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# The enzymatic hydrolysis of 6-acylamino-4-methylumbelliferyl-β-D-glucosides: identification of a novel human acid β-glucosidase

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**Abstract**

Fluorogenic 6-acylamino-4-methylumbelliferyl-β-D-glucosides were found to be poor substrates for the three known human β-glucosidases, i.e., lysosomal and non-lysosomal glucocerebrosidases and cytosolic broad-specificity β-glucosidase. However, homogenates of human tissues and human cell types showed significant enzymatic hydrolysis of 6-ethanoylamino-4-methylumbelliferyl-β-D-glucoside (EMGlc) due to the activity of a hitherto undescribed β-glucosidase, called here EMGlc-ase. It was shown that the isozyme is hardly active towards 4-methylumbelliferyl-β-D-glucoside or glucosylceramide. EMGlc-ase exhibits maximal activity at pH 4.5 and 5.0 in the absence and presence of sodium taurocholate respectively. It is a soluble lysosomal enzyme with a discrete isoelectric point of about 5.0. EMGlc-ase is not inhibited by conduritol B-epoxide, is activated by sodium taurocholate and binds strongly to Concanavalin A. This enzyme is not deficient in relation to Gaucher disease.

*Keywords:* Human β-glucosidases; Gaucher disease; Fluorogenic substrates

## 1. Introduction

β-Glucosidic linkages are rare in mammalian cells. The only known endogenous substrate for β-glucosidases is the glycosphingolipid glucosylceramide (glucocerebroside) which is assumed to be catabolized specifically in lysosomes. Nevertheless, in human tissues and cell types various β-glucosidase activities are observed. The best known enzyme is lysosomal glucocerebrosidase (EC 3.2.1.45); this enzyme is present in all cell types. It is a weakly membrane-associated glycoprotein of 497 amino acids containing 4 *N*-linked glycans. The glucocerebrosidase gene is located on chromosome 1 locus q21. A deficiency in lysosomal glucocerebrosidase forms the basis of an inherited lysosomal storage disorder in man, called Gaucher disease. The manifestation of Gaucher disease is clinically heterogeneous. Three phenotypes of the disease are distinguished on the basis of clinical severity, age of onset and neuronal involvement: type 1 (adult form), type 2 (infantile form) and type 3 (juvenile form) (for recent reviews, see

Abbreviations: MGlc, 4-methylumbelliferyl-β-D-glucoside; EMGlc, 6-ethanoylamino-4-methylumbelliferyl-β-D-glucoside; BMGlc, 6-butanoylamino-4-methylumbelliferyl-β-D-glucoside; OMGlc, 6-octanoylamino-4-methylumbelliferyl-β-D-glucoside; HMGlc, 6-hexadecanoylamino-4-methylumbelliferyl-β-D-glucoside; MGlcNAc, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucoside; TCh, sodium taurocholate; CBE, conduritol B epoxide; SAP-2, sphingolipid activator protein 2

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Refs. [1,2]). The lysosomal glucocerebrosidase is active not only towards the natural lipid substrate glucosylceramide but also to a number of artificial β-D-glucosidic and β-D-xylosidic substrates, such as fluorogenic 4-methylumbelliferyl derivatives [3]. The enzyme has an acid pH optimum (4.5–5.0) [4] and is very heterogeneous in isoelectric point (p*I* values ranging from 3.5 to 7.0) [5]. It is irreversibly inhibited by conduritol B-epoxide (CBE) [6], is activated by sodium taurocholate (TCh) [7] and, also, by phosphatidylserine in combination with the sphingolipid activator protein 2 (SAP-2) [8].

The existence of a second, non-lysosomal tightly membrane-bound glucocerebrosidase has been reported in many tissues and cell types [9]. This enzyme seems to be identical to the enzyme previously described as membraneous non-specific β-glucosidase [10–13]. Like the lysosomal enzyme, non-lysosomal glucocerebrosidase is able to hydrolyze glucosylceramide and 4-methylumbelliferyl-β-D-glucoside, but it differs from lysosomal glucocerebrosidase in several properties. In particular, it is strongly membrane-bound, is not located in lysosomes, has a more neutral pH optimum, is markedly inhibited by detergents, is not inhibited by CBE and is not deficient in Gaucher disease [9]. The physiological role of the enzyme is as yet unknown.

