A New Type of Carbohydrate-deficient Glycoprotein Syndrome Due to a Decreased Import of GDP-fucose into the Golgi*

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Torben Lübke, Thorsten Marquardt‡, Kurt von Figura, and Christian König§

From the Georg-August-Universität Göttingen, Abteilung Biochemie II, Heinrich-Düker-Weg 12, D-37073 Göttingen, Germany and the Klinik und Poliklinik für Kinderheilkunde, 48149 Münster, Germany

The fucosylation of glycoproteins was found to be deficient in a patient with a clinical phenotype resembling that of leukocyte adhesion deficiency type II (LAD II). While in LAD II hypofucosylation of glycoconjugates is secondary to an impaired synthesis of GDP-fucose due to a deficiency of the GDP-D-mannose-4,6-dehydratase, synthesis of GDP-fucose was normal in our patient (Körner, C., Linnebank, M., Koch, H., Harms, E., von Figura, K., and Marquardt, T. (1999) J. Leukoc. Biol., in press). Import of GDP-fucose into Golgi-enriched vesicles was composed of a saturable, high affinity and a nonsaturable component. In our patient the saturable high affinity import of GDP-fucose was deficient, while import of UDP-galactose and the activity of GDPase, which generates the nucleoside phosphate required for antiport of GDP-fucose, were normal. Addition of L-fucose to the medium of fibroblasts restored the fucosylation of glycoproteins. We propose that this new form of carbohydrate-deficient glycoprotein syndrome is caused by impaired import of GDP-fucose into the Golgi.

The glycosylation of proteins and lipids, which mainly takes place in the luminal part of the endoplasmic reticulum and the Golgi apparatus, requires the presence of specific nucleotide sugar transporters. These proteins allow the import of cytoplasmically synthesized nucleotide sugars and function as antiporters by exchanging the nucleotide sugars with the corresponding nucleoside monophosphates. The latter is generated in the organelle lumen by the action of glycosyltransferases and nucleoside diphosphatases (1). Several of these transporters have been identified by cDNA complementation studies using mutant cell lines with impaired nucleotide sugar transport. Examples are the cloning of the yeast and the mammalian transporter for UDP-N-acetylglucosamine (2, 3), the transporter for GDP-mannose from Leishmania donovani (4), the transporter for CMP-N-acetylneuraminic acid from Chinese hamster (5) and the transporter for UDP-galactose from mouse (6).

In the present study we describe for the first time the association of a human disease with a defect in the translocation of a nucleotide sugar. This disease belongs to the group of carbohydrate-deficient glycoprotein syndromes (CDGS), a group of hereditary disorders with impaired glycosylation of newly synthesized glycoproteins. The clinical phenotype of CDGS is heterogeneous. CDGS present mostly with severe psychomotor and mental retardation as in CDGS types Ia, II, III, IV, V and in leukocyte adhesion deficiency type II (LAD II) (7–12). In addition there are forms presenting as a hepatogastrointestinal disorder as in CDGS Ib (13) or as an anemia as in congenital dyserythropoietic anemia II (14). All defects described so far affect enzymes catalyzing the synthesis of GDP-mannose, GDP-fucose, or dolichyl-PP-GlcNAc2Man9Glc or modifying N-linked oligosaccharides in newly synthesized glycoproteins.

We report here the decreased import of GDP-fucose into the Golgi apparatus of skin fibroblasts from a patient who suffers from dysmorphic signs, retarded growth, psychomotor retardation, and severe infections. The biochemical hallmark is a general hypofucosylation of N- and O-glycosylated proteins (for a detailed description of the clinical and biochemical findings in this patient, see Ref. 15). The clinical phenotype and the hypofucosylation of our patient resemble a defect termed LAD II, which was described previously in two other patients (12). In LAD II the conversion of GDP-mannose to GDP-fucose is impaired due to the inactivity of GDP-D-mannose-4,6-dehydratase (16). Since the activity of GDP-D-mannose-4,6-dehydratase was normal in fibroblasts from our patient (17), the hypofucosylation must result from a different defect. Here we report that the hypofucosylation results from a severely decreased import of GDP-fucose into the Golgi.

