Glycoconjugate Journal 15, 499–505 (1998)
© 1998 Kulwer Academic Publishers. Manufactured in the U.K.

Carbohydrate-deficient glycoprotein syndrome type 1: correction of the glycosylation defect by deprivation of glucose or supplementation of mannose

Christian Körner*, Ludwig Lehle[1] and Kurt von Figura

Georg-August-Universität, Abt. Biochemie II, Gosslerstr. 12d, D-37073 Göttingen, Germany
[1] *Universität Regensburg, Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universitätsstr. 31, D-93053 Regensburg*

In the carbohydrate deficient glycoprotein syndrome (CDGS) type 1 glycoproteins with less and shorter N-linked oligosaccharides are synthesized due to a deficiency of phosphomannomutase. Glucose deprivation or mannose addition are shown to partially or fully correct the size of oligosaccharides incorporated into lipid linked oligosaccharides and nascent glycoproteins in skin fibroblasts from CDGS type 1 patients with a phosphomannomutase defect. The corrective effect is ascribed to regulatory mechanisms and/or metabolic pathways that bypass phosphomannomutase.

***Keywords*: CDGS type 1, mannose addition, glucose deprivation**

Introduction

The carbohydrate deficient glycoprotein syndrome (CDGS) comprises a heterogeneous group of inherited disorders, which have in common the synthesis of undersialylated glycoproteins [1–3]. The most frequent form, CDGS type 1, is an autosomal recessive disease with severe neurological involvement and about 20% lethality with the first year of life [2, 4]. CDGS type 1 is genetically heterogeneous [5]. In most cases described so far a defect in the chromosomal locus 16p13 encoding the gene for phosphomannomutase 2 (PMM2; EC 5.4.2.8), the enzyme that converts mannoe 6-phosphate into mannose 1-phosphate, has been identified as the cause for this disorder [6]. Nevertheless some patients with alterations in the glycoprotein pattern typical for CDGS type 1 seem to suffer from a different enzyme defect pointing to a further diversification. The identification of a cohort of different missense mutations in the *PMM2* gene [6] is in agreement with the observation that the PMM activity in CDGS type 1 fibroblasts, leukocytes and liver tissue is less than 10% compared to controls [7]. The biochemical alterations in CDGS type 1 fibroblasts with a defect in *PMM2* show considerable variations [8, 9]. Incorporation of [2-^{3}H] mannose into nascent glycoproteins was consistently found to be reduced, while incorporation of [2-^{3}H] mannose into mannose phosphates, GDP-mannose, dolicholphosphomannose (Dol-P-Man) and lipid linked oligosaccharides (LLO) were found to be normal, reduced or even elevated [9–12]. A particular variability was observed for the size of oligosaccharides in LLO and nascent glycoproteins, which was found to vary between normal size and severe truncation when CDGS type 1 fibroblasts were repeatedly examined under apparently identical conditions. This biochemical variability has made it difficult to assess the metabolic alterations resulting from phosphomannomutase deficiency.

In a recent study we observed in CDGS type 1 fibroblasts with phosphomannomutase deficiency a reduced incorporation of [2-^{3}H] mannose into mannose 1-phosphate, GDP-mannose, GDP-fucose, Dol-P-Man and nascent glycoproteins, while the incorporation into mannose 6-phosphate was normal. Although the incorporation of [2-^{3}H] mannose into LLO was only slightly decreased, the size of oligosaccharides in LLO and nascent glycoproteins was severely truncated [9]. Addition of mannose to the culture medium of CDGS type 1 fibroblasts was found to restore the size of oligosaccharides in LLO and nascent glycoproteins and the incorporation of [2-^{3}H] mannose into water soluble mannose-derived metabolites [12]. In the present study we analysed the effect of glucose deprivation and of mannose supplementation on the incorporation of [2-^{3}H] mannose into mannose 1-phosphate, GDP-mannose, GDP-fucose, Dol-P-Man, LLO and nascent glycoproteins in CDGS type 1 fibroblasts with phosphomannomutase deficiency.

* To whom correspondence should be addressed. Tel.: (0049)551-392127; Fax: (0049)551-395979.

Materials and methods

Chemicals, Enzymes and Isotopes

D-[2-^{3}H] mannose (18 Ci mmol^{-1}), [^{35}S] methionine were from Amersham Buchler (Braunschweig). Recombinant endoglycosidase H (Endo H) and N-glycosidase F (PNGase F) were from Boehringer (Mannheim). [^{14}C] Dol-P-Man, [2-^{3}H] mannose-6-phosphate [8] and [2-^{3}H] oligosaccharide standards of known size [13] were prepared as described.

