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# Stabilising normal and mis-sense variant α-glucosidase

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**Abstract** **α-Glucosidase (EC 3.2.1.3) is a lysosomal enzyme that hydrolyses α-1,4- and α-1,6-linkages of glycogen to produce free glucose. A deficiency in α-glucosidase activity results in glycogen storage disorder type II (GSD II), also called Pompe disease. Here, D-glucose was shown to be a competitive inhibitor of α-glucosidase and when added to culture medium at 6.0 g/L increased the production of this protein by CHO-K1 expression cells and stabilised the enzyme activity. D-Glucose also prevented α-glucosidase aggregation/precipitation and increased protein yield in a modified purification scheme. In fibroblast cells, from adult-onset GSD II patients, D-glucose increased the residual level of α-glucosidase activity, suggesting that a structural analogue of D-glucose may be used for enzyme enhancement therapy.**


*Keywords:* α-Glucosidase; Enzyme stability; Pompe disease; Enzyme enhancement

## 1. Introduction

Lysosomal α-glucosidase (EC 3.2.1.3) is responsible for hydrolysing α-1,4- and α-1,6-linkages of glycogen, releasing free glucose [1]. A deficiency in lysosomal α-glucosidase results in glycogen storage disorder type II (GSD II, also known as Pompe disease or acid maltase deficiency) [2]. This reduction or loss of α-glucosidase activity results in the lysosomal accumulation of glycogen in various cell types, with myocytes of cardiac, respiratory and skeletal muscles most severely affected [3]. GSD II patients present within a spectrum of clinical phenotypes, ranging from the severe, rapidly progressive infantile-onset form with cardiac involvement, to a less progressive adult-onset form without cardiac symptoms. The clinical phenotype of GSD II is thought to be primarily determined by the nature of the variant alleles and the amount of residual enzyme activity [4]. Infantile-onset GSD II patients tend to have little or no detectable α-glucosidase activity, while the residual α-glucosidase activity in adult-onset patients is between 7% and 22% of normal controls [5,6].

Enzyme replacement therapy (ERT) using recombinant human α-glucosidase has been evaluated for the treatment of GSD II in human clinical trials [7–10]. Large quantities of α-glucosidase (up to 40 mg/kg) were required for this ERT to be effective in GSD II patients [7–10]. The need for such high amounts of recombinant human α-glucosidase has necessitated efficient overexpression systems [11,12] and led to a number of methods for enzyme purification to improve yields [13]. However, problems have been encountered with α-glucosidase aggregation and precipitation during purification [13] (www.worldpompe.org./synpac.html).

The inability to completely clear lysosomal glycogen storage in ERT-treated GSD II patients, despite high doses of α-glucosidase, has stimulated interest in alternative therapies. Another potential approach to treat GSD II is enzyme enhancement therapy (EET, or chemical chaperone therapy), which has recently been investigated for other LSD (Gaucher disease/β-glucosidase [14], MPS IVB/β-galactosidase [15]; Fabry disease/α-galactosidase A [16,17]; Tay-Sachs and Sandhoff diseases/β-hexosaminidase [18]). This therapy is based on the ability of chemical reagents/active site inhibitors to stabilise the variant protein in patient cells [19]. Protein stabilisers are thought to assist the folding of variant protein within the rough endoplasmic reticulum, increasing the amount of protein that passes the quality control process, thereby enhancing the residual enzyme activity in affected cells. EET could be used to stabilise the mis-sense variant α-glucosidase in GSD II patients, particularly in adult-onset patients who have significant residual enzyme activity. This therapy would aim to shift the dynamic balance between the residual enzyme activity and substrate turnover in the patient [20], to reduce the level of substrate accumulation below the critical threshold and avoid the onset of pathogenesis.

D-Glucose is the product of α-glucosidase glycogen hydrolysis and is an inhibitor of the enzyme. It was hypothesised that D-glucose could interact with the α-glucosidase catalytic site to improve the stability of both normal and mis-sense variant α-glucosidase protein. In this study we have investigated the ability of D-glucose to increase α-glucosidase production in CHO-K1 expression cells, to improve purification yields of the recombinant protein from culture medium and to increase the amount of α-glucosidase in adult-onset GSD II patient fibroblasts.

