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# Increased α3-fucosylation of $\alpha_1$-acid glycoprotein in patients with congenital disorder of glycosylation type IA (CDG-Ia)[1]

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**Abstract Increased fucosylation of the type (sialyl) Lewis$^x$ was detected on the acute-phase plasma protein $\alpha_1$-acid glycoprotein (AGP) in patients with the congenital disorder of glycosylation type IA. This is remarkable, because in these patients the biosynthesis of guanosine 5′-diphosphate (GDP)-D-mannose is strongly decreased, and GDP-D-mannose is the direct precursor for GDP-L-fucose, the substrate for fucosyltransferases. The concomitantly occurring increased branching of the glycans of AGP and the increased fucosyltransferase activity in plasma suggest that a chronic hepatic inflammatory reaction has induced the increase in fucosylation.** 

*Key words:* Congenital disorder of glycosylation type IA; $\alpha_1$-Acid glycoprotein; Sialyl Lewis$^x$; Fucosylation; Inflammation; Branching

## 1. Introduction

Congenital disorder of glycosylation type IA (CDG-Ia) is a genetic multisystem disorder, characterized by hypoglycosylation of *N*-linked glycans of glycoproteins due to phosphomannomutase deficiency, a key enzyme in the biosynthesis of guanosine 5′-diphosphate (GDP)-D-mannose (reviewed in [1]). The decreased availability of GDP-D-mannose results in a hypo-*N*-glycosylation of numerous plasma glycoproteins, e.g. transferrin, α1-anti-trypsin, haptoglobin β-chain and $\alpha_1$-acid glycoprotein (AGP) showing cathodical shifts upon electrophoresis. This is caused by the lack of one or more complete *N*-linked glycans and the resulting reduction in the number of sialic acid residues [1,2].

GDP-D-mannose is the precursor for the biosynthesis of GDP-L-fucose [3–7], the L-fucose donor for fucosyltransferases. Therefore, an impairment of the fucosylation of glycoproteins is to be expected in CDG-Ia patients. Fucosylated glycan structures play prominent roles, for example, in the development of tissues and in the selectin-mediated leukocyte–endothelial interactions. In this regard, especially α3-linked fucose residues in the sialyl Lewis$^x$ configuration are important as counter-receptors for selectins [8].

In this study we have investigated the fucosylation of various plasma acute-phase glycoproteins, and in particular AGP, in patients with CDG-Ia. Crossed affinity immunoelectrophoresis (CAIE) with concanavalin A (Con A) and *Aleuria aurantia* lectin (AAL) was used to characterize the glycosylation of the plasma glycoproteins. High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of the PNGase-F released glycans was applied to confirm the CAIE findings for AGP. In addition, the plasma α3-fucosyltransferase activity was measured as a marker for the hepatic α3-fucosylation capacity [9].

## 2. Materials and methods

### 2.1. Materials

Con A Type V was obtained from Sigma (St. Louis, MO, USA) and AAL and the AAL-HiTrap column from Biomed Labs (Newcastle-upon-Tyne, UK). The Carbopac PA-100 column was obtained from Dionex (Sunnyvale, CA, USA) and the Mono-Q column and 1 ml desalting columns from Pharmacia (Uppsala, Sweden). Monospecific goat anti-human AGP anti-serum was a kind gift of Dr. A. Mackiewicz (Poznan, Poland). Rabbit anti-human $\alpha_1$-anti-chymotrypsin and anti-$\alpha_1$-protease inhibitor were purchased from Dakopatts (Glostrup, Denmark). GDP-[$^{14}$C]fucose, specific radioactivity 5.15 Ci/ml, was obtained from NEN (Boston, MA, USA). All other materials used were of analytical grade and obtained from commercial sources.

### 2.2. Sources of plasma

Eight typical CDG-Ia patients were studied; six of these patients have been described [10,11]. Control blood plasma was taken from healthy volunteers. Informed consent was obtained for all plasma samples.

### 2.3. CAIE with Con A or AAL

In order to determine the reactivities of AGP, $\alpha_1$-anti-chymotrypsin and $\alpha_1$-protease inhibitor with Con A and AAL, total plasma was subjected to CAIE as described previously [12]. In short, 2 μl of plasma was subjected to electrophoresis in a polyacrylamide gel containing Con A or AAL to fractionate the glycoproteins into a non-retarded and various retarded glycoforms. The glycoforms were detected by immunoelectrophoresis in a second dimension agarose gel containing mono-specific precipitating antibodies against one of the glycoproteins and the precipitates were visualized as described [12].

