Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I

Emile Van Schaftingen[a], Jaak Jaeken[b]

[a]*Laboratory of Physiological Chemistry, ICP and University of Louvain, Louvain, Belgium*
[b]*Department of Pediatrics, University of Leuven, Leuven, Belgium*

Received 13 November 1995

Abstract Carbohydrate-deficient glycoprotein (CDG) syndromes are genetic multisystemic disorders characterized by defective *N*-glycosylation of serum and cellular proteins. The activity of phosphomannomutase was markedly deficient (≤ 10% of the control activity) in fibroblasts, liver and/or leucocytes of 6 patients with CDG syndrome type I. Other enzymes involved in the conversion of glucose to mannose 1-phosphate, as well as phosphoglucomutase, had normal activities. Phosphomannomutase activity was normal in fibroblasts of 2 patients with CDG syndrome type II. Since this enzyme provides the mannose 1-phosphate required for the initial steps of protein glycosylation, it is concluded that phosphomannomutase deficiency, which is first reported here for higher organisms, is a cause, and most likely the major one, of CDG syndrome type I.

Key words: Carbohydrate-deficient glycoprotein syndrome; Phosphomannomutase; Mannose 1-phosphate; Protein glycosylation

1. Introduction

Carbohydrate-deficient glycoprotein (CDG) syndromes are a family of multisystemic genetic diseases in which secretory glycoproteins, lysosomal enzymes and probably also membrane proteins are underglycosylated. Since its first report in 1980 [1], > 100 patients have been identified [2]. Three clinical variants have been described, of which type I is by far the most frequent. Type II is due to a deficiency in *N*-acetylglucosaminyltransferase II, a Golgi enzyme, and is therefore a defect in the late stages of glycosylation [3–4]. The basic defect in type I is still unknown; the fact that the structure of the N-linked oligosaccharides on transferrin is normal but that their total number is decreased points to a disturbance in the early steps of glycosylation, possibly in the formation of the dolichol-pyrophosphate oligosaccharide precursor or in its transfer to the acceptor protein [5–7]. Accordingly, there is a decreased incorporation of [^{3}H]mannose into N-linked oligosaccharides and in their lipid precursors in fibroblasts of patients with CDG syndrome type I [8].

In the present work, we have explored the possibility that a deficiency of one of the enzymes involved in the conversion of glucose to mannose 1-phosphate was responsible for this disease.

*Corresponding author. UCL 7539, avenue Hippocrate 75, B-1200 Brussels, Belgium. Fax: (32) (2) 764 7598.

2. Materials and methods

2.1. Materials

Mannose 6-phosphate, mannose 1-phosphate and yeast phosphomannoisomerase were from Sigma. Other auxiliary enzymes and NADP were from Boehringer Mannheim. Mannose 1,6-bisphosphate was prepared by incubating 0.3 mM mannose 1-phosphate and 0.2 mM glucose 1,6-bisphosphate in the presence of 50 mM Hepes pH 7.1, 5 mM $MgCl_2$, 0.25 mM NADP, 1 mM dithiothreitol, 5 μg/ml yeast glucose 6-phosphate dehydrogenase, and 10 μg/ml muscle phosphoglucomutase for 1 h at 30°C. The reaction was followed by the change in A_{340} and arrested by heating the mixture for 5 min at 80°C.

2.2. Preparation of cell extracts

Fibroblasts (passage 5 to 12) were grown till confluency in Dulbecco's Modified Eagle's Medium supplemented with 10% calf serum. The dishes were washed with 150 mM NaCl and the cells were scraped, pelleted by centrifugation and stored at −80°C until further use. Cell extracts were prepared by resuspending the cell pellet in the homogenization buffer, which contained 20 mM Hepes, 25 mM KCl, 1 mM dithiothreitol and 10 μg/ml each leupeptin and antipain. The suspension was frozen at −50°C, thawed and centrifuged at 10,000 × *g* for 5 min. The resulting extract was used for enzymatic assay and protein determination. Liver samples were homogenized in the same buffer, using a glass homogenizer, and centrifuged as described above. Leucocytes were isolated from ~ 10 ml of heparinized blood by the dextran procedure [9]. The concentration of proteins in the extracts ranged from 3 to 10 mg protein/ml.