## 2. Materials and methods

### 2.1. Materials

Non-protein binding microtitre plates (96-well, flat bottom) were purchased from Interpath Services (Sydney, NSW, Australia). Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, USA). The polyclonal antibody to α-glucosidase was raised against

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purified recombinant protein and affinity-purified as previously described [21]. The α-glucosidase, α-L-iduronidase and 4-sulphatase CHO-K1 expression cell lines were as previously described [11,22,23]. Anhydrous D-glucose, anhydrous D-galactose and sucrose were from Ajax Chemicals (Auburn, NSW, Australia). α-MEM was purchased from Gibco BRL Life Technologies Inc (Grand Island, NY, USA), and BME from ICN Biochemicals Inc. (Aurora, OH, USA). Foetal calf serum and trypsin-versene were purchased from JRH Biosciences (Lenexa, KS, USA). Sephadex-G100 beads for gel filtration were purchased from Sigma and pre-swollen concanavalin A–Sepharose was purchased from Amersham Pharmacia Biotechnology (Uppsala, Sweden). Ultrafiltration membranes YM10 were purchased from Millipore Corporation (Bedford, MA, USA). All other reagents were purchased from Sigma.

### 2.2. Determination of enzyme activities

α-Glucosidase, α-L-iduronidase and 4-sulphatase activities were measured using the fluorogenic substrates 4-methylumbelliferyl α-D-glucoside, 4-methylumbelliferyl-iduronide and 4-methylumbelliferyl sulphate, as previously described [24–26]. Kinetic experiments with the fluorogenic substrates used 0–5 mM of either 4-methylumbelliferyl α-D-glucoside or 4-methylumbelliferyl-iduronide, in the presence of either 0, 6, 25 or 50 g/L D-glucose (0, 33, 139, 278 mM). Lineweaver–Burk plots for both α-glucosidase and α-L-iduronidase were used to determine the Michaelis–Menten constant ($K_m$) and maximum velocity of catalysis ($V_{max}$) for each condition. The inhibitory constant ($K_i$) value was calculated from plots of slope (from the Lineweaver–Burk plots) versus D-glucose inhibitor concentration.

### 2.3. α-Glucosidase purification

Purification of precursor α-glucosidase from culture medium was conducted as previously described for human urine [27] and adapted for CHO-K1 culture medium [11]. For the modified procedure developed here, an equal volume of 50 mM sodium acetate, 20 mM sodium phosphate, 1 M NaCl, pH 5.2 (A-P buffer, as described in [13]) was added to clarified tissue culture medium to stabilise the α-glucosidase activity. This was loaded (2 L) onto a 10 mL concanavalin A–Sepharose column and run under gravity at 4 °C. The column was washed with 50 mL of A-P buffer and eluted with 15 mL of the same buffer containing 1 M methyl-α-D-glucopyranoside. Elution was maximised by circulating this eluate over the column three times and then rinsing the column with 5 mL of fresh elution buffer. The concanavalin-A eluate was adjusted to 125 g/L D-glucose and 0.1% (v/v) Tween 20 then concentrated with a YM10 membrane that had been pre-soaked in 0.1% (v/v) Tween 20 in distilled water. The concentrated eluate was applied to a Sephadex G-100 column (50 × 1.5 cm) in running buffer (20 mM sodium acetate, pH 4.6, containing 25 mM NaCl). The eluate was assayed for α-glucosidase and the active fractions pooled and concentrated five-fold. The concentrated eluate was dialysed against PBS and filtered through a MILLEX-GV® 0.22 μm sterile filter unit that had been pre-soaked in 0.1% (w/v) Tween 20 in distilled water and assayed for protein by the Lowry method [28].