In most tissues, especially in liver, kidney and spleen, there occurs a cytosolic broad-specificity β-glucosidase (EC 3.2.1.21) with near neutral pH optimum [14–18]. This enzyme does not hydrolyze glucosylceramide but displays a broad specificity towards β-D-glucosidic, β-D-galactosidic, β-D-fucosidic, β-D-xylosidic and α-L-arabinosidic derivatives of 4-methylumbelliferone and *p*-nitrophenol [14,15]. The physiological substrate for the cytosolic broad-specificity β-glucosidase is yet unknown. This enzyme is not a glycoprotein; it is located in the cytosol and shows a discrete isoelectric point of 4.6 [15]. It is not inhibited by CBE, is inhibited by TCh and phosphatidylserine [15,17] and is not deficient in Gaucher disease [19].

4-Methylumbelliferyl-β-D-glucoside is a fluorogenic substrate generally used for detection of β-glucosidase activities. We have investigated the ability of various known β-glucosidase to hydrolyze a series of newly synthesized fluorogenic 6-acylamino-4-methylumbelliferyl-β-D-glucosides with variable acyl-chain length [20]. Based on their structural similarity to glucosylceramide, we expected these compounds to be better/or more specific substrates for glucocerebrosidases as compared to 4-methylumbelliferyl-β-D-glucoside. However, this actually was not the case. Nevertheless, the use of the novel substrates led us to the discovery of a hitherto undescribed β-glucosidase of which a number of characteristics are described.

## 2. Materials and methods

### 2.1. Materials

Sodium taurocholate (TCh) was purchased from Fluka (Buchs, Switzerland), 4-methylumbelliferyl-β-D-glucoside, Concanavalin A Sepharose-4B, castanospermine and deoxynojirimycin were from Sigma (St. Louis, MO, USA), 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucoside was from Calbiochem (San Diego, CA, USA), conduritol B epoxide (CBE) was obtained from Biomol Research Laboratories (Philadelphia, PA, USA) and Percoll from Pharmacia (Uppsala, Sweden). All other chemicals were of the purest grade available.

6-Acylamino-4-methylumbelliferyl-β-D-glucosides were synthesized as described previously [20]. The 6-acylamino-4-methylumbelliferone derivatives were obtained by acylation of 6-amino-4-methylumbelliferone [21] with the acyl chlorides of the corresponding carbonic acids and glycosylated by the Koenigs-Knorr method. The structure of the newly synthesized compounds was confirmed by elemental analysis and $^1$H-NMR spectroscopy. The configuration of the 1,2-*trans*-glucoside bond was confirmed by the presence in the $^1$H-NMR spectrum of a doublet at delta 4.9-5.0 ($J_{1,2}$ 7.9-8.0 Hz). The fluorescence of aglycons was similar to that described earlier for 4-methylumbelliferone.

### 2.2. Tissue and cells

Spleen samples were obtained as surgical specimens during therapeutic splenectomy or by autopsy. Liver and kidney were from autopsy material. The phenotype of the Gaucher patients was established by clinical investigation. Type 1 Gaucher fibroblasts were obtained from the Institute of Medical Genetics, Russian Academy of Sciences, and type 2 Gaucher fibroblasts were kindly provided by Dr. Y. Suzuki from Tokyo Metropolitan Institute of Medical Sciences.

Human blood leukocytes were isolated by the dextran method as described in [22].

Tissues were homogenized in Potter homogenizer in 5 vol. (w/v) of 10 mM potassium phosphate/100 mM NaCl (pH 6.0), containing 0.5% (w/v) Triton X-100. All procedures were performed at 4°C.

### 2.3. Purification of β-glucosidases

Lysosomal glucocerebrosidase was purified from human spleen by immunoaffinity chromatography as described in Ref. [23]. The cytosolic broad-specificity β-glucosidase was isolated from type 1 Gaucher disease spleen as described in Ref. [15].

The membrane-bound non-lysosomal glucocerebrosidase was partially purified as follows. Spleen from type 1 Gaucher disease patient was homogenized in 4 vols. of 50 mM sodium citrate buffer (pH 6.0) using an Ultraturrax homogenizer. The homogenate was centrifuged for 1 h at 80 000 × *g*. The supernatant was discarded and the pellet redissolved in the same buffer by sonication. The suspension was again subjected to ultracentrifugation and the resulting pellet resuspended in sodium citrate buffer. In order to eliminate the contribution of residual lysosomal glucocerebrosidase to the acid β-glucosidase activity, the suspension was incubated for 30 min with 5 mM CBE.