2.3. Enzyme and protein assays

All enzymes were assayed spectrophotometrically at 30°C by the reduction of NADP to NADPH; the change in A_{340} was measured during 60 min (phosphomannose isomerase, phosphomannomutase) or 10–15 min (other enzymes). The assay mixtures contained 50 mM Hepes pH 7.1, 5 mM $MgCl_2$, 0.25 mM NADP and 10 μg/ml yeast glucose 6-phosphate dehydrogenase. Other additions were: for the hexokinase assay, 1 mM glucose, 1 mM ATP·Mg and 5 μl extract; for the phosphoglucomutase assay, 0.5 mM glucose 1-phosphate, 1 μM glucose 1,6-bisphosphate and 2 μl extract; for the phosphoglucose isomerase assay, 1 mM fructose 6-phosphate and 2 μl extract; for the phosphomannoseisomerase assay, 0.5 mM mannose 6-phosphate, 10 μg/ml phosphoglucose isomerase, 2 (instead of 10) μg/ml glucose 6-phosphate dehydrogenase and 5 μl extract; for the phosphomannomutase assay, 0.1 mM mannose 1-phosphate, 1 μM mannose 1,6-bisphosphate, 10 μg/ml phosphoglucose isomerase and 3.5 μg/ml phosphomannose isomerase. All incubations were carried out with or without substrate, and the difference between the two values was taken as the enzymic activity. 1 U enzyme is the activity corresponding to the formation of 1 μmol NADPH/min under the assay conditions. Protein was measured according to Bradford [10] with bovine gamma globulin as a standard. Results are expressed as individual values or as mean ± SD.

3. Results and discussion

Phosphomannomutase was much less active than other enzymes involved in the conversion of glucose to mannose 1-phosphate in control fibroblasts and ~50× less active than

0014-5793/95/$9.50 © 1995 Federation of European Biochemical Societies. All rights reserved.
SSDI 0014-5793(95)01357-1

Table 1
Enzymic activities in fibroblasts of controls and patients with CDG syndromes types I and II

	Phosphomannose isomerase (mU/mg protein)	Phosphomannomutase (mU/mg protein)	Phosphoglucomutase (mU/mg protein)
Controls*	14.6 ± 4.1	1.32 ± 0.37	72 ± 14
CDG syndrome type I**			
HS	13.2, 10.2	≤0.1, ≤0.1	61, 42, 37
PA	6.3, 10.0	≤0.1, ≤0.1	66, 68
PS	16.4, 10.0	≤0.1, ≤0.1	41, 53, 53
BJ	14.8, 10.5	≤0.1, ≤0.1	65, 63
CDG syndrome type II**			
BM	19.6, 11.4	1.4, 1.3	65, 87
VJ	17.3, 11.7	1.9, 1.1	76, 66

*Mean values ± SD for 10–12 fibroblasts pellets derived from 5 controls.
**Individual values: PA and PS, the patients first reported [1] are homozygotic twin girls, aged 19 in 1995; HS and BJ are also girls with a typical CDG syndrome type I; both died around the age of 2 years.

phosphoglucomutase (Table 1). The activities of hexokinase (19.2 ± 6.7 mU/mg protein in controls, $n = 9$), phosphoglucose isomerase (300 ± 68 mU/mg protein in controls, $n = 10$), phosphomannose isomerase, and phosphoglucomutase (Table 1) were not altered in fibroblasts from patients with CDG syndrome type I, in agreement with the results reported by Paneerselvam and Freeze [11], and in fibroblasts of 2 patients with CDG syndrome type II. In sharp contrast, phosphomannomutase activity was undetectable (≤ 10% of the mean control value) in fibroblasts of 4 patients with CDG syndrome type I. It was normal, however, in fibroblasts of patient with CDG syndrome type II (Table 1), indicating that the decrease of activity was not the consequence of deficient glycoprotein synthesis.

Liver biopsy specimens were available from 2 patients with CDG syndrome type I (BJ and HS). Phosphomannomutase was also deficient in this tissue, its activity being ≤0.1 mU/mg protein in patients as compared to 0.94 ± 0.37 in 4 controls, whereas phosphoglucose isomerase, phosphomannose isomerase and phosphoglucomutase were normal. To rule out the possibility that phosphomannomutase deficiency was due to the presence of an inhibitor, assays were carried out in mixtures of liver homogenates from patient HS and from a control. No inhibition of the control enzyme was observed.

Phosphomannomutase deficiency was also found in leucocytes of 2 additional patients (female, 10 years old, named LA and SF) with CDG syndrome type I, the activity being ≤0.05 mU/mg protein as compared to 0.67 ± 0.29 mU/mg protein for 7 controls. The 3 parents of these children who were available for testing had an intermediate activity (≤0.32 ± 0.02 mU/mg protein). This result indicates that phosphomannomutase deficiency is due to a mutation in the gene coding for this enzyme rather than in a regulatory gene.