### 2.4. Determination of α-glucosidase protein in GSD II patient skin fibroblasts

Confluent skin fibroblast cells were treated either with (6 g/L) or without D-glucose for three days then harvested and cell lysates prepared by sonication. Cell lysates were evaluated for α-glucosidase protein using a Luminex bead system (BioRad, USA). Wells of a 96-well microtitre filter plate were pre-wet with 100 μL of assay buffer (0.05% (v/v) Tween 20, 0.5% (w/v) BSA, 0.05% (w/v) γ-globulin in 0.01 M $NaH_2PO_4$/NaOH, pH 7.4, with 138 mM NaCl plus 0.05% (w/v) sodium azide) and the buffer was then suctioned off before the addition of 50 μL α-glucosidase polyclonal antibody-coupled beads (5000 beads, coupled according to the manufacturer's instructions; BioRad, USA), to each well. The buffer was suctioned off and then either α-glucosidase standards serially diluted in assay buffer were added (0–1000 pg in 100 μL, assayed in duplicate) or 100 μL of assay buffer added as blanks or GSD II patient skin fibroblast cell lysates (2 μL in 100 μL of assay buffer) added to the microtitre wells. The α-glucosidase reporter antibody (biotinylated: Biotinylation of antibodies was performed with a FluoReporter®Biotin-XX Labeling Kit according to the manufacturer's instructions; Molecular Probes Inc. Eugene, OR, USA) was diluted in assay buffer (320 μg/L) and 50 μL added to each well. The plates were then covered in foil and shaken at room temperature (150 rpm for 1 h on the Thermoline Orbital Shaker; Thermoline Scientific Equipment Pty. Ltd, Australia) and then incubated overnight at 4 °C. Wells were allowed to reach room temperature by shaking at 150 rpm for 1 h and then washed three times with 100 μL of wash buffer (0.01 M $NaH_2PO_4$/NaOH, pH 7.4, with 138 mM NaCl, containing 0.05% (w/v) Tween 20). The buffer was suctioned off and 125 μL of streptavidin phycoerythrin (1.2 mg/L in assay buffer) was added at 50 ng/well (400 μg/L), then the plate sealed and shaken at 150 rpm for 10 min at room temperature. The plate was uncovered and fluorescence measured on a Bio-Plex array system (Bio-Rad, USA). All results were interpolated through a standard curve to calculate the concentration of α-glucosidase.

*2.4.1. GSD II patients.* Infantile-onset ($n = 6$) and adult-onset ($n = 4$) GSD II patient skin fibroblasts were from patient referrals to the National Referral Laboratory for Lysosomal, Peroxisomal and Related Genetic Disorders, within the Department of Genetic Medicine, Children Youth and Women's Health Service, Adelaide, Australia. The unaffected control fibroblasts were from healthy human donors. All fibroblast samples were de-identified in accordance with human ethics approval and administered by an independent operator (Dr. Michael Fietz, Head of the National Referral Laboratory).

## 3. Results

### 3.1. The effect of D-glucose concentration on α-glucosidase expression in CHO-K1 cells

The expression of α-glucosidase in α-MEM (1.0 g/L D-glucose) resulted in an initial increase in α-glucosidase activity in the culture medium for up to 24 h of culture, but this was followed by a progressive loss of activity for additional culture time points (Fig. 1). The addition of extra D-glucose to α-MEM (3.1, 4.0 and 6.0 g/L final concentrations) resulted in a concentration-dependent increase in α-glucosidase activity over a 72 h time course. Following the 72 h time point, a plateau in α-glucosidase activity was observed for the 3.1 g/L D-glucose treated culture. However, the 4.0 and 6.0 g/L D-glucose treated cultures continued to increase in α-glucosidase activity for up to 144 h. The increase in α-glucosidase activity in these culture media was associated with a similar increase in the