**EXPERIMENTAL PROCEDURES**

[2-3H]Mannose (636 GBq/mmol), GDP-[-14C]fucose (11.5 GBq/mmol), UDP-[3H]galactose (359 GBq/mmol), and methyl-[-14C]glucosinolates (0.33 MBq/g) were purchased from Amersham Pharmacia Biotech (Braunschweig, Germany). Cell Culture—Human primary fibroblast cultures from controls, parents, and the patient were obtained from upper arm skin biopsies. Cells were grown at 37 °C in the presence of 5% CO₂ on Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Pansystems, Aidenbach, Germany).

**Metabolic Labeling with [2-3H]Mannose—**Labeling of fibroblasts with 125 μCi of [2-3H]mannose was carried out for 30 min or 6 h as described previously (18).

Analysis of GDP-[2-3H]mannose and GDP-[3H]fucose—GDP-[3H]mannose and GDP-[3H]fucose were separated from other labeled metabolites by descending paper chromatography. [3H]Mannose and [3H]fucose were released from nucleotide sugars by mild acid hydrolysis, separated by thin layer chromatography (TLC), and quantified by TLC scanner (Berthold) as described previously (18).

**Preparation of Radiolabeled Glycopeptides and Analysis of Glycopeptide-bound [3H]Mannose and [3H]Fucose—**Glycopeptides of control and patient fibroblasts labeled with [2-3H]mannose were sequentially extracted as described previously (18) followed by a digestion with 100 μg/ml of Pronase (Roche Molecular Biochemicals) for 24 h at 50 °C in a buffer containing 0.1 M Tris/HCl, pH 7.5, and 0.5% SDS. [3H]Mannose and [3H]fucose were released from the resulting glycopeptides by acid hydrolysis in 1 M trifluoroacetic acid at 100 °C for 4 h and analyzed by TLC as described above.

* The abbreviations used are: CDGS, carbohydrate-deficient glycoprotein syndrome(s); LAD II, leukocyte adhesion deficiency type II.

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**Table I**

| Incorporation of radioactivity into fucose bound to nucleotide sugars or glycoproteins |
|-----------------------------------|---------------------------------|
| GDP-[3H]fucose | Protein-bound GDP-[3H]fucose | Protein-bound GDP-[3H]mannose |
| % of GDP-[3H]fucose and GDP-[3H]mannose | % of protein bound GDP-[3H]radioactivity |
| Control fibroblasts | 17.7 ± 2.0 | 28.7 ± 4.6 |
| (n = 3) | (n = 3) | (n = 4) |
| Patient fibroblasts | 3.1 ± 0.2 | 25.5 ± 2.8 |
| (n = 3) | (n = 3) | (n = 3) |

**Table II**

| Correction of hypofucosylation by addition of fucose to patient derived fibroblasts |
|----------------------------------|----------------|
| Addition | Flow-through | Eluate | % bound to Lens lectin |
| cpm [3H]glycopeptides |
| Control | 160,354 | 27,341 | 14.57 |
| 1 mM mannose | 33,843 | 3664 | 9.77 |
| 1 mM fucose | 147,962 | 22,132 | 13.01 |
| Patient | 113,880 | 131 | 0.11 |
| 1 mM mannose | 38,747 | 63 | 0.16 |
| 1 mM fucose | 135,158 | 10,225 | 7.04 |

In the cytoplasmic synthesis of GDP-fucose, of its import in the Golgi, or of a common cofactor of fucosyltransferases. *De novo* synthesis of GDP-mannose to GDP-fucose is catalyzed by GDP-D-mannose-4,6-dehydratase together with the FX protein, which has an epimerase and reductase activity. Extracts from fibroblasts and leukocytes of our patient catalyzed the synthesis of GDP-fucose from GDP-mannose at a normal rate (17).

