Biochemical and Biophysical Research Communications 441 (2013) 838–843

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cholesterol glucosylation is catalyzed by transglucosylation reaction of β-glucosidase 1

Hisako Akiyama [a,b], Susumu Kobayashi [c], Yoshio Hirabayashi [b], Kimiko Murakami-Murofushi [a,d,*]

[a] Graduate School of Humanities and Sciences, Department of Life Science, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan
[b] Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[c] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[d] Endowed Research Division of Human Welfare Sciences, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan

ARTICLE INFO

Article history:
Received 17 October 2013
Available online 6 November 2013

Keywords:
β-Glucosidase 1 (GBA1)
Cholesteryl glucoside
Gaucher disease
Glycolipid
Sterol glucosyltransferase
Transglucosylation

ABSTRACT

Cholesteryl glucoside (β-ChlGlc), a monoglucosylated derivative of cholesterol, is involved in the regulation of heat shock responses. β-ChlGlc, which is rapidly induced in response to heat shock, activates heat shock transcription factor 1 (HSF1) leading to the expression of heat shock protein 70 (HSP70) in human fibroblasts. Identification and biochemical characterization of the enzyme responsible for β-ChlGlc formation is important for a complete understanding of the molecular mechanisms leading to HSP70-induction following heat shock. Recently, we demonstrated that β-ChlGlc synthesis is not dependent on UDP-Glucose but glucosylceramide (GlcCer) in animal tissue and human fibroblasts. In this study, we examined the possibility of glucocerebrosidase, a GlcCer-degrading glycosidase, acting as β-ChlGlc-synthesizing enzyme. Overexpression of β-glucosidase 1 (GBA1, lysosomal acid β-glucocerebrosidase) led to an increase in cholesterol glucosylation activity in human fibroblasts. Using a cell line generated from type 2 Gaucher disease patients with severe defects in GBA1 activity, we found that cholesterol glucosylation activity was very low in the cells and the overexpression of GBA1 rescued the activity. In addition, purified recombinant GBA1 exhibits conduritol B-epoxide-sensitive cholesterol glucosylation activity. The optimum pH and temperature for cholesterol glucosylation by GBA1 were at about 5.3 and 43 °C, respectively. Short chain C8:0-GlcCer was the most effective donor for cholesterol glucosylation activity among GlcCer containing saturated fatty acid (C8:0 to C18:0) tested. GlcCer containing mono-unsaturated fatty acid was more preferred substrate for cholesterol glucosylation when compared with GlcCer containing same chain length of saturated fatty acid. These results demonstrate, for the first time, a novel function of GBA1 as a β-ChlGlc-synthesizing enzyme. Therefore, our results also reveal a new pathway for glycolipid metabolism in mammals.

1. Introduction

Steryl glucoside (SG) is the most abundant membrane-bound sterol derivative found in many organisms [1]. Previously, we reported that heat shock rapidly induces SG in mold cells [2], human fetal lung fibroblast cells (TIG-3) [3], and rat gastric mucosa [4]. Cholesteryl glucoside (1-*O*-cholesteryl-β-D-glucopyranoside, β-ChlGlc), a glucosylated derivative of cholesterol, is a member of SG family. Heat shock causes rapid accumulation of β-ChlGlc in cells, followed by HSF1 activation and HSP70 induction [4,5]. We have previously reported that the addition of exogenous β-ChlGlc induces HSF1 activation and HSP70 induction in human fibroblasts [5]. Thus, β-ChlGlc may serve as a heat-sensing signal molecule that is required for HSP70 gene activation. However, the precise functions of β-ChlGlc remain unclear.

The formation of SG is known to be catalyzed by a membrane-bound enzyme, namely UDP-glucose:sterol glucosyltransferase (UDPG-SGTase, EC 2.4.1.173), and UDPG-SGTases from a number of organisms have been cloned [1]. Additionally, UDPG-SGTase expressed in a slime mold, *Physarum polycephalum*, has been characterized [6,7]. However, a sterol glucosyltransferase (SGTase) has not yet been identified in animals. Therefore, in order to understand the molecular mechanisms leading to HSP70-induction in animals, it is essential to identify the SGTase responsible for the

Abbreviations: β-ChlGlc, cholesteryl glucoside; GBA1, β-glucosidase 1, lysosomal acid β-glucocerebrosidase; GBA2, β-glucosidase 2, non-lysosomal β-glucocerebrosidase; GBA3, cytosolic glucocerebrosidase; GCase, glucocerebrosidase; GlcCer, glucosylceramide; LPH, lactase-phlorizin hydrolase; SG, steryl glucoside; SGTase, sterol glucosyltransferase.

* Corresponding author at: Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan. Fax: +81 3 5978 5362.
E-mail address: murofushi.kimiko@ocha.ac.jp (K. Murakami-Murofushi).

0006-291X
http://dx.doi.org/10.1016/j.bbrc.2013.10.145

synthesis of β-ChlGlc. Previously, we developed a sensitive assay for SGTase using fluorescence-labeled cholesterol (25-NBD-Cholesterol). Using this assay, we demonstrated that SGTase of animal origin is a novel enzyme, since glucosylceramide (GlcCer), not UDP-glucose, functioned as a glucose donor for β-ChlGlc formation [8]. Supporting this we found that GM-95 cells, a mutant B16 melanoma cell line that does not express GlcCer synthase (GlcT-1, UDP-glucose:ceramide glucosyltransferase, EC 2.4.1.80), were unable to synthesize β-ChlGlc without the addition of exogenous GlcCer.