The fact that phosphomannomutase is deficient whereas phosphoglucomutase is normal confirms the finding that these two enzymes are distinct entities in mammalian tissues [12]. A deficiency in the former enzyme is expected to deprive the cells from the mannose 1-phosphate and the GDP-mannose that are needed for the synthesis of dolichol-pyrophosphate-oligosaccharides. Accordingly, mutations in the phosphomannomutase gene result in defective glycosylation and in a lethal phenotype in yeast [13]. Our results indicate that phosphomannomutase deficiency is the cause of hypoglycosylation in many if not all patients with CDG syndrome type I.

A linkage study in 25 European families has enabled to map the CDG1 gene to chromosome 16p13.3-p13.12 [14]. The authors of this work stressed that there was no evidence for heterogeneity among the families they tested. Since 3 cases of phosphomannomutase deficiency (PS, PA and HS; see Table 1) were included in the study of Martinsson et al. [14], it is likely that the CDG1 gene mapped by these authors encodes phosphomannomutase.

GDP-mannose is also required for the synthesis of other mannosylated conjugates, such as the glycosylphosphatidylinositol membrane anchors [15] or some O-mannosylated brain proteoglycans [16]. Phosphomannomutase deficiency should also alter these biosynthetic processes, which could explain, at least in part, the clinical differences between CDG syndromes types I and II [2]. Knowledge of the enzymatic lesion should now allow prenatal diagnosis and the identification of the mutations. It is worth mentioning in this respect that phosphomannomutase is expressed in amniocytes, its activity amounting to 0.70 ± 0.02 mU/mg protein in 3 samples.

Acknowledgements: We are indebted to Dr. J.-J. Cassiman for providing the fibroblasts and to G. Berghenouse for her competent technical help. Supported by the Belgian National Fund for Scientific Research, by the Actions de Recherche Concertées and by the Belgian Federal Service for Scientific, Technical and Cultural Affairs.

References

[1] Jaeken, J., Vanderschueren-Lodeweyckx, M., Casaer, P., Snoeck, L., Corbeel, L., Eggermont, E. and Eeckels, R. (1980) Pediatr. Res. 14, 179.

[2] Jaeken, J., Carchon, H. and Stibler, H. (1993) Glycobiology 3, 423–428.

[3] Jaeken, J., Schachter, H., Carchon, H., De Cock, P., Coddeville, B. and Spik, G. (1994) Arch. Dis. Childhood 71, 123–127.

[4] Charuk, J.H.M., Tan, J., Bernardini, M., Haddad, S., Reithmeier, R.A.F., Jaeken, J. and Schachter, H. (1995) Eur. J. Biochem. 230, 797–805.

[5] Wada, Y., Nishikawa, A., Okamoto, N., Inui, K., Tsukamoto, H., Okada, S. and Taniguchi, N. (1992) Biochem. Biophys. Res. Commun. 189, 832–836.

[6] Yamashita, K., Ideo, H., Ohkura, T., Fukushima, K., Yuasa, I., Ohno, K., and Takeshita, K. (1993) J. Biol. Chem. 268, 5783–5789.

[7] Krasnewich, D.M., Holt, G.D., Brantly, M., Skovby, F., Redwine, J. and Gahl, W.A. (1995) Glycobiology 5, 503–510.
[8] Powell, L.D., Paneerselvam, K., Vij, R., Diaz, S., Manzi, A., Buist, N., Freeze, H. and Varki, A. (1994) J. Clin. Invest. 94, 1901–1909.
[9] Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, Suppl. 97, 77–89.
[10] Bradford M.M. (1976) Anal. Biochem. 72, 248–254.
[11] Panneerselvam, K. and Freeze, H.H. (1995) Biochem. Biophys. Res. Commun. 208, 517–522.
[12] Guha S.K. and Rose Z.E. (1985) Arch. Biochem. Biophys. 243, 168–173.
[13] Kepes, F. and Schekman, R. (1988) J. Biol. Chem. 263, 9155–9161.
[14] Martinsson, T., Bjursell, C., Stibler, H., Kristiansson, B., Skovby, F., Jaeken, J., Blennow, G., Strömme, P., Hanefeld, F. and Wahlström, J. (1994) Hum. Mol. Genet. 3, 2037–2042.
[15] Stevens, V.L. (1995) Biochem. J. 310, 361–370.
[16] Krusius, T., Reinhold, V.N., Margolis, R.K. and Margolis, R.U. (1987) Biochem. J. 245, 229–234.