### 2.4. Enzyme assays

To measure β-glucosidase activities, the following fluorogenic substrates were used: 4-methylumbelliferyl-β-D-glucoside (MGlc), 6-ethanoyl-, 6-butanoyl-, 6-octanoyl- and 6-hexadecanoylamino-4-methylumbelliferyl-β-D-glucosides (EMGlc, BMGlc, OMGlc and HMGlc, respectively). If not indicated otherwise, the reaction mixture (final volume 0.2 ml) contained 2.2 mM MGlc or 1 mM EMGlc, BMGlc, OMGlc or HMGlc, 50/100 mM citrate/phosphate (pH 5.0), 0.6% (w/v) TCh and the enzyme preparation. (0.05–0.2 mg of protein). After 15–180 min at 37°C, the reaction was terminated by addition of 2 ml of 0.4 M glycine-NaOH (pH 10.5) with MGlc as substrate. When EMGlc, BMGlc, OMGlc or HMGlc were used as substrates, the reaction terminating solution also contained 66% (v/v) ethanol. The fluorescence of the liberated 4-methylumbelliferone was measured in Shimadzu RF-5000 spectrofluorometer using an excitation wavelength of 365 nm and emission wavelength of 450 nm and the fluorescence of 6-acylamino-4-methylumbelliferones, using an excitation wavelength of 385 nm and emission wavelength of 450 nm.

β-Hexosaminidase was measured with 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucoside (MGlcNAc) at a final concentration of 1.6 mM in 0.1 M acetate buffer (pH 5.0). The reaction was stopped by adding 2.0 ml of 0.4 M glycine-NaOH (pH 10.5) and the 4-methylumbelliferone formed was determined fluorometrically.

### 2.5. Subcellular fractionation

Rat liver, previously perfused with cold 250 mM sucrose, 5 mM Tris/HCl (pH 7.0) and 1 mM EDTA, was homogenized in a Potter homogenizer in 6 vol. of the same solution for 1 min at 0°C. Homogenate was centrifuged for 5 min at 700 × *g* and the resulting postnuclear supernatant was layered on Percoll material with a starting density of 1.09 g/ml. Continuous gradient was generated in situ by centrifugation at 30000 × *g* for 60 min at 4°C using Beckman 50 Ti fixed angle rotor. Fractions of 0.3 ml each were collected from gradient.

### 2.6. Isoelectric focusing

Preparative flat-bed isoelectric focusing in Ultrodex containing 1% (v/v) Triton X-100 was performed overnight at 500 V using a LKB 2117 Multiphor apparatus as described by the manufacturer. The gel was fractionated and the fractions of gel material were extracted with distilled water.

## 3. Results

### 3.1. Comparison of the rates of hydrolysis of various fluorogenic substrates by purified β-glucosidases from human spleen

The specific activity of known β-glucosidases (lysosomal glucocerebrosidase, non-lysosomal glucocerebrosidase and cytosolic broad-specificity β-glucosidase) from human spleen towards MGlc, EMGlc, BMGlc, OMGlc and

Table 1
Specific activity of purified β-glucosidases towards artificial substrates

| Substrate | Spec. act. (nmol/h per mg protein) | | |
|---|---|---|---|
| | Lysosomal glucocerebrosidase | Soluble broad-specificity β-glucosidase [a] | Non-lysosomal glucocerebrosidase [b] |
| MGlc | 2 300 000 | 77.3 | 4.9 |
| EMGlc | 48 300 | 1.6 | 0.25 |
| BMGlc | 22 800 | 0.8 | 0.17 |
| OMGlc | 13 200 | 0.2 | 0.10 |
| HMGlc | 2 200 | 0.05 | 0 |
| MGlc/EMGlc [c] | 47.6 | 48.0 | 19.6 |

[a] Activity was measured in the absence of TCh at pH 6.0.
[b] Activity was measured in the absence of TCh at pH 5.5.
[c] The ratio of specific activities.

Table 2
β-Glucosidase activities in human tissues and cell extracts

| Source | Spec. act. (nmol/h per mg protein) | | | | | |
|---|---|---|---|---|---|---|
| | MGlc | EMGlc | BMGlc | OMGlc | HMGlc | MGlc/EMGlc [a] |
| Liver | 47 | 8.0 | 3.8 | 1.7 | 0.06 | 5.9 |
| Kidney | 46 | 7.3 | 3.5 | 1.5 | 0.06 | 6.3 |
| Spleen | 10.5 | 1.9 | 0.5 | 0.3 | 0.01 | 5.5 |
| Fibroblasts | 130 | 12.3 | 4.6 | 2.5 | 0.7 | 10.6 |
| Leukocytes | 7.0 | 3.9 | 1.6 | 0.6 | 0.2 | 1.8 |

[a] The ratio of specific activities.