GlcCer is degraded in an enzymatic reaction involving the cleavage of β-glucosyl linkages between ceramide and glucose by glucocerebrosidase (GCase, GlcCer-degrading glycosidase). Because the unidentified SGTase of animal origin transfers the glucose moiety from GlcCer to cholesterol [8], we examined the possibility that this SGTase may also be a GCase. To date, four mammalian GCases, β-glucosidase 1 (GBA1, lysosomal acid GCase, EC 3.2.1.45), β-glucosidase 2 (GBA2, non-lysosomal GCase, EC 3.2.1.45), cytosolic GCase (GBA3, EC 3.2.1.21), and lactase-phlorizin hydrolase (LPH, EC 3.2.1.62/108) have been identified. In mammals, catabolism of GlcCer primarily takes places in lysosome by GBA1 [9,10]. Gaucher disease, caused by an inherited deficiency of GBA1, is the most common lysosomal storage disorder and is characterized by the accumulation of GlcCer in the lysosomal compartment of macrophages [11,12]. GBA1 is specifically and irreversibly inhibited by conduritol B epoxide (CBE) [13]. GBA2, which is insensitive to CBE, is a non-integral membrane-associated protein that localizes at the cytosolic surface of the ER and Golgi apparatus [14]. GBA2 knockout mice exhibited GlcCer accumulation in the testis, brain, and liver. The mice showed male infertility [15] and delayed liver regeneration [16]. GBA3, a cytosolic, Klotho-related protein (KLrP) whose function is unknown, is insensitive to CBE [17,18]. LPH, which is sensitive to CBE, is exclusively present in the plasma membrane of the small intestine and is possibly involved in the digestion of dietary GlcCer [19]. In the presence of detergent, GCase activities of GBA1, GBA2, GBA3, and LPH are maximum at pH 5.0–6.0, pH 5.5–6.5 [14], pH 6.0–7.0 [18], and pH 5.0–6.0 [19], respectively. In the study reported here, we investigated whether or not these four GCases had SGTase activity. Here we report that, among the four GCases tested, overexpression of GBA1 in TIG-3 cells led to an increase in SGTase activity in the corresponding cell lysates. We demonstrated that the SGTase activity of purified recombinant GBA1 is CBE-sensitive. Our studies reveal for the first time that, GBA1 represents a previously unidentified β-ChlGlc-synthesizing SGTase. We have characterized the optimum *in vitro* conditions for the synthesis of β-ChlGlc by GBA1.

2. Materials and methods

2.1. Materials

25-[*N*-[(7-Nitro-2-1,3-benzoxa-diazol-4-yl)methyl]amino]-27-norcholesterol (25-NBD-Cholesterol), *N*-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-D-glucosyl-ß1-1′-sphingosine (C6-NBD-GlcCer), D-glucosyl-ß-1,1′ *N*-octanoyl-D-*erythro*-sphingosine (C8:0-GlcCer), D-glucosyl-ß-1,1′ *N*-lauroyl-D-*erythro*-sphingosine (C12:0-GlcCer), D-glucosyl-ß-1,1′ *N*-palmitoyl-D-*erythro*-sphingosine (C16:0-GlcCer), D-glucosyl-ß-1,1′ *N*-stearoyl-D-*erythro*-sphingosine (C18:0-GlcCer), D-glucosyl-ß-1,1′ *N*-oleoyl-D-*erythro*-sphingosine (C18:1-GlcCer), and D-glucosyl-ß-1,1′ *N*-nervonoyl-D-*erythro*-sphingosine (C24:1-GlcCer) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 1-*O*-cholesteryl-β-D-glucopyranoside (β-cholesteryl glucoside, β-ChlGlc) was chemically synthesized according to reported methods [20]. Conduritol-B-epoxide (D, L-1,2-anhydro-*myo*-inositol; CBE) was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Cerezyme®, a recombinant human GBA1 used in enzyme replacement therapy in Gaucher disease [21], was purchased from Genzyme Japan (Tokyo, Japan).

2.2. Cloning of cDNAs encoding GCases

Using specific primers containing restriction sites and extended Kozak sequence in front of the start codon, the complete open reading frames for GBA1 (NM_008094.4), GBA2 (NM_172692.3), transcript variant 1 of GBA3 (GBA3-tv1, NM_020973.3), transcript variant 2 of GBA3 (GBA3-tv2, NM_001128432.1), and LPH (NM_001081078.1) were PCR amplified from mouse kidney, mouse testis, GBA3-tv1-pcDNA3.1/Myc-His(+) [18], cDNA clone of GBA3-tv2 (IMAGE ID; 4612206, GenBank accession number; BC109377.1), and mouse small intestine, respectively. GBA3-tv1-pcDNA3.1/Myc-His(+) was generously provided from Dr. Makoto Ito of Kyusyu University. cDNA clone of GBA3-tv2 was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The PCR product was subcloned into p3xFLAG-CMV-14 (Sigma–Aldrich, St. Louis, MO, USA).