We therefore analyzed the synthesis of GDP-fucose and the fucosylation of glycoproteins in fibroblasts from our patient in more detail. When fibroblasts were incubated for 30 min in the presence of [2-3H]mannose the incorporation of radioactivity into GDP-fucose was almost six times lower than in controls (Table I). When the incubation period was prolonged to 6 h, the incorporation of radioactivity into GDP-fucose was close to normal, while the incorporation into glycoproteins was about one-fourth of control (Table I). One possibility to explain these observations is the assumption that the import of cytosolic GDP-fucose into the Golgi is impaired, leading to an accumulation of GDP-fucose in the cytosol, a deficiency of GDP-fucose in the Golgi and a hypofucosylation of glycoproteins. The increase of cytosolic GDP-fucose can explain the decreased rate of GDP-mannose to GDP-fucose conversion by the known feed

**RESULTS AND DISCUSSION**

A biochemical hallmark in leukocytes and fibroblasts of our patient is the absence of α1,2-, α1,3-, and α1,6-linked fucose residues in glycoconjugates (15). This suggests either a defect
back inhibition of GDP-\(\alpha\)-mannose-4,6-dehydratase by GDP-fucose (26).

To verify the hypofucosylation of glycoproteins, fibroblasts were metabolically labeled with \([2-^{3}H]\)mannose for 6 h. When labeled glycopeptides prepared from these cells were subjected to \(L.\ culinaris\) affinity chromatography, which retains a 1,6-fucosylated glycopeptides, the bound fraction was less than 1% of that in controls (Table II), thus confirming the severe hypofucosylation of glycoproteins. Moreover, we could demonstrate that incubation of the patients fibroblasts in the presence of 1 mM fucose, but not in the presence of 1 mM mannose overcomes by large the fucosylation defect (Table I). One explanation for the correction of the defect by addition of L-fucose to the medium would be a \(K_m\) defect in the GDP-fucose transport. The elevated amount of GDP-fucose in the cytoplasm would increase the transport into the Golgi to an extent that ensures nearly normal fucosylation of glycoproteins. The corrective effect renders the possibility unlikely that the hypofucosylation results from the deficiency of a cofactor common to all fucosyltransferases.

Next we assayed the import of GDP-\([14C]\)fucose into preparations enriched in Golgi vesicles. The import of GDP-\([14C]\)fucose under standard assay conditions was linear with time for up to 10 min, leveled off after 20 min, and was proportional to the amount of vesicles added for up to 110 \(\mu\text{g}\) of protein per assay. Determination of import as a function of GDP-fucose revealed that import of GDP-fucose is composed of a saturable and a nonsaturable component (Fig. 1 A). The \(K_m\) for the saturable component was 0.7 \(\pm\) 0.3 \(\mu\text{M GDP-fucose}\). At standard assay conditions (3 \(\mu\text{M GDP-fucose}\)) import via the saturable mechanism accounted for about 85% of total GDP-fucose import. GDP-fucose import by the patients’ vesicles at standard assay conditions was 20% of controls (Fig. 1 B). Following import for up to 60 min revealed that GDP-fucose import into patients’ vesicles reached its maximum within 5 min. When import was determined at 0.5–30 \(\mu\text{M GDP-fucose}\), it became apparent that the residual import was accounted largely if not exclusively by the nonsaturable import mechanism (Fig. 1 A).

As a control we determined the activity of galactosyltransferase, a marker of trans Golgi membranes (Fig. 1 C). The activity of galactosyltransferase was similar in membranes from controls (37 \(\pm\) 11 pmol \([3H]\)galactose/min \(\times\) mg protein, \(n = 9\)) and the patient (25 \(\pm\) 9 pmol \([3H]\)galactose/min \(\times\) mg protein, \(n = 7\)). Furthermore we determined the import of a second nucleotide sugar as a control for the integrity of the vesicles. The import rate for UDP-\([3H]\)galactose in control and patients’ membranes was comparable (Fig. 1 D). This indicates that the patients’ vesicles were import competent for nucleotide import of GDP-[14C]fucose and UDP-[3H]galactose into and activity of galactosyltransferase in Golgi-enriched vesicles. A shows the import of GDP-[14C]fucose in the presence of 0.5–30 \(\mu\text{M}\) GDP-fucose by vesicle preparations from two control fibroblast lines (open symbols) and from patient’s fibroblasts (filled squares). Assays at 0.5–10 \(\mu\text{M}\) GDP-fucose were done at constant specific radioactivity of GDP-[14C]fucose and that at 30 \(\mu\text{M}\) GDP-fucose at a 3-fold lower specific radioactivity. B summarizes GDP-fucose import by Golgi-enriched vesicles from fibroblasts of controls (six cell lines, open column), the patient (filled column), and his parents (striped columns: left, mother; right, father) under standard conditions. The mean, the S.D., and the number of independent determinations (\(n\)) are given. C summarizes the activity of galactosyltransferase and D the import of UDP-galactose. Symbols are the same as described for B.