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*2.4. Characterization of AGP and its glycans*

AGP was isolated from 0.5 ml of plasma [13]. SDS–PAGE of purified AGP, blotting on nitrocellulose and detection with anti-AGP antibodies was performed as detailed elsewhere [12]. Treatment of 100 μg of pure preparations of AGP with PNGase F and recovery of the released glycans was performed as described earlier [13]. The lyophilized glycans were dissolved in 200 μl phosphate buffered saline (PBS; pH 8.6) and subjected to affinity chromatography on a 5 ml AAL-HiTrap column in PBS (1 ml/min). Non-retarded glycans (V0) were collected in the void volume of the column and bound glycans were eluted with 10 mM *L*-fucose in PBS (Vf). After desalting over a Bio-Gel P2 column in milliQ water the Vf fraction was further subfractionated according to charge by Mono-Q chromatography into Vf-1/2, Vf-3 and Vf-4 (see scheme in Fig. 1). The Vf-1/2 fraction was discarded because the amount of glycans was below detectable levels on HPAEC-PAD. After desalting and lyophilization the Fractions V0, Vf-3 and Vf-4 were dissolved in milliQ water and analyzed by HPAEC-PAD using a Dionex PA-100 column (0.4×25 cm) [13]. The column was calibrated with non-fucosylated as well as α6- or α3-fucosylated di-, tri- and tetrasialylated complex type oligosaccharides that had been characterized by $^1$H-nuclear magnetic resonance.

*2.5. Assay of α3-fucosyltransferase activity*

The activity of plasma α3-fucosyltransferase in plasma was determined using calf fetuin as an acceptor [14]. All assays were performed in duplicate for 16 h in 10 μl of plasma.

## 3. Results

*3.1. Molecular weight composition and lectin reactivity of AGP*

At least two additional bands were detected for AGP of all CDG-Ia patients on nitrocellulose blots of SDS–PAGE gels in comparison to AGP obtained from controls (Fig. 2). The bands showed a comparable cathodical shift to normal AGP after desialylation (Fig. 2, lanes b). So, the lower molecular weight bands do not represent undersialylation of the glycans, but instead represent the lack of one or more glycans in accordance with the defect of CDG-Ia [1].

Analysis of the lectin reactivity of AGP with CAIE revealed that the reactivity with Con A was decreased (Fig. 3) and with AAL was increased in all patients (Fig. 4) in comparison to normal AGP. The decrease in Con A reactivity was about the same for all patients indicating a reduction of diantennary complex type *N*-linked glycans on AGP [15]. In six out of the eight patients studied a very strong increase in reactivity with the fucose-specific AAL was found (Fig. 4d); the two other patients showed an intermediate and a low increase in AAL reactivity (Fig. 4b,c). The increased AAL reactivities

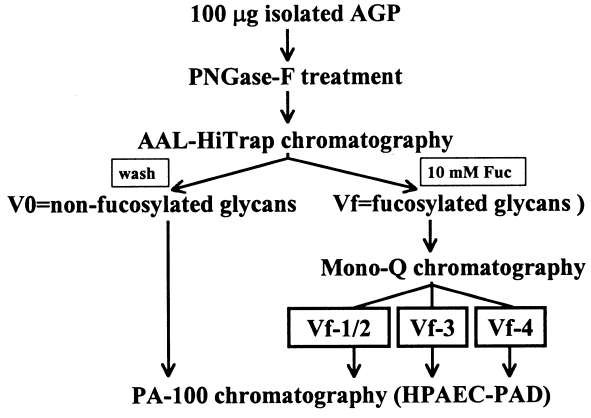


Fig. 1. Scheme of the subfractionation of PNGase-F released glycans from AGP prior to HPAEC-PAD analysis.