Cell culture

Human primary fibroblast cultures obtained from upper arm skin biopsies of CDGS type 1 patients during the first 5 years of life were kindly provided by Professor F. Hanefeld (Department of Pediatrics, University Hospital Göttingen, Göttingen, Germany) and Professor J. Jaeken (Department of Pediatrics, University Hospital Gasthuisberg, Leuven, Belgium). Diagnosis of CDGS type 1 was based on the clinical symptoms, the isoelectric focusing pattern of serum transferrin and in part by linkage analysis. Cells were grown at 37 °C in the presence of 5% CO_2 on Dulbeccos modified Eagles medium (DMEM; GibcoBRL) supplemented with 10% fetal calf serum (PANSYSTEMS) and passaged by trypsinization.

Metabolic labelling with [2-^{3}H] mannose and [^{35}S] methionine

6.6×10^4 fibroblasts per cm^2 were plated onto culture dishes and grown for 60 h in DMEM containing 5 mM glucose. In case of glucose deprivation cells were grown in DMEM supplemented with 0.5 mM glucose 12 h prior to labelling. Cells were metabolically labelled for 30 min in methionine free MEM containing 0.5 mM glucose, supplemented with either 20 to 125 μCi [2-^{3}H] mannose or 2 μCi [^{35}S] methionine, and 1 mM mannose when indicated. Cells were then washed three times with icecold 10 mM phosphate buffered saline pH 7.4 (PBS), scraped into 10 mM PBS/0.1% Triton X-100 or buffers as indicated and lysed by sonification. Aliquots were used for protein determination [14].

Determination of radioactivity incorporated into proteins

Aliquots of cell lysates were spotted onto Whatman 3 MM paper filters and dried for 1 h. The filters were fixed for 10 min in 10% icecold TCA, boiled for 5 min in 5% TCA and subsequently washed in 5% icecold TCA followed by a short wash in ethanol/diethyl ether (1 : 1). Radioactivity was determined by liquid scintillation counting.

Digestion with Endo H and PNGase F

Cells metabolically labelled with [2-^{3}H] mannose were suspended in water supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), lysed by sonification and dialysed overnight against water. An aliquot of the dialysed material was used for protein determination. For Endo H treatment 20 μg of protein lysate were dried under vacuum, resuspended in 50 μl 50 mM 2-mercaptoethanol, 0.1% SDS, sonified and heated for 5 min at 95 °C. The sample was supplemented with 15 μl 0.5 M sodium citrate, ph 5.5, 5 μl 10% PMSF, 5 μl 05 U ml^{-1} Endo H and 75 μl of water. After incubation for 12 h at 30 °C the sample was heated for 5 min at 95 °C. For PNGase F digestion 100 μg of protein were dried under vacuum, resuspended in 25 μl of 0.1 M 2-mercaptoethanol, 0.5% SDS and heated for 5 min at 95 °C. After incubation in 25 μl 0.5 M Tris-HCl, pH 8.0, 10 μl 0.1 M 1,10-phenanthroline, 10 μl 10% Triton X-100 and 5 μl of PNGase F (250 mU ml^{-1}) the reaction was stopped as above. Released oligosaccharides were separated from protein bound oligosaccharides by elution with water from C18 Sep Pak cartridges (Waters), dried under vacuum, resuspended in 100 μl of water and analysed by HPLC (see below).

Metabolic labelling and extraction of Dol-P-Man and LLO

Cells were labelled with [2-^{3}H] mannose as described, scraped into 2 ml of icecold methanol and lysed by sonification. After addition of 4 ml chloroform the material was again sonified, followed by centrifugation for 10 min at 5000 rpm at 4 °C. Supernatants were collected and the pellets extracted two more times with chloroform/methanol (3 : 2). The combined supernatants containing Dol-P-Man and LLOs of small size were dried under nitrogen, dissolved in 3 ml chloroform/methanol (3 : 2), washed and analysed by thin layer chromatography on Silica gel 60 in chloroform/methanol/water (65 : 25 : 4) as described previously [13]. The remaining pellet containing the large size LLOs were washed and extracted with chloroform/methanol/water (10 : 10 : 3) as described [13]. Corresponding aliquots of the chloroform/methanol and chloroform/methanol/H_2O extracts were combined and dried under nitrogen and resuspended in 35 μl 1-propanol. To release the oligosaccharides by mild acid hydrolysis 500 μl 0.02 N HCl were added followed by an incubation for 30 min at 100 °C. The hydrolysed material was dried under nitrogen, resuspended by sonification in 200 μl H_2O and cleared by centrifugation. The supernatant containing the released oligosaccharides was used for HPLC analysis (see below).