Table 3
β-Glucosidase activities in extracts of fibroblasts and spleens from control subjects and Gaucher disease patients

| Source | Spec. act. (nmol/h per mg protein) | | | | |
|---|---|---|---|---|---|
| | MGlc | EMGlc | BMGlc | OMGlc | HMGlc |
| Fibroblasts | | | | | |
| Control (5) mean | 124.6 | 12.0 | 4.3 | 2.1 | 0.6 |
| Range | 94.5–143.8 | 9.3–14.1 | 3.4–4.8 | 1.3–2.5 | 0.4–0.7 |
| Type 1 patient | 10.5 | 11.9 | 4.3 | 1.9 | 0.5 |
| Type 2 patient | 5.6 | 9.1 | 3.4 | 1.4 | 0.6 |
| Spleen | | | | | |
| Control (3) mean | 10.5 | 1.8 | 0.6 | 0.4 | 0.02 |
| Range | 9.8–11.3 | 1.4–2.2 | 0.4–0.7 | 0.3–0.6 | 0.01–0.03 |
| Type 1 patient | 2.0 | 1.5 | 0.8 | 0.6 | 0.03 |
| Type 2 patient | 1.3 | 1.7 | 0.7 | 0.4 | 0.02 |

HMGlc was investigated. Table 1 shows that lysosomal glucocerebrosidase, cytosolic broad-specificity β-glucosidase and, to a lesser extent, non-lysosomal glucocerebrosidase are able to hydrolyze MGlc much better than 6-acylamino-4-methylumbelliferyl-β-D-glucosides. The rate of hydrolysis of the best substrate from this series, EMGlc, by lysosomal glucocerebrosidase and by cytosolic broad-specificity β-glucosidase was approximately 50 times, and by non-lysosomal glucocerebrosidase, 20 times lower than that of MGlc. Moreover it was noted that 6-acylamino-4-methylumbelliferyl-β-D-glucosides were very poor inhibitors of MGlc hydrolysis by various β-glucosidases (not shown). It has to be concluded that the novel substrates are not an attractive alternative to MGlc for the measurement of activity of the above mentioned enzymes.

### 3.2. Comparison of the rates of hydrolysis of various fluorogenic substrates by homogenates of human tissues and cell types

The hydrolysis of different fluorogenic substrates by homogenates of various human tissues and cell types was investigated. As seen in Table 2, most materials exhibit a significant hydrolytic activity towards 6-acylamino-4-methylumbelliferyl-β-D-glucosides. In fact, the observed hydrolysis rates of these substrates by homogenates are higher than would be expected based on activities of the three known β-glucosidases (compare Tables 1 and 2). In this case the ratios of MGlc/EMGlc activities ranged from 2 to 11 depending on tissue or cell types. It should be noted, that the activity towards 6-acylamino-4-methylumbelliferyl-β-D-glucosides in all tissues and cell types investigated was not reduced in the presence of CBE (not shown).

These findings suggest the presence of another enzyme with β-glucosidase activity, further referred to as EMGlcase.

Table 4
Distribution of MGlc and EMGlc activities between particulate and soluble fraction of human kidney

| Fraction | Recovery of β-glucosidase activities | |
|---|---|---|
| | MGlc act. (%) | EMGlc act. (%) |
| Soluble | 39 | 92 |
| Particulate | 58 | 7 |

Human kidney was homogenized in 4 vol. (w/v) 50 mM potassium phosphate (pH 6.0), centrifuged for 1 h at 80000× *g* and supernatant collected. Membrane pellet was resuspended in the same buffer by sonication. Activities were measured as described in Section 2.

### 3.3. The hydrolysis of various fluorogenic substrates by enzyme preparations from Gaucher disease patients

The β-glucosidase activities towards MGlc and 6-acylamino-4-methylumbelliferyl-β-D-glucosides were determined in homogenates of skin fibroblasts and spleens from normal subjects and patients with different phenotypes of Gaucher disease. Table 3 shows that in all homogenates from Gaucher disease patients the activity towards MGlc was severely reduced, being always less than 20% of control values. The rate of hydrolysis of EMGlc, as well as other 6-acylamino-4-methylumbelliferyl-β-D-glucosides, by homogenates from Gaucher disease patients was not significantly reduced irrespective of clinical phenotype of the disorder.

### 3.4. Comparison of distribution of MGlc-ase and EMGlc-ase activities after ultracentrifugation

Table 4 shows the distribution of MGlc-ase and EMGlc-ase activities in the 80 000 × *g* supernatant and in particulate fraction, derived from human kidney homogenate. The results indicate that the activity towards EMGlc is predominantly recovered in the soluble protein fraction, whereas about 60% of MGlc-ase activity is present in particulate fraction.

