:0.5, v/v/v) to separate Bodipy-ceramide from Bodipy-sphingomyelin and illuminated with a UV lamp.

FIG. 1. TLC on silicic acid-coated plates was performed on three samples. The left lane demonstrates the formation of ceramide after the incubation of POPC/DMPG/C16:0-sphingomyelin/Bodipy-sphingomyelin (0.7:0.1:0.15:0.05, mole fraction; total lipid 22.5 μM) LUVs with isolated human plasma LDL (at a final protein concentration of 0.125 mg/ml) for 24 h at 37 °C in 5 mM Hepes, 150 mM NaCl, 2 mM CaCl_2, 2 mM MgCl_2, pH 7.4. The middle lane shows identical liposomes incubated in the absence of LDL. The right lane shows the mobility of Bodipy-ceramide. The TLC plate was developed with 1,2-dichloroethane/methanol/water (90:20:0.5, v/v/v) to separate Bodipy-ceramide and Bodipy-sphingomyelin (0.7:0.8, mole fraction).

We have recently demonstrated using LUVs that in contrast to sphingomyelin, ceramide forms microdomains in both fluid and gel state phosphatidylcholine membranes (30, 33, 34). We have also shown that subjecting sphingomyelin containing GUVs to the action of externally applied sphingomyelinase resulted first in the rapid formation of ceramide-enriched microdomains, with subsequent formation of “endocytotic” vesicles in the internal cavity of the giant liposome (21). It was thus of interest to explore the possibility that a similar process could be induced by the sphingomyelinase activity associated with LDL. Giant SOPC/C16:0-sphingomyelin (0.75:0.25 molar ratio) liposomes were visualized by fluorescence microscopy of the included fluorescent lipid tracer Bodipy-sphingomyelin (x = 0.15; Fig. 3). Uniform distribution of Bodipy-sphingomyelin is evident at the resolution of the optical microscope (Fig. 3A), in accordance with the miscibility of sphingomyelin in phosphatidylcholine membranes (30). In order to investigate the effects of LDL on the fluid giant liposome membrane, we attempted to add lipoprotein to the vesicle surface by microinjection. However, LDL was strongly aggregated onto the micropipette tip. The reason for this remains uncertain at this stage. One possibility is that the aggregate formation is due to small vesicles present in solution. Accordingly, the aggregated LDL on the tip was brought by micromanipulator to the GUV surface. Within approximately 90 s, a brightly fluorescent area below the tip of the micropipette become observable (Fig. 3B). Thereafter, ~3 min after the addition of LDL, small vesicles became visible in the interior of the giant liposome (Fig. 3C), their number increasing with time (Fig. 3D). The three-dimensional structures for LDL and bacterial sphingomyelinase are not available and, especially in the case of apoB-100, are hard to predict. Yet, in order to identify a putative region of LDL responsible for its sphingomyelinase activity, we compared the amino acid sequence of human apoB-100 with those of B. cereus, L. interrogans, and S. aureus sphingomyelinase. Three homologous regions were identified by SIM in the sequences of apoB-100 and B. cereus sphingomyelinase consisting of 24, 39, and 43 amino acids and having 38,
36, and 28% identical amino acids, respectively (Fig. 4). Comparison of apoB-100 with three different isoforms of B. cereus sphingomyelinase reveals that out of the total 305 amino acids of this enzyme, 65 (21%) align with the sequence of apoB-100. Previous sequence comparisons have shown DNase I to share common mechanistic characteristics with sphingomyelinase and identified tentatively eight amino acids in the sequence of sphingomyelinase to be important in substrate recognition (35, 36). Interestingly, out of these functionally important amino acids, six are conserved, and one is weakly similar also in the alignment of B. cereus sphingomyelinase and apoB-100 (Fig. 4).

Alignment of human apoB-100 with B. cereus, L. interrogans, and S. aureus sequences gives 30 amino acids that are common in all (Fig. 4).