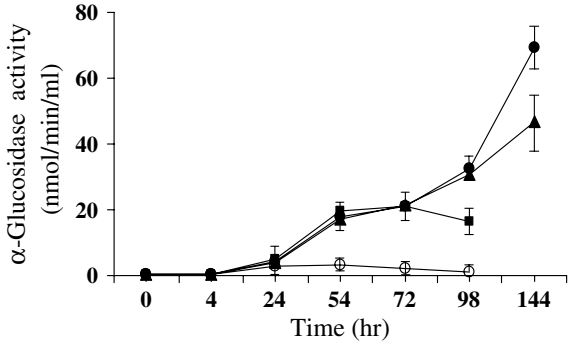


Fig. 1. The effect of D-glucose on α-glucosidase activity in culture medium from CHO-K1 expression cells. CHO-K1 cells expressing α-glucosidase were cultured in either α-MEM (○, 1.0 g/L D-glucose), or in α-MEM supplemented with a final concentration of either 3.1 g/L (■), or 4.0 g/L (▲) or 6.0 g/L (●) of D-glucose. CHO-K1 cells were cultured in the latter conditions for up to 144 h ($n = 6$ per treatment group). The results showed a significant difference for the α-MEM versus glucose supplemented treatment groups at 54, 72 and 98 h time points ($P < 0.001$, $t$-test). The 6.0 g/L treatment showed a significant difference from the 3.1 g/L treatment at 98 h ($P < 0.001$, $t$-test) and the 4.0 g/L treatment at 144 h ($P < 0.001$, $t$-test). The total cell protein for the CHO-K1 cell extracts was 5.0 ± 0.3 mg/mL.

amount of α-glucosidase protein, with mainly precursor α-glucosidase and minor amounts of mature form, evident by immune detection (data not shown).

### 3.2. Effect of sugar and butyric acid on α-glucosidase, α-L-iduronidase and 4-sulphatase expression in CHO-K1 cells

The specificity of the effect of D-glucose on α-glucosidase production was examined using either different saccharides or energy sources and compared to other expression systems. D-Glucose, D-galactose (another monosaccharide), sucrose (a disaccharide) and butyric acid (a non-sugar energy source) were investigated for their effect on CHO-K1 cells expressing either α-glucosidase, α-L-iduronidase or 4-sulphatase (Fig. 2). For α-glucosidase, all of the treatment groups showed an increase in enzyme activity in each culture medium for up to 72 h of culture (Fig. 2A). However, the amount of α-glucosidase activity was higher with D-glucose (6.0 g/L) and D-galactose (6.0 g/L) suggesting an increase in production (synthesis/secretion into the medium) for these two treatment groups. Following this time point only the cultures treated with either D-glucose (6.0 g/L) or D-galactose (6.0 g/L) showed an increase in α-glucosidase activity, whereas the other treatment groups showed a progressive decline in α-glucosidase activity. The reduced activity in the α-MEM, sucrose and butyric acid-treated CHO-K1 cultures indicated a problem with α-glucosidase stability, but this was less evident in the culture treated with D-galactose (6.0 g/L) and not evident in the culture treated with D-glucose (6.0 g/L; Fig. 2A).