2.3. Cell culture and transfection

TIG-3 cells were generously provided from Dr. Kazuhiko Kaji of University of Shizuoka and cultured in Eagle's minimum essential medium (MEM; Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Biowest, Miami, FL, USA) under 5% CO_2 at 37 °C. The population doubling level of TIG-3 cells used in this study was 30.0–40.0. GM00877, a cell line generated from patients with type 2 Gaucher disease, was purchased from Coriel Cell Repositories (Camden, NJ, USA) and was cultured in Eagle's minimum essential medium with Earle's salts (EEMEM; Sigma–Aldrich) supplemented with 10% FBS under 5% CO_2 at 37 °C. Chinese hamster ovary cells (CHO-K1 cells) (RCB0285, established by Puck, T. T.), were purchased from RIKEN BioResource Center (Ibaraki, Japan) and cultured in Ham's F-12 medium (Nissui) supplemented with 10% FBS under 5% CO_2 at 37 °C. cDNA transfection for TIG-3 and CHO-K1 cells were carried out using Lipofectamine® 2000 Transfection Reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h, medium containing transfection reagents was removed, and cells were harvested after washing with PBS. For GM00877 cells, cDNA was transfected using X-tremeGENE HP Transfection Reagent (Roche Applied Science, Basel, Switzerland) according to the manufacturer's instructions. After 48 h, medium containing transfection reagents was removed, cells were washed with PBS and harvested.

2.4. Assay of SGTase activity in vitro

The assay of SGTase activity was carried out according to the method we established previously [8] with slight modifications. The reaction mixture in a total volume of 20 µl contained 40 µM 25-NBD-Cholesterol, 80 µM C16:0-GlcCer, 16 mM citrate–phosphate buffer, pH 5.0, 0.5% 3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS; Dojindo, Kumamoto, Japan), 2% ethanol, and desired amount of enzyme with or without 1 mM ethylenediaminetetraacetic acid (EDTA) and 5 mM $CoCl_2$. For reactions involving purified enzyme, the concentration of citrate–phosphate buffer in the reaction mixture was adjusted to 50 mM to prevent precipitation during the reaction. After incubation at 37 °C for 1 h, the reaction was terminated by adding chloroform/methanol (2:1, v/v), and the lipids were extracted and analyzed as reported before [8].

2.5. Assay of GCase activity in vitro

The GCase assay used in this study was carried out according to the method described by Hayashi et al. [18] with some modifications. The reaction mixture in a total volume of 20 μl contained 100 pmol C6-NBD-GlcCer, 50 mM citrate–phosphate buffer, pH 5.0, 0.25% Triton X-100, 0.6% sodium taurocholate, and a desired amount of enzyme. After incubation at 37 °C for 30 min, the reaction was terminated by adding chloroform/methanol (2:1, v/v), and the lipids were extracted. The extracted lipids were separated by thin layer chromatography on silica gel 60 using chloroform/methanol/water (65:25:4, v/v/v) as eluent. Fluorescence emission of C6-NBD-ceramide on the silica gel plate was detected using a LAS-3000 Luminoimaging Analyzer, and was quantified with Multi Gauge V2.2 software.

2.6. Western blotting

Cells were harvested and lysates were subjected to SDS–PAGE followed by Western blotting to detect the expression of FLAG tagged GCases, GBA1, and β-actin. Typically, proteins present in the cell lysates were separated by SDS–PAGE and transferred to an Immobilon-P Transfer Membrane (Merck Millipore, Billerica, MA, USA). Using anti-FLAG M2 (#F1804, Sigma–Aldrich, 1:1,000 dilution) or anti-GBA1 (#G4171, Sigma–Aldrich, 1:10,000 dilution) or anti-β-actin (#PM053, Medical & Biological Laboratories, Nagoya, Japan, 1:10,000 dilution) as primary antibodies and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (#170–6515, Bio-Rad, Hercules, CA, USA, 1:20,000 dilution) or HRP-conjugated anti-mouse IgG (#55570, Cappel Laboratories, West Chester, PA, USA, 1:20,000 dilution) as secondary antibodies, immunodetection was performed using an enhanced chemiluminescence (ECL) system (GE Healthcare UK Ltd, Amersham Place, Little Chalfont, England).

2.7. Purification of recombinant GBA1

Recombinant mouse GBA1: FLAG tagged mouse GBA1 was 1expressed in CHO-K1 cells and purified using FLAG® immunoprecipitation kit (Sigma–Aldrich) according to the manufacturer's instructions. The expression of GBA1 was tested using immunoprecipitated samples by Western blotting as described earlier.

Recombinant human GBA1: Cerezyme®, was dialyzed and deglycosylated according to reported methods [22]. Typically, 5 ng protein (0.5 μU) was used for each assay. One unit of β-glucosidase activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside per minute at pH 5.0 and 37 °C.

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Mean ± SEM values were calculated for all values. The data were analyzed using one-way analysis of variance (ANOVA) with Dunnett's post hoc-test or unpaired Student's *t*-test. A P value <0.05 was considered as statistically significant.

3. Results

3.1. GBA1 is a candidate for mammalian SGTase

To identify animal SGTase, we attempted to examine SGTase activity of four known GCases (GBA1, GBA2, GBA3, and LPH) overexpressed in TIG-3 cells. Two transcript variants of GBA3 (GBA3-tv1 and GBA3-tv2) differing in their lengths were examined. Our results showed that SGTase activity increased significantly in lysates of TIG-3 cells overexpressing GBA1 (Fig. 1). Almost the same results were obtained at the reaction condition between pH 5.0 and 6.2 (data not shown).