DISCUSSION

The results presented here demonstrate that isolated human plasma LDL possesses sphingomyelinase activity when using sphingomyelin-containing liposomes as a substrate. Whether this activity is an intrinsic property of apoB-100 or is due to an unidentified relatively tightly associated sphingomyelinase that is not detached from LDL during ultracentrifugation and dialysis remains to be established. From a functional point of view, however, this question may not be important, since the sphingomyelinase activity seems to be tightly associated with LDL and should thus be involved in its interactions in vivo as well as in vitro. Based on the sequence comparison between sphingomyelinase and apoB-100, it seems possible that this enzymatic activity could be an intrinsic property of apoB-100. Moreover, electrophoresis of LDL preparations in 1% agarose gel did not reveal impurities (data not shown). To this end, sphingomyelinase activity of LDL is abolished by oxidation, similarly to the phospholipase A2 activity of apoB-100 (37). Interestingly, other lipoproteins, VLDL, HDL2, and HDL3, were inactive. Lack of sphingomyelinase activity in apoB-100 of VLDL does not contradict the notion that sphingomyelinase activity would be contained in the apoB-100 moiety of LDL. Accordingly, evidence for different apoB-100 conformations derives from the studies of Catapano et al. (38). They showed that internalization of VLDL by cultured fibroblasts by the apoB-100 receptor-mediated pathway and the subsequent suppression of hydroxymethylglutaroyl-CoA reductase require prior catabolic processing of isolated VLDL to an in vitro LDL by lipoprotein lipase. This sensitivity of apoB-100 to its lipid environment would unfortunately make ambiguous the use of apo-100 constructs to determine if the sphingomyelinase activity of LDL is inherent to apoB-100.

The phospholipids of LDL consist mostly of sphingomyelin and phosphatidylcholine (approximately 30 and 66 mol %, respectively), and the surface of LDL is further ordered into lipid microdomains (39, 40). It has been suggested that the apoB-100 moiety interacts preferentially with phosphatidylcholine, and it has also been shown that the acyl chains of fluorescent phosphatidylcholine are cleaved by apoB-100, revealing the presence of both phospholipase A1 and A2 activities (39–41). The surface of LDL displays compositional microheterogeneity that may affect LDL metabolism in vivo (40) as well as the folding of apoB-100. In keeping with the above, it seems feasible to suggest that the sphingomyelin present on the LDL surface is protected from the autocatalytic hydrolysis by the sphingomyelinase activity associated with apoB-100. Treating LDL with B. cereus sphingomyelinase results in its profound aggregation; however, this process was very slow compared with the formation of ceramide (9). Our previous work (30) showed that in POPC/C16:0-sphingomyelin LUVs the hydrolysis of sphingomyelin was nearly complete within ~2.5 min, whereas the formation of ceramide-enriched domains was slow (~100 min), thus implying that for LDL the formation of ceramide-enriched lipid domains could mediate particle aggregation.

Exposure of human blood macrophages to LDL, especially its oxidized or acetylated form, induced a 40% elevation of the endogenous level of ceramide by increasing both neutral and acidic sphingomyelinase activity (42). Both native and oxidized

![Fig. 3](http://www.jbc.org/)

Transformations of a single SOPC/C16:0-sphingomyelin/Bodipy-sphingomyelin (0.75:0.2:0.05, mole fraction) giant vesicle, induced by the addition of human plasma LDL into the vicinity of the vesicle outer surface. Still fluorescence images were taken before (A) and 1.5 min (B), 3 min (C), and 10 min (D) after the administration of LDL. D, scale bar, 20 μm.
LDL have been demonstrated to induce sphingomyelin hydrolysis in smooth muscle cells (43). While the above observations reveal a link between LDL and ceramide formation in cells, the possibility that sphingomyelinase activity would be an intrinsic property of LDL was not considered. In this respect it is of interest to note that Parthasarathy et al. (44) demonstrated that apo-B-100 possesses phospholipase A₂ activity, which is reduced upon oxidative modifications (37). Using a fluorescent phosphatidylcholine derivative incorporated into LDL, it was further shown that both phospholipase A₁ and A₂ activities were present (41). In addition, human plasma PAF acetylhydrolase activity has been demonstrated to be associated with this enzyme in the sequence of apoB-100 (45).

Although the identification of the putative active site of LDL remains uncertain at present, the alignment of apo-B-100 with sphingomyelinase from B. cereus, L. interrogans, and S. aureus is intriguing (Fig. 4). In keeping with the suggested functionally important residues (35, 36) in sphingomyelinase, it is tempting to speculate that this catalytically active His-Ser-Asp triad common to a wide range of lipolytic enzymes in the sequence of apo-B-100 are marked with shading. Multiple alignment was constructed using the CLUSTAL W program.