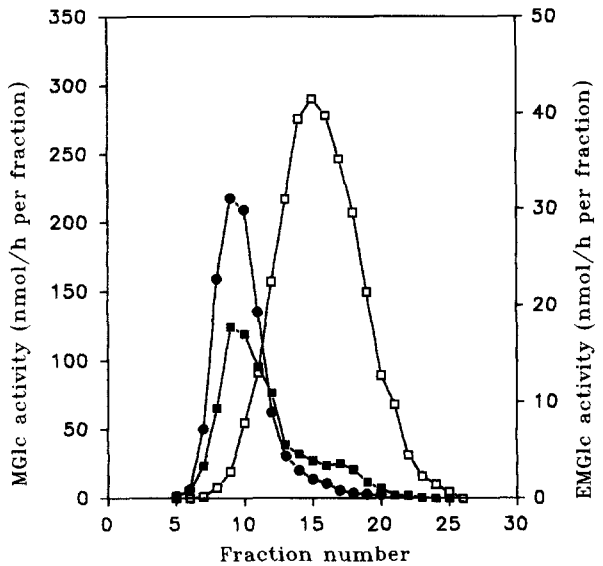


Fig. 1. Elution profiles of the β-glucosidase activities in an extract of human kidney on Sephadex G-100. Bed dimensions: 1×40 cm, eluent: 0.1 M citrate buffer, pH 6.0. Activities of β-glucosidases were measured as follows: lysosomal glucocerebrosidase (■) as CBE-sensitive hydrolysis of MGlc in the presence of 0.6% (w/v) TCh; soluble broad-specificity β-glucosidase (□) as CBE-insensitive hydrolysis of MGlc without further additions; EMGlc-ase (●) as CBE-insensitive hydrolysis of EMGlc in the presence of 0.6% (w/v) TCh.

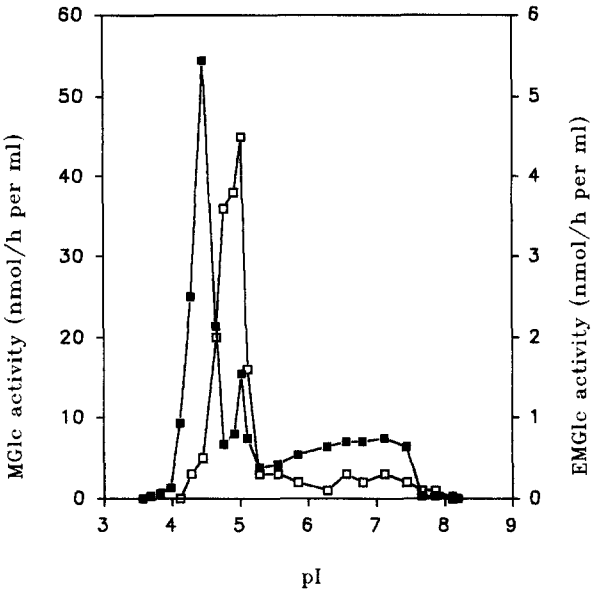


Fig. 2. Isoelectric focusing of β-glucosidases from human kidney. Activities were measured with MGlc (■) and with EMGlc (□) in the presence of 0.6% (w/v) TCh.

### 3.5. Gel filtration

A soluble protein fraction prepared from human kidney was subjected to Sephadex G-100 gel filtration. Fig. 1 shows that EMGlc-ase activity was separated from soluble broad-specificity β-glucosidase activity but not from lysosomal glucocerebrosidase activity (both enzymes showing an apparent molecular mass of about 120–140 kDa).

### 3.6. Isoelectric focusing

A soluble protein fraction prepared from human kidney was subjected to isoelectric focusing as described in Section 2. After fractionation of the gel, the β-glucosidase activities in the individual fractions were determined with MGlc and EMGlc as substrates (Fig. 2). EMGlc-ase activity shows a discrete p*I* of about 5.0, whereas the MGlc-ase activity profile, reflecting the distribution of soluble broad-specificity β-glucosidase and lysosomal glucocerebrosidase, is very heterogeneous in isoelectric point (with p*I* values ranging from 4.0 to 7.6).