Size fractionation of oligosaccharides by HPLC

The separation of LLOs was performed on a Supelcosil LC-NH_2 column (25 cm × 4.6 mm; 5 μm; Supelco) including a LC-NH_2 (2 cm × 4.6 mm) precolumn. A linear gradient of acetonitril from 70% to 50% in H_2O (total volume 75 ml) was applied at a flow rate of 1 ml min^{-1}. Eluate fractions were analysed by liquid scintillation counting.

Preparation of mannose 6-phosphate, mannose 1-phosphate, GDP-mannose and GDP-fucose

After labelling with [2-^{3}H] mannose cells were harvested by scraping into 2 ml of icecold 50% methanol, 10 mM Tris, pH 7.4, 1 mM EDTA. The suspension was boiled for 3 min and sonified. After centrifugation the supernatant was collected. The pellet was extracted two more times. Combined supernatants were dried under N_2, suspended in methanol/water (1 : 1) and desalted by descending paper chromatography on Whatman No. 1 paper in methanol/1 M ammonium acetate, pH 7.5 (5 : 2) for 16 h. The radioactivity was determined by a TLC-analyser. Mannose-phosphates were separated from nucleotide sugars (GDP-mannose, GDP-fucose) and free mannose. [^{3}H] mannose-phosphates were eluted with water, dried by lyophilization and subjected to high voltage electrophoresis at 65 V cm^{-1} for 50 min on Whatman 3 MM paper in a buffer containing 80 mM pyridine adjusted to pH 5.5 with acetic acid. [^{3}H] Mannose-phosphates were eluted with water, dried by lyophilization and subjected to descending paper chromatography on Whatman No. 1 paper in butanol/acetic acid/water (3 : 3 : 2) for 16 h. [^{3}H] Mannose-phosphates were eluted with water, dried by lyophilization and subjected to mild acid hydrolysis in 0.02 N HCl at 95 °C for 30 min. [2-^{3}H] Mannose released from [2-^{3}H] mannose 1-phosphate and [2-^{3}H] mannose 6-phosphate was separated by high voltage electrophoresis as above, eluted and quantified by liquid scintillation counting.

The nucleotide sugars were eluted from the paper chromatogram (see above) and analysed by high voltage electrophoresis, paper chromatography and acid hydrolysis as described for the mannose phosphates, except that acid hydrolysis products ([^{3}H] mannose and [^{3}H] fucose) were separated by thin layer chromatography in acetone/butanol/water (70 : 15 : 15) on Silica gel 60.

Analysis of phosphomannomutase

Cells were grown as described above, scraped into 20 mM HEPES pH 7, 1/150 mM NaCl and collected by centrifugation. Pellets were resuspended in homogenization buffer (20 mM HEPES, 25 mM KCl, 1 mM dithiothreitol, 10 μg ml^{-1} each of leupeptin and antipain). Homogenization was achieved by one freeze-thaw cycle followed by sonification. Phosphomannomutase activity was determined by following the conversion of mannose 6-phosphate into mannose 1,6-bisphosphate in the presence of glucose 1,6-bisphosphate. The assay mixture contained 50 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 25 000 dpm [2-^{3}H] mannose 6-phosphate, 1 mM glucose 1,6-bisphosphate and 2 μg of protein in a total volume of 25 μl. After incubation for 30 min at 37 °C the supernatants were collected by centrifugation and [2-^{3}H] mannose 6-phosphate was separated from [2-^{3}H] mannose 1,6-bisphosphate by high voltage paper electrophoresis as described above.

Results

Corrective effect of glucose deprivation and mannose addition on glycoprotein and lipid-linked oligosaccharides

Several studies in CDGS type 1 fibroblasts had reported that in a notable number of experiments the size of oligosaccharides in LLO and nascent glycoproteins was found to be normal contrary to the majority of experiments, where the oligosaccharides are found to be truncated [10–12]. Glucose starvation is known to induce the synthesis of truncated oligosaccharides in LLO and the incorporation of truncated oligosaccharides into nascent glycoproteins [15]. We examined therefore whether a reduction of glucose concentration in the medium would produce a more consistent phenotype in CDGS type 1 fibroblasts.

Contrary to our expectation we observed that reducing the glucose concentration from 5 mM to 0.5 mM prior to labelling, increased the incorporation of [2-^{3}H] mannose into glycoproteins in CDGS type 1 fibroblasts. In cells maintained under standard conditions (5 mM glucose) prior to labelling, the incorporation of [2-^{3}H] mannose relative to that of [^{35}S] methionine is 30% of the control (Figure 1). The incorporation of [2-^{3}H] mannose increased to about two thirds of control when the cells had been maintained in 0.5 mM glucose for 12 h prior to labelling.