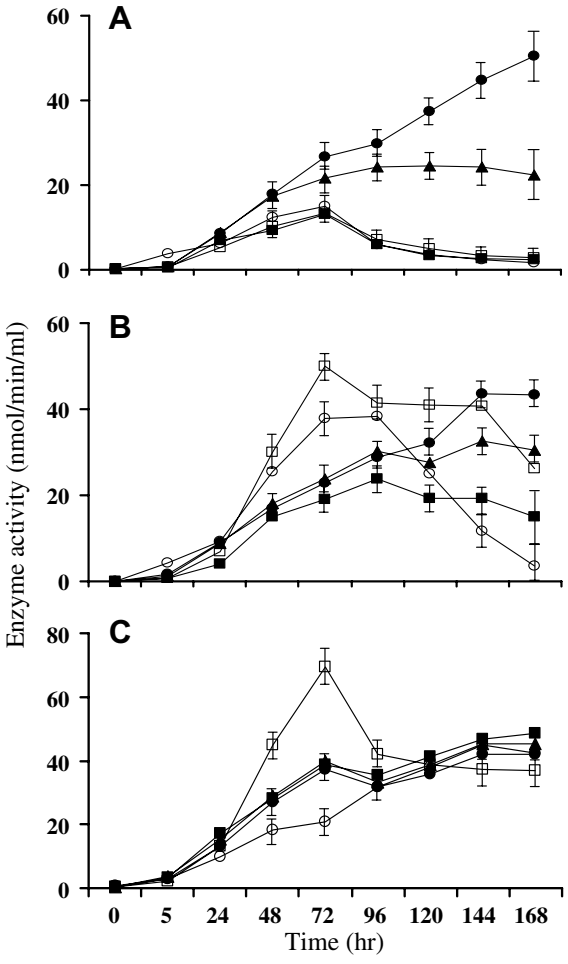


Fig. 2. The effect of either different saccharides or energy sources on lysosomal enzyme expression in CHO-K1 cells. CHO-K1 cells expressing either α-glucosidase (A) α-L-iduronidase (B) and 4-sulphatase (C) were cultured in either α-MEM (○) or α-MEM containing either 6.0 g/L of D-glucose (●), 6.0 g/L D-galactose (▲), 6.0 g/L sucrose (■) or 0.1 M butyric acid (□). CHO-K1 cells were cultured in the latter conditions for up to 168 h ($n = 3$ per treatment group). All points where error bars showed no overlap were significantly different ($P < 0.001$, $t$-test). The total cell protein for the CHO-K1 cell extracts was 4.4 ± 0.3 mg/mL. The apparently lower α-glucosidase expression in the figure compared to Fig. 1 was due to a lower number of expression cells as indicated by the total protein in the cell extracts.

The effect of D-glucose and D-galactose on the amount of α-glucosidase produced by CHO-K1 expression cells (Fig. 2A) was not observed for CHO-K1 cells expressing α-L-iduronidase (Fig. 2B). Instead, the addition of either D-glucose, D-galactose or sucrose resulted in a lower amount of α-L-iduronidase activity when compared to the control medium (Fig. 2B). Butyric acid resulted in an increase in the level of α-L-iduronidase but this was not stable after 72 h and showed a decline of α-L-iduronidase comparable to control medium. However, there was a sugar-dependent effect on α-L-iduronidase stability in long-term 168 h cultures, with 6.0 g/L D-glucose then 6.0 g/L D-galactose having the most effect (Fig. 2B). The stability effect observed for 6.0 g/L D-glucose and 6.0 g/L D-galactose on both α-glucosidase (Fig. 2A) and α-L-iduronidase (Fig. 2B) was not evident for 4-sulphatase (Fig. 2C), despite a slight increase in the production of 4-sulphatase with either D-glucose, or D-galactose or sucrose addition (Fig. 2C). This slight increase in 4-sulphatase production with sugar addition was less than that observed for butyric acid.

### 3.3. Enzyme kinetic analysis of α-glucosidase and α-L-iduronidase

Inhibition experiments showed that D-glucose was a relatively weak inhibitor of α-glucosidase ($K_i = 44$ mM, Fig. 3A). Lineweaver–Burk plots showed that the $K_m$ and $V_{max}$ values for recombinant α-glucosidase (purified α-glucosidase from CHO-K1 culture medium) were 2.4 mM and 5 nmol/min, respectively, with the 4-methylumbelliferyl α-glucoside substrate (Fig. 3B). In the presence of D-glucose, the $K_m$ for α-glucosidase increased in proportion to the concentration of D-glucose, but the $V_{max}$ remained constant. This indicated that D-glucose was acting as a competitive inhibitor of α-glucosidase (Fig. 3B). The inhibitory constant ($K_i$) value was calculated as 45 mM from plots of slope versus D-glucose inhibitor concentration (from the Lineweaver–Burk plots, data not shown). The $V_{max}$ (47.6 nmol/min) and $K_m$ (0.14 mM) values for α-L-iduronidase (using the 4-methylumbelliferyl-iduronide substrate) were both altered by the addition of D-glucose, indicating that it was acting as an uncompetitive inhibitor (Fig. 3C).

### 3.4. Improved yield of recombinant α-glucosidase from CHO-K1 cell cultures with D-glucose

Purification of α-glucosidase from CHO-K1 cell culture using a previously reported method [11] resulted in a visible precipitate during concentration and gel filtration, leading to purification yields of around 10% (Table 1A). The addition of 128 mg/mL of D-glucose (almost completely inhibiting α-glucosidase, Fig. 3) to the concanavalin A column eluate, prior to concentration and loading onto the Sephadex G-100 gel filtration column, resulted in the elimination of the previously