### 3.7. Concanavalin A-Sepharose chromatography

EMGlc-ase from human kidney, was separated from glucocerebrosidase and soluble broad-specificity β-glucosidase by chromatography on Con A-Sepharose (Fig. 3). Tissue was homogenized in 4 vols. of 50 mM acetate

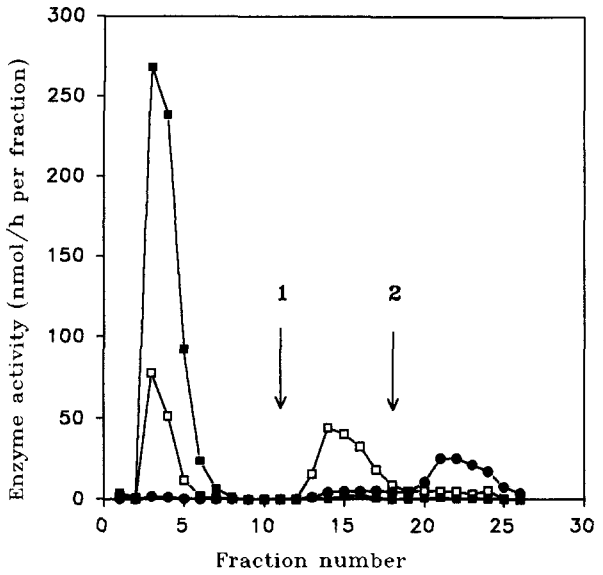


Fig. 3. Chromatography β-glucosidases from human kidney on Con A-Sepharose 4B. Activities of β-glucosidases were measured with MGlc without (■) or with (□) 0.6% (w/v) TCh and with EMGlc in the presence of 0.6% (w/v) TCh (●). At the points indicated by arrows 1 and 2 elution was started with buffer A, containing 0.1 M and 0.4 M α-D-methylmannoside, respectively.

buffer (pH 5.3), containing 0.9% NaCl (buffer A) and the homogenate was subjected to ultracentrifugation. The soluble fraction was applied to a Con A-Sepharose column. The EMGlc-ase activity was completely bound. Next, the column was washed with buffer A until no β-glucosidase activity was detected in the eluant. Bound glucocerebrosidase activity was then eluted with this buffer, containing 0.1 M α-D-methylmannoside (fractions 13–18). EMGlc-ase activity was eluted with the same buffer containing 0.4 M α-D-methylmannoside. Fractions with EMGlc-ase activity were pooled (fractions 20–25), incubated for 30 min with 5 mM CBE to eliminate the residual glucocerebrosidase activity and used in subsequent experiments to study EMGlc-ase properties. Control experiments show that α-D-methylmannoside in any concentrations used has no effect on EMGlc-ase activity.

The possibility cannot be excluded that a small amount (1–2%) of an unidentified impurity in the EMGlc preparation is a substrate for a hypothetical glycosidase X differing from EMGlc-ase. Then the activity of fractions 20–25 would be associated with glycosidase X rather than with our enzyme. If this is the case then the amount of produced aglycon would not exceed 1–2% of the substrate added irrespective of incubation time. To exclude this possibility additional experiments were carried out and it was found that fractions 20–25 was able to hydrolyze up to 50% of the substrate, indicating that just EMGlc was hydrolyzed by this enzyme preparation.

### 3.8. pH optimum and effect of detergents

The effect of pH on EMGlc-ase activity (fractions 20–25) was investigated. As seen in Fig. 4, the enzyme has a pH optimum at pH 4.5 when activity was determined in the absence of detergents. In the presence of 0.6% (w/v) TCh, the enzyme activity maximum was shifted to pH 5.0.

The effect of TCh and Triton X-100 on EMGlc-ase activity was examined at pH 5.0. Fig. 5 shows that EMGlc-ase activity is strongly stimulated by TCh. Maximal stimulation, representing a threefold increase in activity, occurred at detergent concentrations 0.6–0.7% (w/v). Triton X-100 did not produce appreciable stimulation of EMGlc-ase activity at any of the concentrations studied.

### 3.9. Substrate specificity and inhibitor sensitivity

The substrate specificity of EMGlc-ase (fractions 20–25) is shown in Table 5. The activity of the enzyme towards EMGlc was about 20-fold higher as compared to MGlc. With elongation of the fatty acid's acyl chain length from 2 to 16 carbon atoms the rate of hydrolysis of 6-acylamino-4-methylumbelliferyl-β-D-glucosides was significantly decreased. The enzyme is not active towards glucosylceramide (data not shown).