FIG. 4. Alignment of the sequences of human apo-B-100 and sphingomyelinase from B. cereus, S. aureus, and L. interrogans. Residue conservation is denoted by asterisks, and the suggested functionally important residues for sphingomyelinase (Refs. 35 and 36) are marked with boldface type. Residue conservation is denoted by shading. Alignment was constructed using the CLUSTAL W program.
higher than plasma LDL (9). However, unaggregated lesional LDL was not enriched in ceramide, suggesting that the aggregation of LDL was necessary for the formation of this lipid. Accordingly, the conversion of sphingomyelin to ceramide could be in part due to the sphingomyelinase activity associated with LDL itself.

Acknowledgments—We thank professor Marja-Riitta Taskinen (Department of Medicine, University of Helsinki) for kindly providing preparations used in the preliminary stages of this study.

REFERENCES

1. Ross, R. (1986) *N. Engl. J. Med.* **314**, 488–500
2. Ross, R. (1993) *Nature* **362**, 801–809
3. Brown, M. S., and Goldstein, J. L. (1986) *Science* **232**, 34–47
4. Berliner, J. A., and Heinecke, J. W. (1996) *Free Radiol. Biol. Med.* **20**, 707–727
5. Heinecke, J. W. (1997) *Curr. Opin. Lipidol.* **8**, 268–274
6. Parthasarathy, S., Santanam, N., Ramachandran, S., and Meilhac, O. (1999) *J. Lipid. Res.* **40**, 2143–2157
7. Guyton, J. R., and Klemp, K. F. (1996) *Thromb. Vasc. Biol.* **8**, 1345–1353
8. Khoo, J. C., Miller, E., McLoughlin, P., and Steinberg, D. (1988) *Arteriosclerosis* **8**, 348–358
9. Schissel, S. L., Tweedie-Hardman, J., Rapp, J. H., Graham, G., Williams, K. J., and Tabas, I. (1996) *J. Clin. Invest.* **98**, 1455–1464
10. Srinivasan, S. R., Dolan, P., Radhakrishnamurthy, B., and Berenson, G. S. (1972) *Atherosclerosis* **16**, 95–104
11. Camejo, G., Lalaguna, F., Lopez, F., and Starosta, R. (1980) *Atherosclerosis* **35**, 367–320
12. Lauraeus, S., Holopainen, J. M., Taskinen, M.-R., and Kinnunen, P. K. J. (1998) *Biochim. Biophys. Acta* **1373**, 147–162
13. Jeong, T.-S., Schissel, S. L., Tabas, I., Pownall, H. J., Tall, A. R., and Jiang, X.-C. (1998) *Arteriosclerosis, Thromb. Vasc. Biol.* **18**, 1–38
14. Jarvis, W. D., Grant, S., and Kolesnick, R. N. (1996) *Clin. Cancer Res.* **2**, 1–6
15. Hannan, Y. A., and Obeid, L. M. (1995) *Trends Biol. Sci.* **20**, 73–77
16. Kolesnick, R. N. (1991) *Proc. Lipid Res.* **30**, 1–38
17. Geng, Y. J., and Libby, P. (1995) *Am. J. Pathol.* **147**, 251–266
18. J. M., Angelova, M., and Kinnunen, P. K. J. (2000) *Biophys. J.* **80**, 34–47
19. Havel, R. J., Eder, H. A., and Bragdon, J. R. (1955) *J. Clin. Invest.* **34**, 1345–1353
20. Basso, S. K., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 3178–3182
21. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
22. Esterbauer, H., Gebicki, J., Puhl, H., and Jurgens, G. (1992) *Free Radiol. Biol. Med.* **13**, 341–390
23. Angelova, M. I., and Dimitrov, D. S. (1986) *Faraday Discuss. Chem. Soc.* **81**, 303–311
24. Sehnsorg, M., Potrykus, I., and Neuhaus, G. (1994) *Exp. Cell Res.* **210**, 260–267
25. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680
26. Huang, X., and Miller, W. A. (1991) *Adv. Appl. Math.* **12**, 337–357
27. Holopainen, J. M., Subramanian, M., and Kinnunen, P. K. J. (1998) *Biochemistry* **37**, 17962–17970
28. Fanani, M. L., and Maggio, B. (1997) *J. Mol. Biol. Med.* **14**, 25–29