To verify whether GBA1 had SGTase activity, we used the GM00877 cell line generated from patients with type 2 Gaucher disease. Protein levels of GBA1, GCase activity, and SGTase activity in the lysates of GM00877 cells transfected with empty vector were lower than in those of TIG-3 cells (Fig. 2). Interestingly, overexpression of GBA1 in GM00877 cells resulted in an increase in the protein level of GBA1, leading to elevated GCase and SGTase activities. Two other cell lines, GM00372 and GM00852, generated from patients with type 1 Gaucher disease (Coriel Cell Repositories), also showed low expression level of GBA1 protein, as well as low GCase and SGTase activities compared to TIG-3 cells (Fig. S1). However, low transfection efficiency prevented us from confirming the effect of GBA1 overexpression in GM00372 and GM00852 cells.

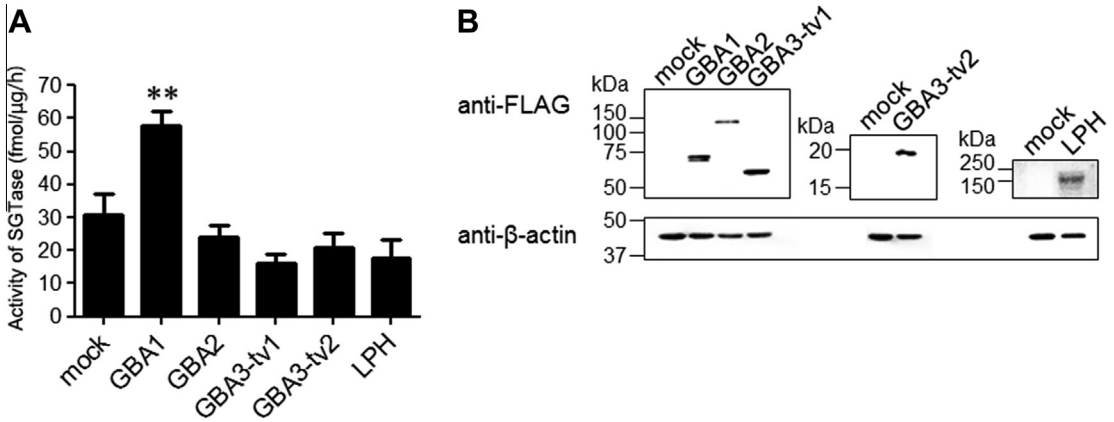

Fig. 1. SGTase activities in the lysates of TIG-3 cells transfected with cDNA encoding GCase. (A) SGTase activity of cell lysates prepared from TIG-3 transfectants. *GBA1*, *GBA2*, *GBA3-tv1*, *GBA3-tv2*, *LPH*, and *mock* represent the transfectants with cDNA encoding the respective GCase or empty vector (mock). Differences were established using one-way ANOVA with Dunnett's post hoc test, $n = 4$. $^{**}P < 0.01$ compared with SGTase activity of *mock*. (B) Western blotting of GCase expressed in TIG-3 cells. Protein levels of FLAG tagged GCase and β-actin were determined using anti-FLAG M2 or anti-β-actin as primary antibodies.

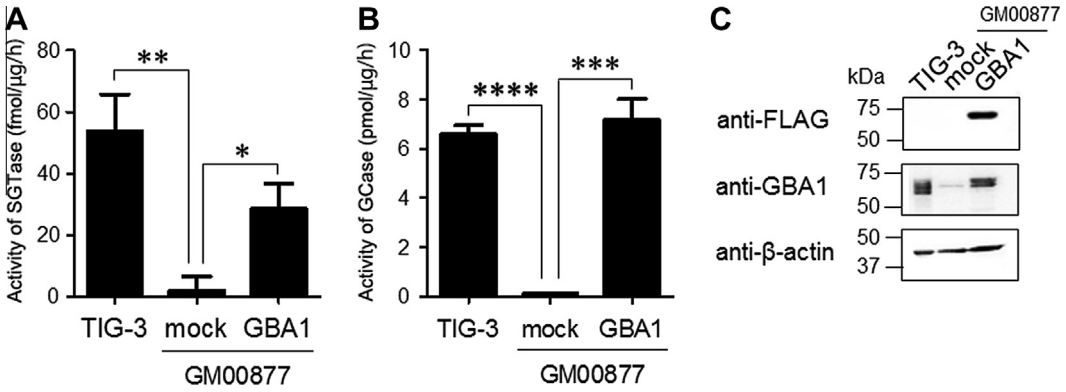

Fig. 2. SGTase activities in the lysates of GM00877 cells transfected with cDNA encoding GBA1. (A) SGTase activity in the lysates of GM00877 transfectants and TIG-3 cells. *GBA1* and *mock* represent the cells transfected with cDNA encoding GBA1 or empty vector (mock). (B) GCase activity in the lysates of GM00877 transfectants and TIG-3 cells. Differences were established using unpaired Student's *t*-test, $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. (C) Western blotting of GBA1 expressed in GM00877 transfectants and TIG-3 cells. Protein levels of FLAG tagged GCase, GBA1, and β-actin were determined using anti-FLAG M2, anti-GBA1, and anti-β-actin, respectively, as primary antibodies.