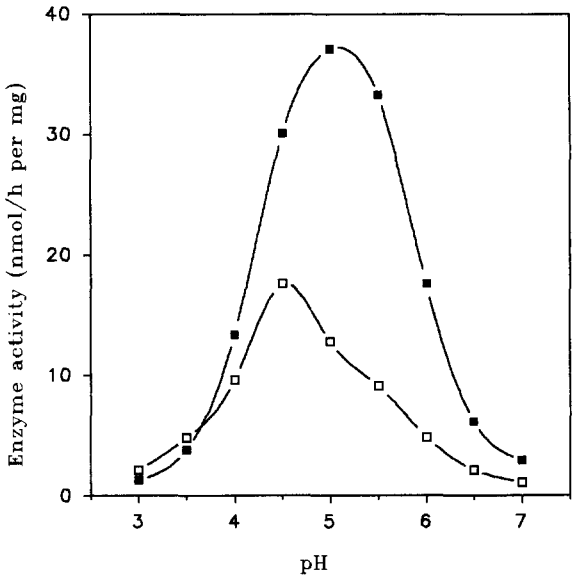


Fig. 4. pH-Profile of EMGlc-ase activity. EMGlc-ase activity was measured as described in Section 2 without further additions (□) and with 0.6% (w/v) TCh (■).

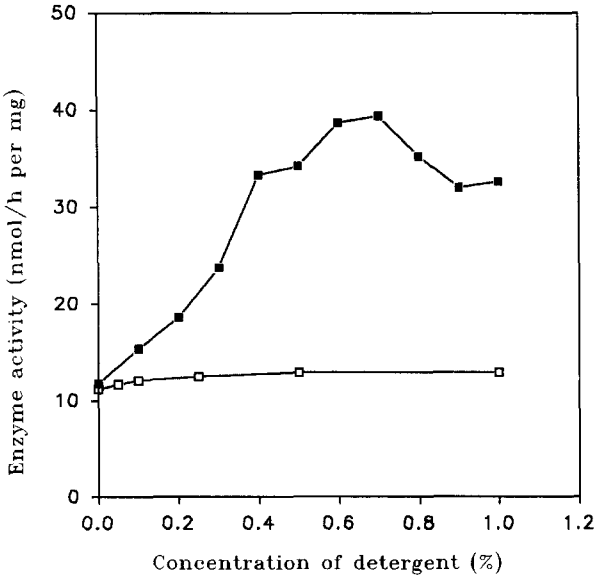


Fig. 5. Effect of TCh (■) and Triton X-100 (□) at varying concentrations on EMGlc-ase activity.

The effect of various inhibitors of β-glucosidases on EMGlc-ase was investigated. As shown in Table 6, castanospermine and deoxynojirimycin, which are known to be potent competitive inhibitors of lysosomal glucocerebrosidase [24,25], are much less effective with EMGlc-ase. methyl-D-Glucoside, methyl-D-xyloside and D-(1,5)-gluconolactone have no effect on EMGlc-ase activity. Besides, this activity is not inhibited by CBE.

### 3.10. Subcellular localization of EMGlc-ase

The subcellular localization of EMGlc-ase and other β-glucosidases was determined by Percoll density gradient fractionation. For practical reasons, rat liver was used. Fig. 6 shows that distribution of EMGlc-ase activity is similar to the distribution of lysosomal β-hexosaminidase and glucocerebrosidase while the non-lysosomal glucocerebrosidase is localized in compartments with a considerably lower density.

Table 5
Hydrolysis of various fluorogenic glucosides by EMGlc-ase from human kidney

| Substrate | Spec. act. (nmol/h per mg protein) |
|---|---|
| MGlc | 1.6 |
| EMGlc | 39.6 |
| BMGlc | 15.8 |
| OMGlc | 5.9 |
| HMGlc | 2.0 |

Table 6
Effect of various inhibitors on EMGlc-ase from human kidney

| Addition | Final concn. (mM) | EMGlc-ase activity (% of control) |
|---|---|---|
| CBE | 5 | 99.8 |
| methyl-D-Xyloside | 50 | 97.9 |
| methyl-D-Glucoside | 50 | 108.5 |
| D-(1,5)-Gluconolactone | 50 | 114.9 |
| Castanospermine | 5 | 56.7 |
| Deoxynojirimycin | 5 | 66.0 |

## 4. Discussion

The results obtained show that 6-acylamino-4-methylumbelliferyl-β-D-glucosides are unattractive substrates for the detection of lysosomal and non-lysosomal glucocere-