The abnormal size distribution of oligosaccharides released from newly synthesized glycoproteins by PNGase F digestion was also partially corrected. In glycoproteins synthesized under standard conditions, less than 20% of the [2-^{3}H] mannose is found in oligosaccharides with eight or more mannose residues (Figure 2A). This fraction increases to 65% after glucose deprivation (Figure 2B), while in control fibroblasts it represents more that 90% of the [^{3}H] oligosaccharides (Figure 2D).

The truncated oligosaccharides found in newly synthesized glycoproteins of CDGS type 1 fibroblasts are resistent to Endo H [9, 12]. The larger average size of N-linked oligosaccharides after glucose deprivation was also reflected in an increased susceptibility to Endo H. The fraction of [^{3}H] oligosaccharides sensitive to Endo H increased from 32 ± 8% to 58 ± 13% after glucose deprivation for 12 h. In control fibroblasts 80 to 85% of [^{3}H] oligosaccharides are sensitive to Endo H. It should be noted that glucose deprivation in control fibroblasts had neither an effect on the incorporation of [2-^{3}H] mannose into glycoproteins (see Figure 1) nor on the size of the N-linked oligosaccharides (data not shown).

Glucose deprivation has also a corrective effect on the size of oligosaccharides in LLO. While in controls more than 90% of the [2-^{3}H] mannose is recovered in $GlcNAc_2Man_9Glc_3$ oligosaccharides (Figure 3D), in CDGS type 1 fibroblasts this species represents only a minor fraction (Figure 3A). More than 50% of the [2-^{3}H] mannose is recovered in oligosaccharides with less than five mannose

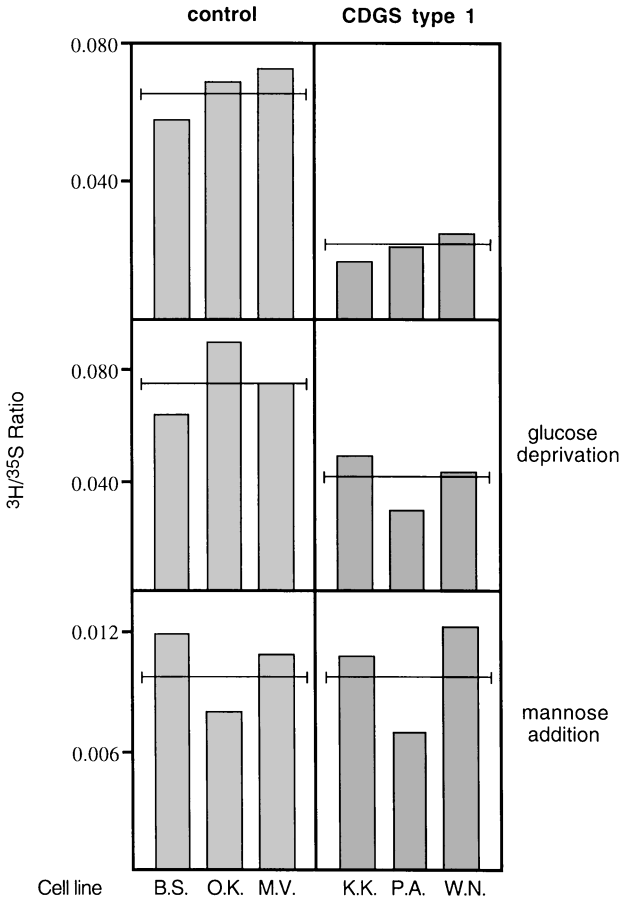

Figure 1. Effect of glucose deprivation and mannose addition on glycoprotein synthesis. Parallel dishes of fibroblasts from control (bright columns) and CDGS type 1 (dark columns) were metabolically labelled for 30 min with [2-^{3}H] mannose or [^{35}S] methionine. The $^3H/^{35}S$ ratio of the TCA-insoluble material of the cell extracts was determined. The upper panel shows the $^3H/^{35}S$ ratio for cells labelled under standard conditions (5 mM), the middle panel the ratio for cells that had been maintained for 12 h in medium containing 0.5 mM glucose, the lower panel the ratio for cells labelled in the presence of 1 mM mannose. The horizontal bars indicate the mean for control and CDGS type 1 fibroblasts, respectively.