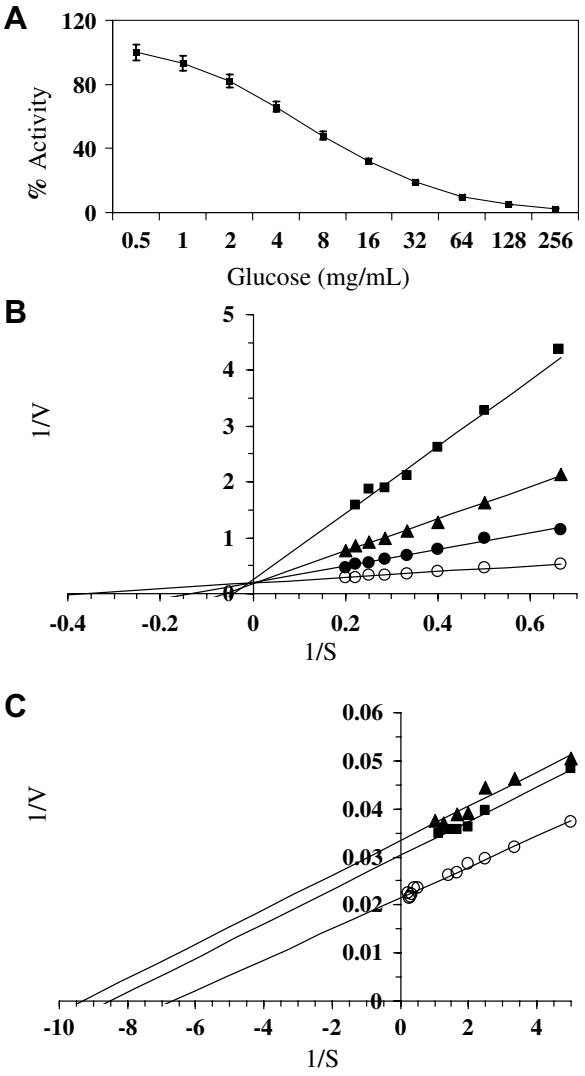


Fig. 3. Kinetics analysis of α-glucosidase and α-L-iduronidase in the presence of D-glucose. α-Glucosidase enzyme inhibition curve for D-glucose ($n = 3$, A) and Lineweaver–Burk plots of α-glucosidase (B) and α-L-iduronidase (C) in the presence of the inhibitor D-glucose at concentrations of either 0 (○), 6.0 (●), 25 (▲) or 50 (■) g/L. The $K_i$ for the D-glucose inhibition of α-glucosidase was 44 mM from the inhibition curve and 45 mM from the Lineweaver–Burk plots.

visible precipitate and improved the yield (Table 1B). Yields were also improved by using A-P buffer in the culture medium (Table 1B), as previously reported by Van Hove and colleagues [13]. Recirculation of the methyl-glucopyranoside elution buffer over the concanavalin A column increased α-glucosidase recovery from the column. The modified purification protocol resulted in a 3.5-fold increase in final yield when compared to the previously reported method (Table 1). Immune analysis of purified α-glucosidase indicated the presence of predominantly precursor α-glucosidase (110 kDa) with minor quantities of the 95 and 76 kDa mature forms (data not shown).

### 3.5. Effect of D-glucose on α-glucosidase activity in GSD II patient skin fibroblasts

Either BME or BME supplemented with D-glucose (6.0 g/L) was added to confluent GSD II skin fibroblasts and incubated for 72 h. An increase in the amount of α-glucosidase protein was observed for normal control and adult-onset GSD II patient skin fibroblasts treated with 6.0 g/L D-glucose (Fig. 4), when compared to the control BME medium. The increase in α-glucosidase protein corresponded to a similar increase in α-glucosidase activity (data not shown). In contrast, only two of the six infantile-onset GSD II patient cell lines showed a detectable increase in α-glucosidase protein with D-glucose treatment (Fig. 4), but this protein had no detectable activity. In all of the fibroblasts tested (GSD II and normal control), four other lysosomal markers β-glucuronidase, Saposin C, Lamp 1 and iduronate-2-sulphatase showed either no change or a slight reduction in protein after D-glucose treatment (data not shown).