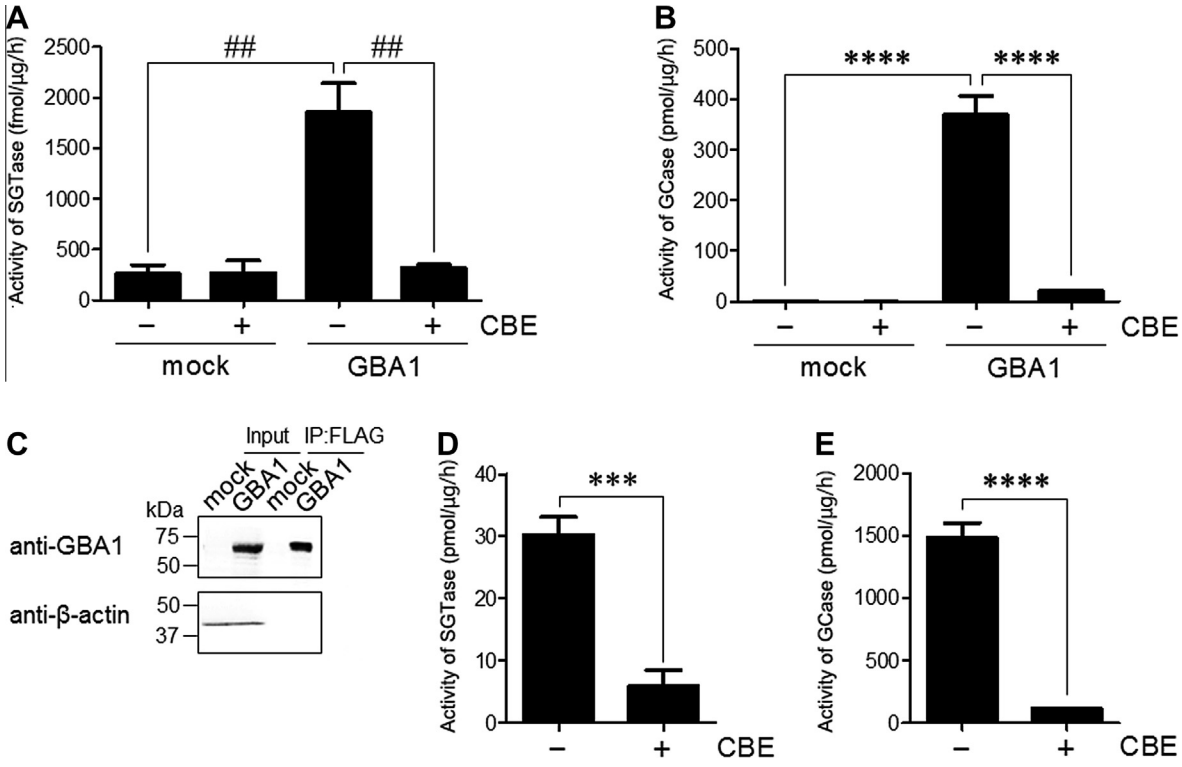

Fig. 3. SGTase and GCase activities of purified recombinant GBA1. (A and D) SGTase activity of purified recombinant GBA1 from mouse (A) and human (D). The SGTase activity was measured with or without 0.5 mM CBE. (B and E) GCase activity of purified recombinant GBA1 from mouse (B) and human (E). The activity of GCase was measured with or without 0.5 mM CBE. In (A) and (B), *GBA1* and *mock* represent the purified proteins from CHO-K1 transfectants with cDNA encoding mouse GBA1 or empty vector (mock). Differences were established using unpaired Student's *t*-test, $n = 4$. $^{***}P < 0.001$; $^{\#\#}P < 0.005$; $^{****}P < 0.0001$. (C) Western blot analysis of GBA1 purified from CHO-K1 transfectants. Protein levels of GBA1 in the cell lysates of CHO-K1 transfectants (*Input*) and in the purified products (*IP:FLAG*) were determined using anti-GBA1 or anti-β-actin as primary antibodies.

3.2. Purified recombinant GBA1 has CBE-sensitive SGTase activity

To verify whether GBA1 catalyzes cholesterol glucosylation, we purified recombinant human and mouse GBA1 as described under the "Materials and methods" section. Results of our *in vitro* assays using purified proteins showed that recombinant mouse GBA1 had GCase and SGTase activities, which were sensitive to CBE. Further, we found that these activities were not observed in products purified from CHO-K1 cells transfected with empty vector (Fig. 3A–C). Additionally, we found that deglycosylated-Cerezyme, when used as the purified recombinant human counterpart of mouse GBA1, exhibited CBE-sensitive GCase and SGTase activities (Fig. 3D and E). These results demonstrate that GBA1 acts as a β-ChlGlc-synthesizing enzyme. We also found that NBD-ChlGlc formed during the *in vitro* reactions had glycosidic bonds in the β-configuration (Fig. S2).

3.3. Characterization of optimum conditions for the synthesis of β-ChlGlc by human GBA1 in vitro

Using deglycosylated-Cerezyme, we determined the optimum conditions required for SGTase activity of human GBA1 *in vitro*.

We found that the SGTase activity was increased gradually and continuously up to 24 h examined (Fig. 4A). Using enzymatically synthesized NBD-ChlGlc [8] as a substrate, we confirmed that human GBA1 has β-ChlGlc-degrading activity (Fig. S2). We observed that the SGTase activity of human GBA1 was maximum at about pH 5.3 (Fig. 4B) and 43 °C (Fig. 4C). Divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Ba^{2+}) at 1–10 mM concentrations had no effect on increase of the SGTase activity (data not shown). As shown in Fig. 4D, among the GlcCer containing saturated fatty acids (C8:0 to C18:0) tested, short chain C8:0-GlcCer was found to be most effective glucose donor for SGTase. We also found that C18:1-GlcCer was more suitable as a SGTase substrate than C18:0-GlcCer. Therefore, GlcCer containing mono-unsaturated fatty acid is considered to be more preferred substrate for cholesterol glucosylation when compared with GlcCer containing same chain length of saturated fatty acid.