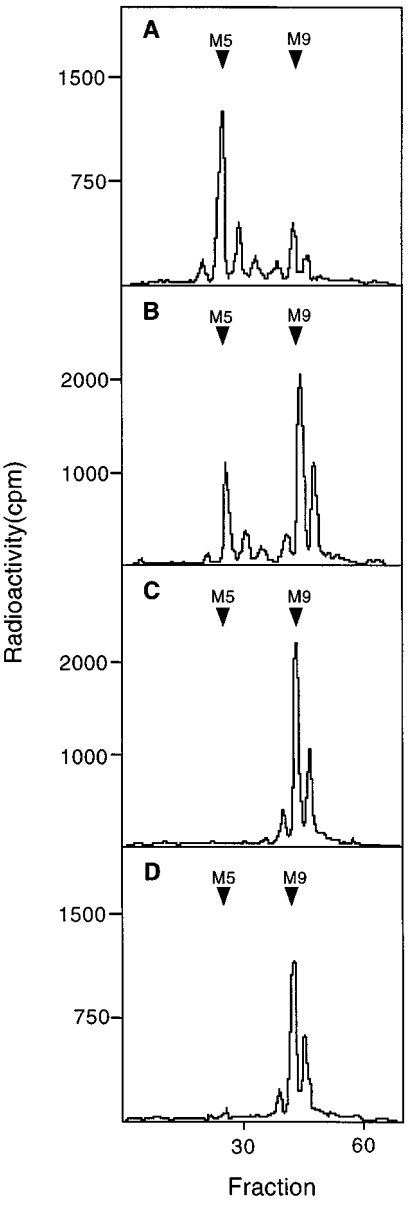

Figure 2. Effect of glucose deprivation or mannose addition on glycoprotein linked oligosaccharides. CDGS type 1 (cell line K.K., panel A–C) and control (cell line O.K., panel D) fibroblasts were metabolically labelled for 30 min with [2-^{3}H] mannose under standard conditions (A, D), after maintaining for 12 h in medium reduced to 0.5 mM glucose (B) or in the presence of 1 mM mannose (C). [^{3}H] Oligosaccharides (3600–8900 cpm) released from glycoproteins with PNGaseF were subjected to size fractionation. M5 and M9 refer to the elution of $GlcNAc_2$ Man_5 and $GlcNAc_2$ Man_9 standards.

residues. After glucose deprivation, more than 90% of [2-^{3}H] mannose is incorporated into $GlcNAc_2Man_9Glc_3$ oligosaccharides (Figure 3B).

While this study was in progress a corrective effect of mannose on glycoprotein synthesis in CDGS type 1 fibroblasts was reported [12]. Addition of 1 mM mannose to the labelling medium reduced in control fibroblasts the relative incorporation of [2-^{3}H] mannose into glycoproteins about seven-fold (Figure 1). In CDGS type 1 fibroblasts the relative incorporation of [2-^{3}H] mannose increased to the value observed in control fibroblasts (see Figure 1) indicating that not only the size but also the number of N-linked oligosaccharides in glycoproteins was restored to the normal situation. In fact, the size of glycoprotein linked oligosaccharides (Figure 2C), their sensitivity to Endo H (82%) and the size of lipid linked oligosaccharides was indistinguishable from that in controls (Figure 3C). It should be noted that addition of 1 mM mannose to control fibroblasts had no effect on the size of N-linked oligosaccharides and their sensitivity to endoglucosaminidase H (not shown).

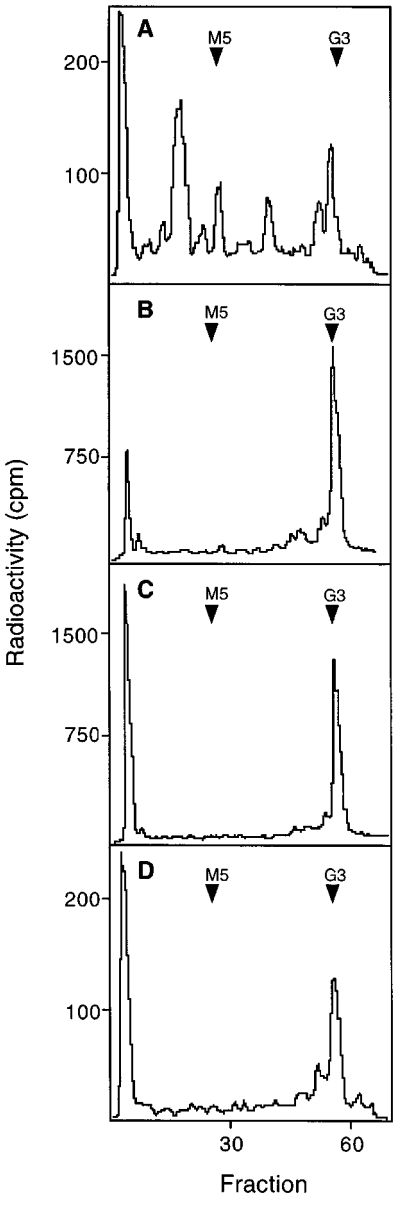

Figure 3. Effect of glucose deprivation or mannose addition on oligosaccharides in LLO. CDGS type 1 (cell line K.K., panel A–C) and control (cell line O.K., panel D) fibroblasts were metabolically labelled for 30 min with [2-^{3}H] mannose under standard conditions (A, D), after maintaining for 12 h in medium reduced to 0.5 mM glucose (B) or in the presence of 1 mM mannose (C). Oligosaccharides (500–5100 cpm) released from LLO fraction by mild acid hydrolysis were subjected to size fractionation. $GlcNAc_2Man_5$ and $GlNac_2Man_9G_3$ were used as standards.The LLO fraction contained variable amounts of [2-^{3}H] mannose, which eluted in the flowthrough fraction.