## 4. Discussion

Effective ERT in GSD II patients requires extremely large doses of recombinant α-glucosidase [7–10]. The purification of recombinant human α-glucosidase from CHO-K1 cell culture medium has addressed this need, but there have been problems with aggregation and precipitation of the purified protein [13] (www.worldpompe.org./synpac.html). This led to the investigation of a strategy to stabilise the α-glucosidase catalytic site using substrate product, with the aim of increasing the yield of α-glucosidase protein from expression cells and in an improved purification scheme.

D-Glucose is the product of α-glucosidase hydrolysis and was shown to be a competitive inhibitor, which specifically interacted with the catalytic site of α-glucosidase. D-Glucose at a concentration of 6.0 g/L (33 mM) was able to dramatically increase the production (synthesis/secretion) of α-glucosidase in CHO-K1 expression medium. The concentration of D-glucose, at which this biological effect was observed, was similar to the $K_i$ for α-glucosidase (45 mM), consistent with the D-glucose effect being mediated by binding to the active site of α-glucosidase. In the presence of 6.0 g/L D-glucose or D-galactose, α-glucosidase activity in the culture medium from CHO-K1 cells was maintained for more than 72 h after the initial treatment; but in the absence of these monosaccharides, a progressive drop in α-glucosidase activity was observed following this time point. The increased production of α-glucosidase in the presence of these monosaccharides suggested that either they provided a better energy source for protein expression or possibly improved the folding/yield of α-glucosidase in the biosynthetic compartment. However, preventing the loss of α-glucosidase activity for cultures that were extended past 72 h, indicated a stability effect and this was most evident for D-glucose treatment. The D-glucose stability effect was not observed for 4-sulphatase, and only partially evident for another glycosidase α-L-iduronidase.

A previous study reported increased production and stabilisation of α-glucosidase in the presence of D-glucose and butyrate [12], which was explained by a decrease in pH of culture conditions resulting from the increase in lactic acid production. Here, this stabilisation of α-glucosidase was shown to be most likely due to D-glucose rather than butyrate. D-Glucose also prevented aggregation and precipitation of α-glucosidase during purification from CHO-K1 cell culture medium and improved the yield compared to a previously described

Table 1
Purification of α-glucosidase

| Sample | Activity (nmol/min/ml) | α-Glucosidase (μg/ml) | Volume (ml) | Total α-glucosidase (μg) | Yield (%) |
|---|---|---|---|---|---|
| *A* | | | | | |
| Media | 51.04 | 85.07 | 55.00 | 4678.85 | 100.00 |
| Con A flowthrough | 6.50 | 10.83 | 54.00 | 584.98 | 12.50 |
| Concentrated eluate | 450.00 | 750.00 | 3.00 | 2250.00 | 48.10 |
| Sephadex eluate | 29.85 | 49.75 | 18 | 895.50 | 19.10 |
| Concentrated eluate | 98.20 | 163.67 | 2.80 | 458.28 | 9.80 |
| *B* | | | | | |
| Media | 6.69 | 11.20 | 1000.00 | 11200.00 | 100.00 |
| Media + AP buffer | 4.50 | 7.60 | 2000.00 | 15200.00 | 137.70 |
| Con A flowthrough | 0.17 | 0.28 | 1970.00 | 551.60 | 4.90 |
| Recirculated Con A eluate | 319.90 | 533.20 | 9.50 | 5065.40 | 45.20 |
| Con A eluate + glucose | 225.90 | 376.40 | 10.00 | 5332.00 | 47.60 |
| Concentrated eluate | 1050.90 | 1751.66 | 2.00 | 3503.32 | 31.20 |
| Sephadex eluate | 82.50 | 138.00 | 30.00 | 4140.00 | 36.90 |
| Concentrated eluate | 704.30 | 1173.80 | 3.50 | 4108.30 | 36.70 |

Sample A shows the purification of CHO-K1 α-glucosidase medium using a previously described method [11]. Sample B shows CHO-K1 α-glucosidase medium purification with a modified method as described in Section 2. Con A = concanavalin A–Sepharose column.