4. Discussion

SGTases in a number of organisms are known to use UDP-glucose as the glucose donor and have been identified and cloned [1]. However, the identity of the animal counterparts of these SGTases remained elusive. The results presented here demonstrate that, in human and mouse, GBA1 is responsible for the synthesis of β-ChlGlc from GlcCer and cholesterol. Therefore, we suggest that GBA1 represents a previously unidentified mammalian SGTase.

As shown in Fig. 3, CBE inhibited the SGTase activity of GBA1 *in vitro*. The crystal structure of GBA1 with bound CBE revealed that CBE binds to the active site of GCase [23]. Therefore, we hypothesized that the inhibitory effect CBE on β-ChlGlc formation may have been caused by reduced GlcCer degradation followed by a decrease in the amount of transposable glucose.

Previously, GBA1 has been found to catalyze not only GlcCer hydrolysis but also transglucosylation reactions. Formation of β-ChlGlc catalyzed by GBA1 is considered to be a transglucosylation reaction between GlcCer and cholesterol. Partially purified GBA1 from the calf brain has been found to catalyze transglucosylation reactions between an artificial glucose donor, 4-methylumbelliferone β-D-glucoside and ceramide [24]. Additionally, it was reported that human placental GBA1 catalyzes the reaction between GlcCer and two alcohols, namely *n*-pentanol and retinol [25], but the products of the reaction, *N*-pentyl glucoside and retinyl glucoside are not naturally occurring substances. Compared to these reactions, β-ChlGlc formation we demonstrated here is novel GBA1-mediated transglucosylation reaction because the molecules involved in β-ChlGlc formation are naturally occurring ones. The relationship between GBA1 mutations and transglucosylation activity has been reported. Thus, N370S mutation, a common GBA1 mutation in non-neuropathic Gaucher disease (type 1) patients, altered the activity of both hydrolysis and transglucosylation [26]. Since it is not known as to whether or not GBA1 catalyzes transglucosylation reactions *in vivo*, further studies are needed to elucidate the mechanisms responsible for the biosynthesis of β-ChlGlc in organisms.

Using the purified recombinant human GBA1, we characterized the optimum conditions for SGTase activity *in vitro* (Fig. 4). The optimum pH and temperature requirements for maximal SGTase activity of GBA1 were similar to those reported for its GCase activity [14,27,28]. It has been reported that, among GlcCer containing