Corrective effect of glucose deprivation and mannose addition on [2-^{3}H] mannose derived metabolites

The incorporation of [2-^{3}H] mannose into mannose 1-phosphate, GDP mannose and GDP fucose is decreased in CDGS type 1 fibroblasts, while that into mannose 6-phosphate is not affected (see Table 1 and [9]). Deprivation of glucose increased the incorporation of [2-^{3}H] mannose into mannose 1-phosphate. The ratio of mannose 1- versus mannose 6-phosphate increased from 0.06 to 0.10, a value closer to that found in controls (0.14–0.16). Presence of 1 mM mannose during the labelling normalized the mannose 1-versus mannose 6-phosphate ratio (Table 1).

The incorporation of [2-^{3}H] mannose into GDP mannose and GDP fucose, which is about four- to five-fold lower in CDGS type 1 fibroblasts, was restored to normal by the addition of 1 mM mannose (Table 1).

The markedly reduced incorporation of [2-^{3}H] mannose into Dol-P-Man in CDGS type 1 fibroblasts is normalized both after glucose deprivation or in the presence of 1 mM mannose (Figure 4).

Phosphomannomutase activity is not affected by glucose deprivation or mannose addition

Cell extracts were prepared after maintaining the cells for 12 h in medium supplemented with 0.5 mM glucose or for 30 min in medium supplemented with 1 mM mannose. Neither pretreatment affected the residual activity of phosphomannomutase, which was measured by following the conversion of [^{3}H] mannose 6-phosphate into [^{3}H] mannose 1,6-bisphosphate in the presence of an excess of glucose 1,6-bisphosphate (Figure 5). The residual activity of phosphomannomutase was less than 5% of control.

Discussion

The present study confirms and extends the observation of Pannerselvam and Freeze [12] that addition of mannose corrects the synthesis of N-linked glycoproteins in CDGS type 1 fibroblasts. Addition of mannose corrects the size of oligosaccharides in LLO and nascent glycoproteins. Also, the number of oligosaccharides transferred onto glycoproteins, which is reduced in secretory glycoproteins of the liver [16] and of fibroblasts (Poppen and Körner, unpublished observations) appears to become restored to normal. This is indicated by the correction of the [2-^{3}H] mannose incorporation into newly synthesized glycoproteins.

The biochemical abnormalities in CDGS type 1 can be fully explained by the severe deficiency of phosphomannomutase, which reduces the availability of mannose 1-phosphate [7, 9]. The latter is the precursor for GDP-mannose which serves as the donor for the synthesis of mannose containing glycoconjugates and as precursor for GDP-fucose.

The corrective effect of mannose implies that exogenous mannose can improve the availability of GDP-mannose for the synthesis of LLO and N-linked glycoproteins. Mannose can enter mammalian cells via specific transporters with a K_M for uptake of 30–70 mM [17, 18]. Cytosolic mannose is thought to be converted into mannose 6-phosphate. The

Table 1. Incorporation of [2-^{3}H] mannose in mannose 6-phosphate, mannose 1-phosphate, GDP-mannose and GDP-fucose.

Fibroblasts		*Radioactivity (cpm mg^{-1})*[a] *in*				*Ratio of M1P/M6P*
		M6P	*M1P*	*GDP-Man*	*GDP-Fuc*	
A	Control	9733 ± 1915	1406 ± 185	167	103	0.14
	CDGS	11 088 ± 4043	676 ± 258	32	23	0.06
B	Control	9272 ± 1377	1511 ± 543	ND[b]	N.D.	0.16
	CDGS	11 883 ± 1067	1129 ± 142	ND	N.D.	0.10
C	Control	6495	916	137	69	0.14
	CDGS	9792 ± 121	1773 ± 514	136	72	0.18

[a] Control (O.K.) and CDGS type 1 (K.K) fibroblasts were metabolically labelled for 30 min with [2-^{3}H] mannose under standard conditions (A) after maintaining for 12 h in medium supplemented with 0.5 mM glucose (B) or in the presence of 1 mM mannose (C). Radioactivity associated with mannose 6-phosphate (M6P), mannose 1-phosphate (M1P), GDP-Man and GDP-Fuc were quantified as described in Materials and Methods. The values for M6P and M1P refer to the mean and range of two independent determinations.
[b] Not determined.