29. Fong, L. G., Parthasarathy, S., Witztum, J. L., and Steinberg, D. (1987) *J. Lipid. Res.* **28**, 1466–1477
30. Holopainen, J. M., Lehtonen, J. Y. A., and Kinnunen, P. K. J. (1997) *Chem. Phys. Lipids* **88**, 1–13
31. Holopainen, J. M., Lemmich, J., Richter, F., Mournit, O. G., Rapp, G., and Kinnunen, P. K. J. (1990) *Biophys. J.* , in press
32. Weston, S. A., Lahm, A., and Suck, D. R. (1992) *J. Mol. Biol.* **226**, 1237–1256
33. Mateus, Y., Yamada, A., Tsukamoto, K., Tamura, H.-O., Ikezawa, H., Nakamura, H., and Nishikawa, K. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 3488–3492
34. Parthasarathy, S., and Barnett, J. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 9741–9745
35. Catapano, A. L., Gianturco, S. H., Kinnunen, P. K. J., Eisenberg, S., Gotto, A. M. Jr., and Smith, L. C. (1979) *J. Biol. Chem.* **254**, 1007–1009
36. Sommner, A., Prenderg, E., Gorges, R., Stutz, H., Grillhofer, H., Konstner, G. M., Pulda, F., and Hermetter, A. (1992) *J. Biol. Chem.* **267**, 24217–24222
37. Murphy, H. C., Ala-Korpela, M., White, J. J., Burns, S. P., and Isel, R. A. (1997) *Biochem. Biophys. Res. Commun.* **234**, 733–737
38. Reisfeld, N., Lichtenberg, D., Dagan, A., and Yedgar, S. (1993) *FEBS Lett.* **315**, 267–270
39. Kinscherf, R., Claus, R., Deignier, H. P., Nauen, O., Gehrke, C., Hermetter, A., Ruwurm, S., Danel, V., Hack, V., and Metz, J. (1997) *FEBS Lett.* **405**, 55–59
40. Aygu, N., Andrieu, N., Negr-Salvayre, A., Thiers, J.-C., Levade, T., and Salvayre, R. (1996) *J. Biol. Chem.* **271**, 19251–19255
41. Parthasarathy, S., Steinbrecher, U. P., Barnett, J., Witztum, J. L., and Steinberg, D. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 3000–3004
42. Stafforini, D. M., McIntyre, T. M., Carter, M. E., and Prescott, S. M. (1987) *J. Biol. Chem.* **262**, 4215–4222
43. Reaven, E., Chen, Y. D., Spicher, M., Hwang, S. F., Mondon, C. E., and Aza, S. (1986) *J. Clin. Invest.* **77**, 1971–1984
44. Zhang, W. Y., Jaynor, P. M., and Kruth, H. S. (1998) *J. Biol. Chem.* **273**, 31700–31706
45. Hurt-Camejo, E., Olsso, U., Wiklund, O., Bondjers, G., and Camejo, G. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17**, 1011–1017
46. Grasswe, H., Gultinz, E., Breen, B., Forlin, K., Sandhoff, K., Harzer, K., Lang, F., and Meyer, T. F. (1997) *Cell* **91**, 605–615
47. Maratho, S., Schissel, S. L., Yellin, M. J., Beatin, M., Mintser, R., Williams, K. J., and Tabas, I. (1998) *J. Biol. Chem.* **273**, 4081–4088
48. Xu, X.-X., and Tabas, I. (1991) *J. Biol. Chem.* **266**, 24849–24858
49. Tabas, I., Li, Y., Broca, R. W., Xu, S. W., Swenson, T. L., and Williams, K. J. (1993) *J. Biol. Chem.* **268**, 20419–20432
50. Schissel, S. L., Jiang, X., Tweedie-Hardman, J., Jeong, T., Camejo, E. H., Najib, J., Rapp, J. H., Williams, K. J., and Tabas, I. (1998) *J. Biol. Chem.* **273**, 2738–2746
Sphingomyelinase Activity Associated with Human Plasma Low Density Lipoprotein: POSSIBLE FUNCTIONAL IMPLICATIONS
Juha M. Holopainen, Oula Penate Medina, Antti J. Metso and Paavo K. J. Kinnunen

J. Biol. Chem. 2000, 275:16484-16489.
doi: 10.1074/jbc.275.22.16484

Access the most updated version of this article at http://www.jbc.org/content/275/22/16484

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 20 of which can be accessed free at http://www.jbc.org/content/275/22/16484.full.html#ref-list-1