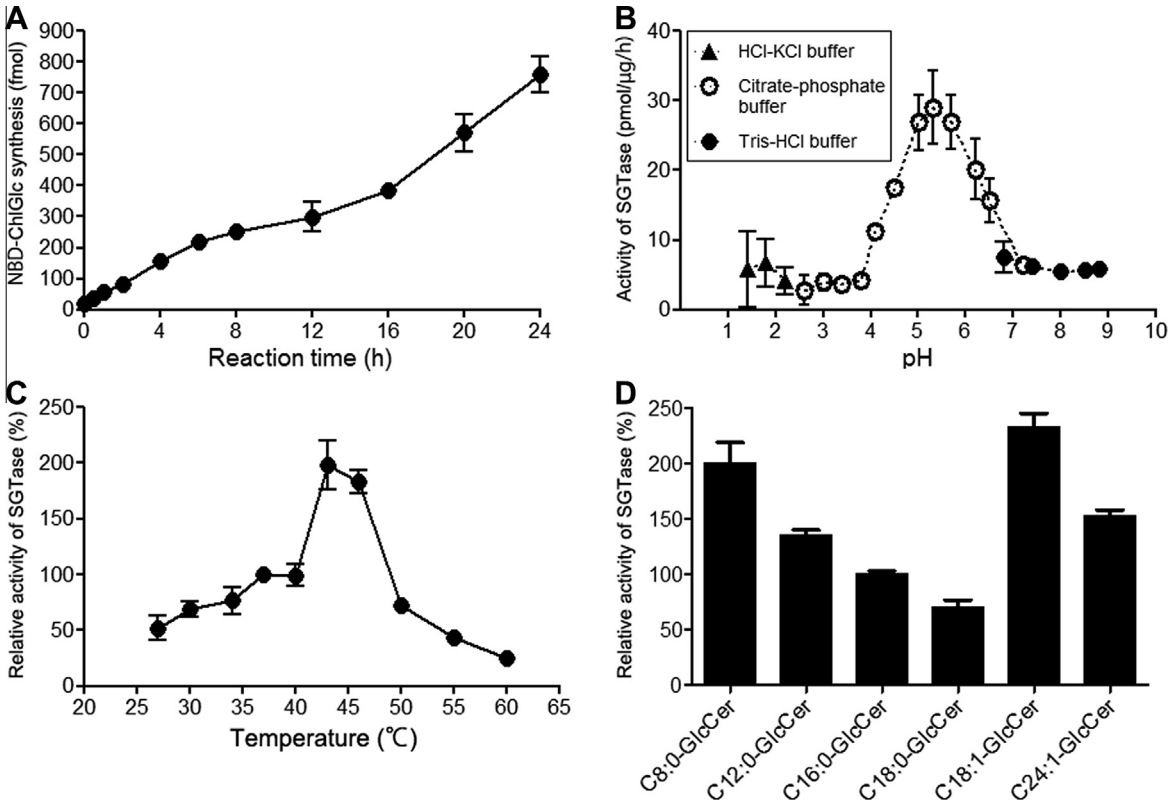

Fig. 4. Characterization of optimum conditions for β-ChlGlc synthesis by purified human GBA1 *in vitro*. (A) Time course of β-ChlGlc synthesis by human GBA1 at 37 °C, pH 5.0 (n = 4–6). (B) pH dependence of SGTase activity of human GBA1. The activity of SGTase was measured at 37 °C for 1 h in a 50 mM concentration of the various buffers shown in the *inset* of *B* (n = 3). (C) Temperature dependence of SGTase activity of human GBA1. The activity of SGTase was measured within a temperature range of 27–60 °C for 1 h at pH 5.3 (n = 4–6). SGTase activity at 37 °C was arbitrarily set as 100%. (D) Substrate specificity of SGTase activity present in human GBA1. The activity of SGTase was measured at 37 °C for 1 h at pH 5.3 using various GlcCer (C8:0-GlcCer, C12:0-GlcCer, C16:0-GlcCer, C18:0-GlcCer, C18:1-GlcCer, and C24:1-GlcCer) as the glucose donor (n = 5–6). The activity of SGTase measured using C16:0-GlcCer was arbitrarily set as 100%.

saturated fatty acids (C1:0 to C18:0), GCase activity of GBA1 showed substrate preference toward C8:0-GlcCer [29]. In our experiments, we found that among the GlcCer containing saturated fatty acids (C8:0 to C18:0) tested, C8:0-GlcCer was the most effective glucose donor for SGTase. For GCase activity of GBA1, substrate specificity among GlcCer containing unsaturated fatty acid has not been studied and reported. Here, we demonstrated that GlcCer containing mono-unsaturated fatty acid was more preferred substrate for cholesterol glucosylation when compared with GlcCer containing same chain length of saturated fatty acid. It is possible that transfer glucose from ceramide to cholesterol becomes easier in the bent conformation, *cis*-double bond, of the unsaturated fatty acid.

We have reported that SGTase activity is present in the lipid raft fraction of TIG-3 cells [30]. Because GBA1 is a lysosomal enzyme, we suggested that β-ChlGlc is probably synthesized in lipid rafts present in the lysosomes. Although the physiological significance of cholesterol glucosylation in animals is not known, glucosylation alters the physical properties of cholesterol, due to the reduction of the depth of sterol moiety embedded in the bilayer caused by the large polar head group [31]. A comparison of models of artificial liposomes before and after the formation of β-ChlGlc suggested that, transfer of glucose moiety from GlcCer to cholesterol results in a change in membrane physical properties, forming thermostable solid-ordered domains [30]. Therefore, it is likely that β-ChlGlc may be involved in modulating the physical properties of lysosomal membrane. Further studies are necessary to understand the function of β-ChlGlc in lysosomes. Our identification of GBA1 as the mammalian enzyme responsible for β-ChlGlc formation is a key step towards understanding the *in vivo* roles of β-ChlGlc in mammals.

Acknowledgments

We are grateful to Dr. Makoto Ito of Kyusyu University for providing GBA3-tv1-pcDNA3.1/Myc-His(+), and Dr. Kazuhiko Kaji of University of Shizuoka for providing TIG-3 cells. This work was supported in part by the Mizutani Foundation for Glycoscience (grant to K.M.-M.), and Sanofi Japan Group (grant to K.M.-M.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.145.