FIG. 1. Activity of GDPase in fibroblast membranes. 20 \(\mu\text{g}\) of a crude membrane fraction from fibroblasts of controls (two cell lines) and the patient were incubated with 3 \(\mu\text{M}\) GDP at 37 °C for the time indicated. Phosphate released from GDP was determined as described under “Experimental Procedures.” The values represent the mean and S.D. from three independent experiments.

FIG. 2. Activity of GDPase in fibroblast membranes. 20 \(\mu\text{g}\) of a crude membrane fraction from fibroblasts of controls (two cell lines) and the patient were incubated with 3 \(\mu\text{M}\) GDP at 37 °C for the time indicated. Phosphate released from GDP was determined as described under “Experimental Procedures.” The values represent the mean and S.D. from three independent experiments.
sugars and that the failure to import GDP-fucose represents a specific defect.

Nucleotide sugar transporters operate as antiporters with the corresponding nucleoside monophosphate (1). Impaired GDP-fucose import could therefore also result from a decreased transfer of fucose onto glycoproteins or decreased GDPase, which converts the released GDP into the GMP substrate of the transporter. A deficiency of fucosyltransferase activity as a cause for the reduced import is unlikely, due to the corrective effect of exogenous fucose (see above) and the absence of α1,2-, α1,3-, and α1,6-linked fucose residues, which are all transferred by different fucosyltransferases.

As shown previously, transport of GDP-mannose into the lumen of Saccharomyces cerevisiae Golgi vesicles requires a guanosine diphosphatase. A null mutation (gda1) showed a five times reduced rate of GDP-mannose import (27). Mammalian cells posses a Golgi UDPase showing highest activity with UDP and GDP as substrates (28). The UDP/GDPase is expressed in all human tissues. In order to exclude a defect in the hydrolysis of GDP in our patient, we examined the GDPase activity and found it to be comparable in control and patient’s membranes (Fig. 2). We conclude from these data that in the Golgi vesicles of the patient, GDP-fucose is severely reduced due to a defect in the saturable, high affinity import of GDP-fucose and that under cell culture conditions the deficiency of GDP-fucose in the Golgi can largely be overcome by raising the fucose concentration in the medium to 1 mM.

The defect in our patient is assumed to be recessively transmitted. We therefore examined the import of GDP-fucose and UDP-galactose into vesicles prepared from the fibroblasts of the parents. GDP-fucose and UDP-galactose were imported at a normal rate by vesicles from the mother, while import into vesicles from the father was reduced by about 40% for both nucleotide sugars (Fig. 1, B and D). It should be noted that the activity of galactosyltransferase was comparable in the parents and about 1.5–2-fold higher than in control and patient’s membranes (Fig. 1C). These data would be compatible with the view that the impaired import of GDP-fucose in the patient’s vesicles is secondary to a defect in a gene unrelated to the GDP-fucose transporter and that only homozygosity for a defect in this gene of unknown function affects GDP-fucose import. Such a gene could encode for a cofactor of the transporter. Alternatively the affected gene could encode for one of the transporter subunits if the transporter for GDP-fucose is a heterooligomer. It should be noted, however, that the only sugar nucleotide transporter for which the quaternary structure has been examined is likely to exist as a homodimer (29). The molecular nature of the GDP-fucose transporter is unknown. Cloning of the gene complementing the GDP-fucose import in our patient may help to identify its molecular nature.

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