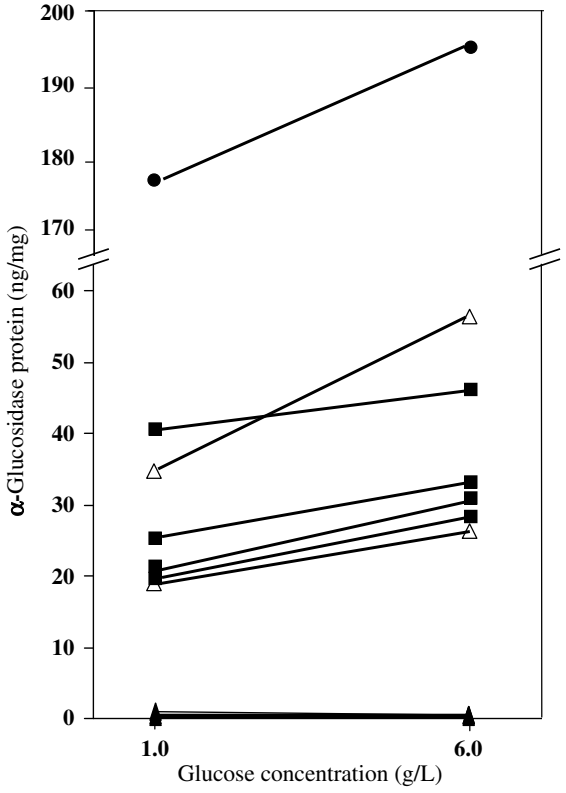


Fig. 4. α-Glucosidase protein levels in GSD II patient skin fibroblasts treated with D-glucose. The effect of D-glucose on α-glucosidase protein levels of normal control (●) and GSD II patient skin fibroblast cell lines from either infantile-onset patients with negligible levels of α-glucosidase protein (▲), infantile-onset patients with variant α-glucosidase protein (△), or adult-onset patients (■). Fibroblast cell lines were cultured either in the presence or absence of D-glucose (6 g/L) for 72 h before determining the level of α-glucosidase protein. The age of the adult-onset patients was similar to the normal controls. After D-glucose treatment, the α-glucosidase activity increased from 101 to 120 nmol/h/mg in the normal control and from 21.8 ± 8.0 to 29.8 ± 9.0 nmol/h/mg in the adult-onset GSD II patient cells, showing the same trend as observed for the α-glucosidase protein values. The infantile-onset GSD II patients had little or no α-glucosidase enzyme activity and this did not change after D-glucose treatment.

method. The increased production and purification yield for α-glucosidase, with D-glucose, could substantially reduce the cost of α-glucosidase preparation for ERT.

The ability of D-glucose to stabilise endogenous α-glucosidase in CHO-K1 cells suggested that a similar strategy could be used to enhance the level of α-glucosidase activity in GSD II cells. In fibroblasts from GSD II patients, only those with significant levels of α-glucosidase protein responded to D-glucose treatment. D-glucose treatment of GSD II skin fibroblasts from adult-onset patients resulted in an increase in residual α-glucosidase protein and activity, providing proof-of-principle for the use of a D-glucose analogue in EET. EET has already shown promising results in preclinical trials of four other LSD: Gaucher [14], mucopolysaccharidosis IVB [15], Fabry [16,17] and Tay-Sachs/Sandhoff patients [18]. Infusion of D-galactose in a 55-year old Fabry patient (deficient in α-galactosidase) increased residual activity of the enzyme 1.4-fold (from 7% to 10% of normal values) in lymphocytes and this was sufficient to significantly ameliorate the patient's condition [29]. Here, the treatment of adult-onset GSD II skin fibroblasts with D-glucose resulted in a 1.5-fold increase in residual α-glucosidase activity consistent with the results in the Fabry patient. However, D-glucose was only a low affinity inhibitor of α-glucosidase ($K_i$ = 45 mM) and high level glucose infusion would not be an appropriate therapy for patients, due to the impact on glucose metabolism. Analogues of D-glucose that are more potent and specific inhibitors of α-glucosidase may provide candidate compounds for EET in GSD II patients, who have residual enzyme activity.

This study demonstrated that D-glucose stabilised α-glucosidase, allowing increased expression and improved purification of the recombinant protein. D-Glucose enhanced the residual α-glucosidase protein and activity in adult-onset GSD II patient cells, providing proof-of-principle for EET in patients with mis-sense mutations (56 of the 109 mutations detected in the GAA gene: http://www.hgmd.cf.ac.uk/).

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