References

[1] S. Grille, A. Zaslawski, S. Thiele, et al., The functions of steryl glycosides come to those who wait: Recent advances in plants, fungi, bacteria and animals, Prog. Lipid Res. 49 (2010) 262–288.
[2] K. Murakami-Murofushi, K. Nishikawa, E. Hirakawa, H. Murofushi, Heat stress induces a glycosylation of membrane sterol in myxoamoebae of a true slime mold, *Physarum polycephalum*, J. Biol. Chem. 272 (1997) 486–489.
[3] S. Kunimoto, T. Kobayashi, S. Kobayashi, K. Murakami-Murofushi, Expression of cholesteryl glucoside by heat shock in human fibroblasts, Cell Stress Chaperones 5 (2000) 3–7.
[4] S. Kunimoto, W. Murofushi, I. Yamatsu, et al., Cholesteryl glucoside-induced protection against gastric ulcer, Cell Struct. Funct. 28 (2003) 179–186.
[5] S. Kunimoto, W. Murofushi, H. Kai, et al., Steryl glucoside is a lipid mediator in stress-responsive signal transduction, Cell Struct. Funct. 27 (2002) 157–162.
[6] Z.A. Wojciechowski, J. Zimowski, J.G. Zimowski, A. Lyznik, Specificity of sterol-glucosylating enzymes from *Sinapis alba* and *Physarum polycephalum*, Biochim. Biophys. Acta 570 (1979) 363–370.
[7] K. Murakami-Murofushi, J. Ohta, Expression of UDP-glucose: poriferasterol glucosyltransferase in the process of differentiation of a true slime mold, *Physarum polycephalum*, Biochim. Biophys. Acta 992 (1989) 412–415.
[8] H. Akiyama, N. Sasaki, S. Hanazawa, et al., Novel sterol glucosyltransferase in the animal tissue and cultured cells: evidence that glucosylceramide as glucose donor, Biochim. Biophys. Acta 1811 (2011) 314–322.
[9] J.A. Barranger, E.I. Ginns, Glucosylceramide lipidoses: Gaucher disease, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic Basis of Inherited Disease, vol. 2, McGraw-Hill Inc., New York, 1989, pp. 1677–1698.
[10] E. Beutler, G.A. Grabowski, Gaucher disease, in: C.R. Scriver, A.L. Beaudet, D. Valle, W.S. Sly (Eds.), The Metabolic and Molecular Bases of Inherited Disease III, McGraw-Hill Inc., New York, 2001, pp. 3635–3668.
[11] R.O. Brady, J.N. Kanfer, D. Shapiro, Metabolism of glucocerebrosides II. Evidence of an enzymatic deficiency in Gaucher's disease, Biochem. Biophys. Res. Commun. 18 (1965) 221–225.
[12] K.S. Hruska, M.E. LaMarca, C.R. Scott, E. Sidransky, Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (*GBA*), Hum. Mutat. 29 (2008) 567–583.
[13] G. Legler, Glycoside hydrolases: mechanistic information from studies with reversible and irreversible inhibitors, Adv. Carbohydr. Chem. Biochem. 48 (1990) 319–384.
[14] H.G. Körschen, Y. Yildiz, D.N. Raju, et al., The non-lysosomal β-glucosidase GBA2 is a non-integral membrane-associated protein at the endoplasmic reticulum (ER) and Golgi, J. Biol. Chem. 288 (2013) 3381–3393.
[15] Y. Yildiz, H. Matern, B. Thompson, et al., Mutation of β-glucosidase 2 causes glycolipid storage disease and impaired male fertility, J. Clin. Invest. 116 (2006) 2985–2994.
[16] M.A. Gonzalez-Carmona, R. Sandhoff, F. Tacke, et al., Beta-glucosidase 2 knockout mice with increased glucosylceramide show impaired liver regeneration, Liver Int. 32 (2012) 1354–1362.
[17] K. Yahata, K. Mori, H. Arai, et al., Molecular cloning and expression of a novel klotho-related protein, J. Mol. Med. 78 (2000) 389–394.
[18] Y. Hayashi, N. Okino, Y. Kakuta, et al., Klotho-related protein is a novel cytosolic neutral β-glycosylceramidase, J. Biol. Chem. 282 (2007) 30889–30900.
[19] T. Kobayashi, K. Suzuki, The glycosylceramidase in the murine intestine. Purification and substrate specificity, J. Biol. Chem. 256 (1981) 7768–7773.
[20] S.G.A. Alivisatos, G. Deliconstantinos, A. Papaphilis, G.P. Theodosiadis, Cooperative nature of the binding of cholesterol on to synaptosomal plasma membranes of dog brain, Biochim. Biophys. Acta 643 (1981) 642–649.
[21] G.A. Grabowski, N.W. Barton, G. Pastores, et al., Enzyme therapy in type 1 Gaucher disease: comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources, Ann. Intern. Med. 122 (1995) 33–39.
[22] H. Dvir, M. Harel, A.A. McCarthy, et al., X-ray structure of human acid-β-glucosidase, the defective enzyme in Gaucher disease, EMBO Rep. 4 (2003) 704–709.
[23] L. Premkumar, A.R. Sawkar, S. Boldin-Adamsky, et al., X-ray structure of human acid-β-glucosidase covalently bound to conduritol-B-epoxide. Implications for Gaucher disease, J. Biol. Chem. 25 (2005) 23815–23819.
[24] R.A. Mumford, S.S. Raghavan, J.N. Kanfer, Hydrolytic and transglucolytic activities of a partially purified calf brain β-glucosidase, J. Neurochem. 27 (1976) 943–948.
[25] D.J. Vanderjagt, D.E. Fry, R.H. Glew, Human glucocerebrosidase catalyses transglucosylation between glucocerebroside and retinol, Biochem. J. 300 (1994) 309–315.
[26] B. Liou, G.A. Grabowski, Participation of asparagine 370 and glutamine 235 in the catalysis by acid β-glucosidase: the enzyme deficient in Gaucher disease, Mol. Genet. Metab. 97 (2009) 65–74.
[27] G.A. Grabowski, S. Gatt, M. Horowitz, Acid β-glucosidase: enzymology and molecular biology of Gaucher disease, Crit. Rev. Biochem. Mol. Biol. 25 (1990) 385–414.
[28] O. Abian, P. Alfonso, A. Velazquez-Campoy, et al., Therapeutic strategies for Gaucher disease: miglustat (NB-DNJ) as a pharmacological chaperone for glucocerebrosidase and the different thermostability of velaglucerase alfa and imiglucerase, Mol. Pharm. 8 (2011) 2390–2397.
[29] K. Osiecki-Newman, D. Fabbro, G. Legler, et al., Human acid β-glucosidase: use of inhibitors, alternative substrates and amphiphiles to investigate the properties of the normal and Gaucher disease active sites, Biochim. Biophys. Acta 915 (1987) 87–100.
[30] H. Akiyama, T. Hamada, Y. Nagatsuka, et al., A possible mechanism of cholesteryl glucoside formation involved in heat shock response in the animal cell membrane, Cytologia 76 (2011) 19–25.
[31] K.K. Halling, B. Ramstedt, J.P. Slotte, Glycosylation induces shifts in the lateral distribution of cholesterol from ordered towards less ordered domains, Biochim. Biophys. Acta 1778 (2008) 1100–1111.