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The carbohydrate moiety of human glycophorin in CDG syndrome*°

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Human glycophorin, the major sialoglycoprotein of erythrocyte membranes, was isolated from erythrocytes of healthy individuals and four patients with CDG syndrome. Sugar analysis revealed lower carbohydrate content in three out of four CDG-glycophorin samples. In order to characterize closer the glycosylation differences between glycophorin samples in health and disease, reaction with four biotinylated lectins was performed, using ELISA procedure on polystyrene microplates. Results obtained so far strongly suggest that both N- and O-glycans of glycophorin are affected in CDG syndrome.

The carbohydrate-deficient glycoprotein syndrome (CDGS), first recognized in 1984 [1], is a group of multisystemic genetic diseases characterized by severe disorders of the nervous system, including growth and psychomotor retardation; it is accompanied with

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Abbreviations: CDGS, carbohydrate deficient glycoprotein syndrome; GPA, glycophorin A from human erythrocyte membranes; GPA-CDG, glycophorin A isolated from erythrocytes of CDG patients; GPA-A, glycophorin A isolated from erythrocytes of blood group A; GPA-OM, glycophorin A isolated from erythrocytes of blood group O with M phenotype; GPA-ON, glycophorin A isolated from erythrocytes of blood group O with N phenotype.

abnormal distribution of adipose tissue, squint and abnormal ocular movements. In most clinical cases described so far, patients represent CDGS type I (untill now less than 200 cases found) [2]; three other much less common types were also described [3–5]. Deficiency of glycoprotein glycosylation in CDGS was evidenced for many serum glycoproteins, e.g. transport proteins, glycoprotein hormones, complement factors, enzymes and enzyme inhibitors. Among these proteins transferrin, one of the transport proteins, is a characteristic and important diagnostic indicator of the disease [6]. A characteristic feature of transferrin in CDGS is that it is not glycosylated in one, or both, of the two N-glycosylation sites. The other glycoproteins in CDGS exhibit analogous property, i.e. they lack oligosaccharides in some of their N-glycosylation sites. The main reason for this not complete N-glycosylation, recognized so far, is phosphomannomutase (PMM) deficiency [7]. PMM is the enzyme responsible for the conversion of Man-6-P into Man-1-P, thus it is involved in the biosynthesis of the dolichol-pyrophosphate oligosaccharide precursor of N-glycosylation.

In numerous papers on CDGS it is suggestd that not only secretory, but also membrane glycoproteins have deficient carbohydrate moiety. In order to get an insight into this problem, we decided to analyze human glycophorin A, which is the major sialoglycoprotein of erythrocyte membranes [8]. Glycophorin contains two types of oligosaccharides, i.e. one N-glycan and about 15 O-glycans. Glycophorin has been the subject of numerous structural investigations, therefore its carbohydrate moiety is fairly well known and may be compared among different samples. In the present investigation we isolated four samples of GPA from CDGS erythrocytes and four samples from erythrocytes of healthy people. These samples were analyzed for total sugar content and were assayed, using the ELISA method, regarding their reaction with different lectins.

MATERIALS AND METHODS

Glycophorins. Glycophorins were isolated from membranes of outdated human erythrocytes by phenol-water extraction [9]. Blood samples of CDGS type I patients were obtained as follows: CDG-① (patient S.F., blood group O) and ②, (patient H.N., blood group A) from Professor J. Jaeken (Department of Pediatrics, University of Leuven, Belgium); CDG-④ (patient T.O., blood-group O) from Professor A. Lundblad (Department of Clinical Chemistry, Linköping University, Sweden). Sample CDG-③ (blood group A) was obtained by us from erythrocytes of a girl (L.M.), living in Białystok (Poland).

Gas liquid chromatography-mass spectrometry (GLC-MS) analysis. The samples of glycophorin were hydrolyzed in 4 M trifluoroacetic acid at 100°C for 4 h. After evaporation to dryness they were supplemented with xylose as an internal standard, then derivatized into alditol acetates and analyzed in a gas-liquid chromatograph as described previously [10].

Lectins. The lectins used in this investigation: *Arachis hypogaea* (PNA), *Phaseolus vulgaris* (PHA-E), *Canavalia ensiformis* (Con A) and *Ulex europaeus* (UEA) were purchased from Sigma (U.S.A.). They were biotinylated according to the described procedure [11], using biotinamidocaproate-*N*-hydroxysuccinimide ester.

ELISA test. It was performed on polystyrene plates as described [11]. The glycophorin was loaded in the wells in 0.05 M carbonate buffer, pH 9.6, at 4°C overnight. Before reaction with the PNA lectin the glycophorin, loaded in the wells, was desialylated with 0.05 M sulfuric acid at 60°C for 4 h. To avoid evaporation the wells were covered with 50 μl aliquots of the liquid hydrocarbon Pristan. For the generation of colour product a conjugate of ExtrAvidin with alkaline phosphatase was used.