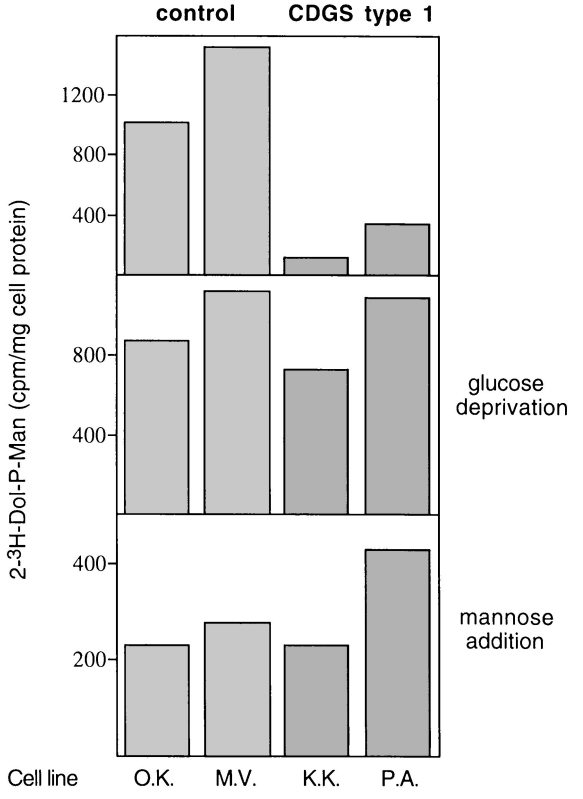

Figure 4. Effect of glucose deprivation or mannose addition on synthesis of Dol-P-Man. Fibroblasts from control (bright columns) and CDGS type 1 (dark columns) were metabolically labelled for 30 min with [2-^{3}H] mannose under standard conditions (upper panel), after maintaining for 12 h in medium containing 0.5 mM glucose (middle panel) or in the presence of 1 mM mannose (lower panel). Radioactivity associated with Dol-P-Man was quantified as described in Methods.

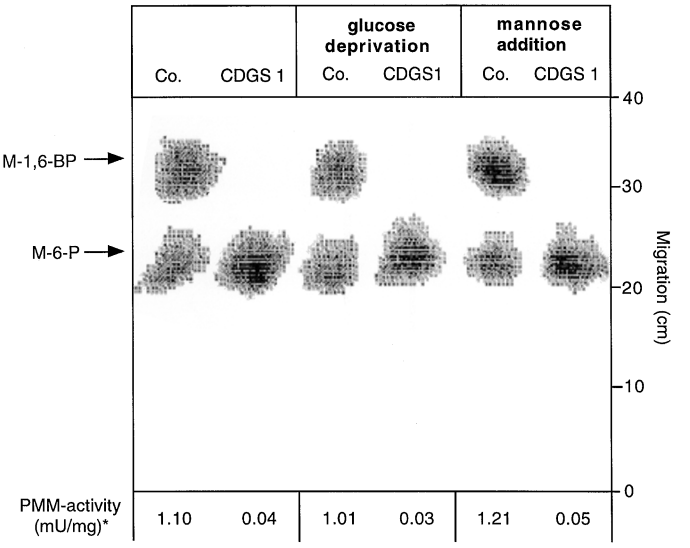

Figure 5. Activities of phosphomannomutase. Extracts were prepared from control (O.K.) and CDGS type 1 (K.K.) fibroblasts grown under standard conditions for 12 h in medium supplemented with 0.5 mM glucose or for 30 min in medium supplemented with 1 mM mannose. Shown is the electrophoretic separation of [^{3}H] mannose 6-phosphate and [^{3}H] mannose 1,6-bisphosphate. The number below the lanes refer to the activity of phosphomannomutase (mU mg^{-1}).

latter can be shuttled into the glycolytic pathway by phosphomannoisomerase or be converted into mannose 1-phosphate by phosphomannomutase [19]. Provided that the missense mutations found in the *PMM2* gene of CDGS type 1 patients [6] reduce the affinity of phosphomannomutase for mannose 6-phosphate, an increase of cytosolic mannose (and of mannose 6-phosphate) could explain the corrective effect of exogenous mannose. However, at present it cannot be excluded that cytosolic mannose is directly converted to mannose 1-phosphate by a mannokinase (not yet detected) thus bypassing the step catalysed by phosphomannomutase.

Glucose starvation produced a similar, albeit only partial corrective, effect on the synthesis of N-linked glycoproteins. As observed for the addition of mannose the size and number of oligosaccharides transferred onto nascent glycoproteins were found to be increased after glucose starvation.