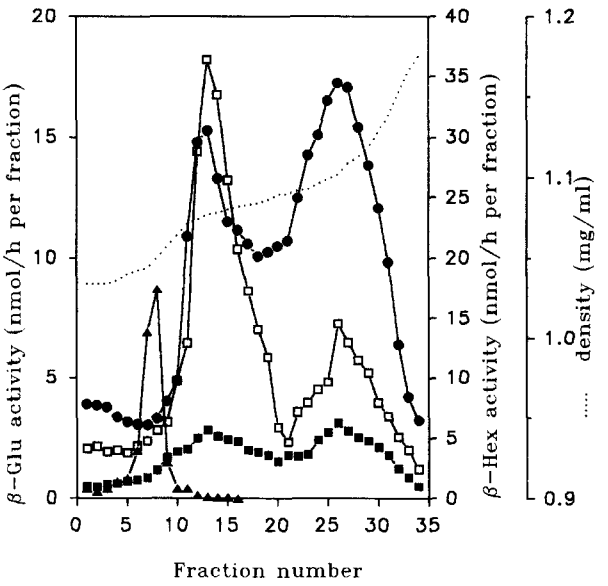


Fig. 6. Subcellular distribution of β-glucosidase activities in rat liver. The enzyme activities were measured as follows: lysosomal glucocerebrosidase (□) as CBE-sensitive hydrolysis of MGlc in the presence of 0.6% (w/v) TCh and 0.1% (v/v) Triton X-100; non-lysosomal glucocerebrosidase (▲) as CBE-insensitive hydrolysis of MGlc without further additions; EMGlc-ase (■) as CBE-insensitive hydrolysis of EMGlc in the presence of 0.6% (w/v) TCh and 0.1% (v/v) Triton X-100; hexosaminidase (●) as hydrolysis of MGlcNAc in the presence 0.1% (v/v) Triton X-100.

Table 7
Comparison of properties of different β-glucosidases

| Parameter | Lysosomal glucocerbrosidase | Non-lysosomal glucocerebrosidase | Cytosolic broad-specificity β-glucosidase | EMGlc-ase |
|---|---|---|---|---|
| Hydrolysis of: | | | | |
| glucosylceramide | yes | yes | no | no |
| MGlc | yes | yes | yes | no |
| EMGlc | no | no | no | yes |
| Deficient in Gaucher disease | yes | no | no | no |
| Inhibition by: | | | | |
| CBE | yes | no | no | no |
| D-(1,5)-gluconolatone | weak [15] | strong [9] | moderate [15] | no |
| deoxynojirimycin | strong | moderate [9] | unknown | weak |
| castanospermine | strong | moderate [9] | strong [29] | weak |
| Effect of TCh | activate | inhibit | inhibit | activate |
| Bound by Con A | yes | unknown | no | yes |
| pH optimum | 4.5 | 5.5 | 6.0 | 4.5 |
| Isoelectric point | heterogeneous (3.5–7.0) | unknown | 4.6 | 5.0 |
| Subcellular localization | lysosomal | non-lysosomal | cytosolic | lysosomal |

brosidases as well as for cytosolic broad-specificity β-glucosidase. The low reactivity of lysosomal glucocerebrosidase towards these substrates is somewhat surprising, since it is known that their chromogenic analog, 2-hexadecanoylamino-4-nitrophenyl-β-D-glucoside, is a good substrate for this enzyme [26,27]. Moreover, 6-acylamino-4-methylumbelliferyl-β-D-galactosides with higher acyl-chain lengths are found to be good substrates for the lysosomal galactocerebrosidase [20,28].

The use of 6-acylamino-4-methylumbelliferyl-β-D-glucosides led us to the identification of a hitherto undescribed β-glucosidase isozyme. The enzyme is clearly distinct in properties from the known β-glucosidases (see Table 7). It is a soluble lysosomal enzyme with a discrete p*I* of 5.0. It is not deficient in Gaucher disease, is not inhibited by CBE and by gluconolactone and is activated by sodium taurocholate. Its substrate specificity is remarkable, showing high affinity to 6-ethanoylamino-4-methylumbelliferyl-β-D-glucoside but decreasing affinity to 6-acylamino-4-methylumbelliferyl-β-D-glucosides with higher acyl chain length and low affinity to 4-methylumbelliferyl-β-D-glucoside. It was impossible to demonstrate the glucocerebrosidase activity for the enzyme. The physiological substrate for the enzyme, that has been shown to be present in all tissues and cell types investigated, is therefore completely unknown at the moment. High affinity to Con A suggests that the enzyme is a glycoprotein. Purification of the enzyme to homogeneity and production of specific antibodies seem warranted to obtain a better insight into its precise composition and clues concerning its physiological function.

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