Protein content. Protein content was determined using bicinchoninic acid method on a micro scale (polystyrene microplates) [12].

RESULTS AND DISCUSSION

Sugar analysis

Four samples of glycophorin were isolated from erythrocytes of four CDGS patients; two of them were of blood-group A and two of blood-group O. For comparison purposes four GPA samples were prepared from erythrocytes of healthy subjects: one of blood-group A, one of blood-group B and two of blood-group O; the latter represented two different phenotypes – one M and one N, which corresponds to the blood-group MN system [8]. These eight samples were analyzed by GLC-MS regarding their carbohydrate content and the results, recalculated for the same amount of protein in each analyzed sample (100 μg), are summarized in Table 1. As can be seen the total sugar content in GPA-CDG samples is lower than in normal GPA samples, with one exception of sample CDG-①, which showed a relatively high sugar content. In GPA samples from normal erythrocytes the lowest total sugar content was found in two samples derived from O erythrocytes (mean value 168 μg), whereas GPA-A and GPA-B samples had substantially more sugars. When one compares CDG-④ sample (derived from O erythrocytes) with GPA-O samples, the content of total sugars in CDG-④ is less by about 30%. Accordingly, CDG-② and CDG-③, both derived from erythrocytes A, have less sugars in comparison with the GPA-A sample by 55% and 58%, respectively. A closer examination of Table 1 reveals also that all CDG samples contain less GalNAc, when compared with GPA samples from normal erythrocytes. This result suggests lower content of O-glycans in CDG samples; it has a direct confirmation in experiments with *Arachis hypogaea* lectin (see below).

CDG-① sample, as can be seen from Table 1, did not exhibit lower sugar content when compared with GPA samples from normal erythrocytes. One explanation of this feature may be the presence in CDG-① of some impurities of non-glycoprotein type, containing carbohydrates (e.g. glycolipids); the other probable explanation refers to a published paper on sugar analysis of IgG preparations derived from two CDG patients [13]. The authors of the paper found that the total sugar content of one of those IgG samples was lower in comparison with controls, whereas the second IgG sample exhibited substantially unchanged total carbohydrates. The only explanation given in that paper was that the first patient had typical manifestations of CDGS type I,

Table 1. Carbohydrate content (μg) of different glycophorin preparations.

The results presented are re-calculated for the same amount of protein in the analyzed samples (100 μg).

Glycoprotein preparation	Fuc	Man	Gal	GlcNAc	GalNAc	Total sugar content
GPA-OM	4.3	7.6	68.6	17.6	70.0	168.1
GPA-ON	5.2	10.5	89.4	17.7	44.9	167.7
GPA-A	6.9	13.8	120.0	55.9	93.6	290.2
GPA-B	7.9	12.1	100.6	50.0	89.7	260.3
CDG-① (O)	16.7	23.5	123.0	40.2	41.3	244.7
CDG-② (A)	5.4	9.9	50.4	23.7	42.0	131.4
CDG-③ (A)	5.4	16.3	45.3	24.8	31.5	123.3
CDG-④ (O)	3.1	5.5	64.5	21.4	23.5	118.0

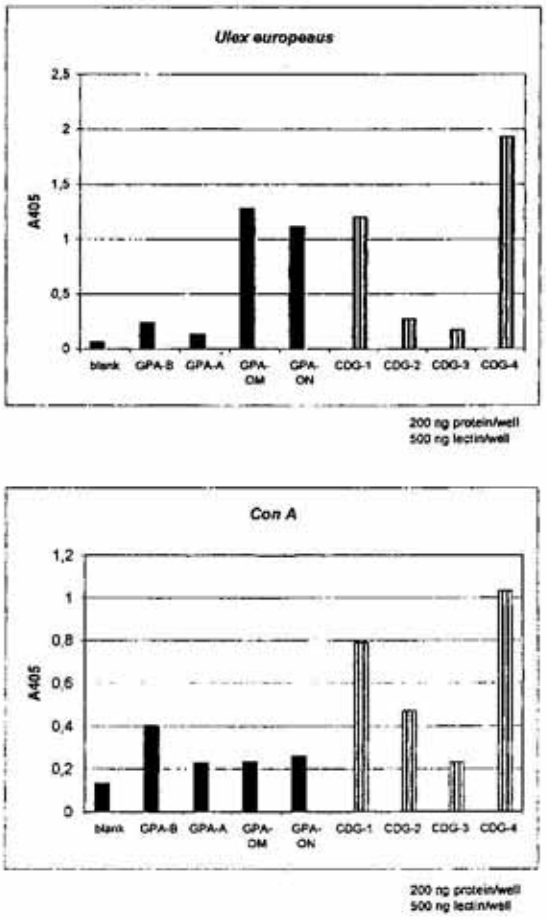

Figure 1.