When glucose was reduced only during the 30 min of labelling (standard conditions), the glycosylation defect was found. When cells were starved for 12 h the corrective effect was seen. Preliminary experiments had indicated that the maximum of correction was obtained after starvation for 12 or 24 h and only partial after starvation for 6 h. While addition of mannose corrected the glycosylation defect immediately [12], the corrective effect of glucose starvation increased with time of starvation.

The partial correction of the glycosylation defect in CDGS type 1 fibroblasts was surprising as several earlier studies had shown that glucose deprivation can induce the synthesis of truncated oligosaccharides in LLO and their transfer onto glycoproteins [15, 20–22]. It is thought that glucose deprivation produces this effect through a regulatory mechanism, rather than by limiting the availability of one of the precursors required for the synthesis of LLO [16].

How glucose starvation can restore the synthesis of N-linked glycoproteins in CDGS type 1 fibroblasts is not clear. We have considered that lowering the glucose concentration in the medium could improve the uptake of exogenous mannose present in the fetal calf serum. However, glucose starvation also produced its corrective effect on the synthesis of N-linked glycoproteins, when a serum-free, chemically defined medium was used (C. Körner, data not shown). We assume, therefore, that a regulatory mechanism improves in CDGS type 1 fibroblasts the supply with precursors from endogenous sources for the synthesis of LLO and N-linked glycoproteins. Such a mechanism could increase the recycling of mannose derived from processing of N-linked oligosaccharides or from the turnover of mannose containing glycoconjugates. Although glucose starvation did not affect the protein synthesis as measured by the incorporation of [^{35}S] methionine into trichloroacetic insoluble material, we cannot exclude that a decreased synthesis of mannose containing glycoconjugates contributed to the synthesis of normally glycosylated N-linked glycoproteins observed under conditions of glucose deprivation.

Acknowledgments

This work was supported by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (SFB 521).

References

1 Jaeken J, Vanderschueren-Lodeweyck M, Casaer P, Snoeck L, Corbeel L, Eggermont E, Eeckels R (1980) *Ped Res* **14**: 1970.
2 Jaeken J, Carchon H (1993) *J Inher Metab Dis* **16**: 813–20.
3 Jaeken J, Carchon H, Stibler H (1993) *Glycobiology* **3**: 423–8.
4 Jaeken J, Matthijs G, Barone R, Carchon H (1997) *J Med Genet* **34**: 73–6.
5 Matthijs G, Leguis E, Schollen E, Vandenberk P, Jaeken J, Barone R, Fiumara A, Visser G, Lambert M, Cassiman JJ (1996) *Genomics* **35**: 597–99.
6 Matthijs G, Schollen E, Pardon E, Veiga-Da-Cunha M, Jaeken J, Cassiman JJ, van Schaftingen E (1997) *Nature Genetics* **16**: 88–92.
7 van Schaftingen E, Jaeken J (1995) *FEBS Lett* **377**: 318–20.
8 Ohkura T, Fukushima K, Kurisaki A, Sagami H, Ogura K, Ohno K, Hara-Kuge S, Yamashita K (1997) *J Biol Chem* **272**: 6868–75.
9 Körner C, Lehle L, von Figura K (1997) *Glycobiology*: **8**(2): 165–71.
10 Powell LD, Paneerselvam K, Vij R, Diaz S, Manzi A, Buist N, Freeze H, Varki A (1994) *J Clin Invest* **94**: 1901–9.
11 Krasnewich DM, Holt GD, Brantly M, Skovby F, Redwine J, Gahl WA (1995) *Glycobiology* **5**: 503–10.
12 Paneerselvam K, Freeze HH (1996) *J Clin Invest* **97**: 1478–87.
13 Lehle L (1980) *Eur J Biochem* **190**: 589–601.
14 Lowry O, Rosebrough N, Farr A, Randall R (1951) *J Biol Chem* **193**: 265–75.
15 Gershman H, Robbins P (1981) *J Biol Chem* **256**: 7774–80.
16 Yamashita K, Ideo H, Ohkura T, Fukushima K, Yuasa I, Ohno K, Takeshita K (1993) *J Biol Chem* **268**: 5783–9.
17 Ogier-Denis E, Blais A, Houri JJ, Voisin T, Trugnau G, Codogno M (1994) *J Biol Chem* **269**: 4285–90.
18 Pannerselvam K, Freeze HH (1996) *J Biol Chem* **271**: 9417–21.
19 Varki A (1991) *FASEB J* **228**: 226–35.
20 Turco SJ (1980) *Arch Biochem Biophys* **205**: 330–9.
21 Rearick JI, Chapman A, Kornfeld S (1981) *JBiol Chem* **256**: 6255–61.
22 Calhoun IV, JC, Chapman AE (1988) *Arch Biochem Biophys* **260**: 320–33.

Received 29 February 1997, revised 5 September 1997, accepted 25 September 1997