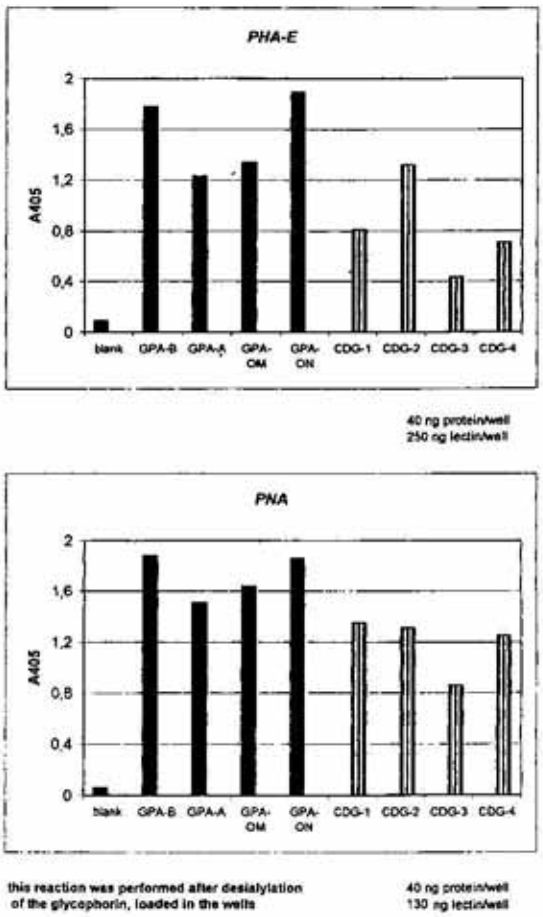

Figure 2.

whereas the second one had a variant form, showing a less clearcut clinical picture. It is possible that a similar case regards our patient, from whom CDG-① sample was derived.

Reaction with lectins

Reaction of four GPA samples derived from healthy persons and four GPA samples derived from CDG patients was performed by the ELISA method, using biotinylated lectins and polystyrene microplates; the results are summarized in Fig. 1. They may be described as follows:

***Arachis hypogaea* lectin (PNA).** This lectin reacts with sugar structures terminating with β-Gal residues, in human glycophorin preferably with O-linked disaccharides Galβ1-3GalNAc [14]. Because these structures are masked in native glycophorin with sialic acid, this determination was performed after desialylation of glycophorin loaded in the wells. As can be seen, the reaction of all four CDG glycophorin samples is lower than the reaction of GPA from normal erythrocytes; the lowest reaction was given by sample CDG-③. Reaction with the PNA lectin is in agreement with sugar analysis, regarding GalNAc content (Table 1). Both results strongly suggest that in GPA-CDG there is a smaller number of O-glycans, in comparison with GPA from normal erythrocytes. Reaction of native GPA-CDG samples with PNA was negligible (data not shown), which indicates that there are no disaccharide chains in native glycophorin from CDG patients.

***Phaseolus vulgaris* lectin (PHA-E).** This lectin is known to recognize biantennary N-

glycans with a bisecting GlcNAc, i.e. a structure found in the erythrocyte glycoproteins [15]. As can be seen, three out of four GPA-CDG samples gave a weaker reaction in comparison with GPA samples from healthy people. A weaker reaction should mean less N-glycans in CDG samples. It is difficult to explain the normal reaction of CDG-② sample with PHA-E lectin.

***Canavalia ensiformis* lectin (Con A).** This lectin reacts, among others, with biantennary N-glycans without the bisecting GlcNAc [15]. From our previous experiments on human glycophorin we know that there is some amount of such oligosaccharide in the GPA (B. Nilsson & H. Krotkiewski, unpublished). Here we have a higher reaction of GPA-CDG samples, with the exception of CDG-③ in comparison with GPA from healthy people (Fig. 2). The only explanation for this effect may be that lower number of O-glycans in CDG samples facilitates the access of Con A to its ligand sugar in the GPA molecule.

***Ulex europaeus* lectin (UEA).** This lectin recognizes H determinant, i.e. non-reducing disaccharide Fucα1-2Galβ1-... [15], which may be present on O-glycans and/or N-glycans. The highest reaction was observed for GPA samples, derived from O erythrocytes, both of healthy people and CDG patients. This experiment did not give any unusual results, CDG-① sample gave as high a reaction as GPA from normal erythrocytes, only CDG-④ sample gave a higher reaction, which could mean a better availability of its sugar ligands for the UEA lectin.

Our preliminary results strongly suggest that human glycophorin A is underglycosylated in CDG syndrome. The observed defect seems to be a consequence of a lower number of O-glycans and, most probably, also of N-glycans. This is one of the first reports on the deficiency of glycosylation of membrane glycoproteins in CDG syndrome. There is a strong need for more experiments, carried out on other glycophorin samples derived from CDG erythrocytes, to confirm the presented results.

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