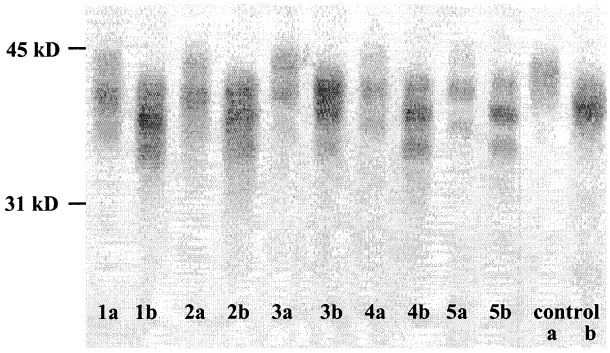


Fig. 2. Molecular weight compositions of native (a) and sialidase treated (b) AGP of CDG-Ia patients and control AGP. AGP was detected on nitrocellulose blots after SDS–PAGE of immunoprecipitated AGP from patients P.A. (1), P.S. (2), dB.E. (3), R.M. (4) and V.A. (5) as well as from control AGP. The same results were obtained for J.I., A.V. and L.D.

indicate an elevated fucosylation of the glycans of AGP in the CDG-Ia patients. Corresponding increases in AAL reactivities were also found for two other acute-phase proteins, $\alpha_1$-anti-chymotrypsin and $\alpha_1$-protease inhibitor I (results not shown).

*3.2. Characterization of the PNGase-F released glycans by HPAEC-PAD*

HPAEC-PAD analysis of the PNGase-F released glycans of purified AGP was performed in order to investigate (a) whether the strong increase in AAL reactivity indeed resulted from an increase in fucosylation and (b) when so, whether α3- or α6-linked fucose residues were responsible. HPAEC-PAD

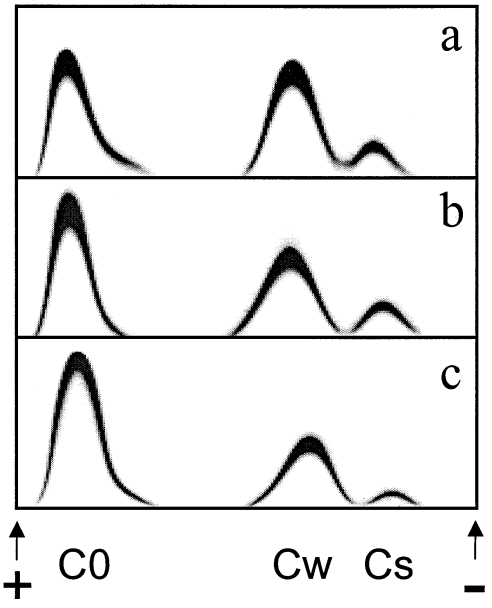


Fig. 3. CAIE with Con A of AGP. a: Control plasma. b: Representative pattern for patients P.A. and P.S. c: Representative pattern for dB.E., R.M., V.A., I.J., D.L. and V.A. CAIE was performed as described in Section 2. The application site for the electrophoresis of plasma samples in the first dimension gel was on the right-hand side of each figure; only the second dimension gels are shown. C0, non-retarded AGP; Cw, weakly Con A-reactive AGP; Cs, strongly Con A-reactive AGP. All AGP glycoforms were recovered at C0 when Con A was omitted from the first dimension gel.

analysis using the PA-100 column is well suited for these aims, because fucosylated structures will elute earlier than the corresponding non-fucosylated structure. α6-Linked fucose on the chitobiose core of *N*-linked glycans induces a shift of −1.8 min, whereas α3-linked fucose on a lactosaminyl unit of a glycan will result in a cumulative shift of −5 to −6 min per fucose residue present [16]. To prevent the occurrence of overlapping peaks, the PNGase-F released glycans were subfractionated according to the presence of fucose residues and subsequently according to charge prior to analysis on HPAEC-PAD (Fig. 1). The analyses were performed for strongly AAL-reactive AGP obtained from the identical twin sisters P.A. and P.S. (cf. Fig. 4d) and for low AAL-reactive control AGP. In the case of the patients, the majority of the free glycans bound strongly to the AAL-HiTrap column (fraction Vf), in accordance with their AAL reactivity in CAIE, whereas for control AGP no more than 20% of the glycans were recovered (not shown). The great majority of the Vf fraction consisted of fucosylated tri- and tetrasialylated glycans (Vf-3, respectively, Vf-4), in view of the fact that mono- and disialylated glycans were undetectable after Mono-Q chromatography in this fraction. The HPAEC-PAD profiles obtained for V0, Vf-3 and Vf-4 of P.A. (Fig. 5, left) were identical to those of her twin sister P.S. (not shown), but differed markedly from control (Fig. 5, right). CDG-Ia AGP appeared to have a higher content of tri- and tetraantennary glycans with two or three fucose residues than the control. This is concluded from the much lower recovery of materials at elution positions of standard non-fucosylated tri- and tetrasialylated glycans in V0 than in control AGP, and the increased recovery at positions of standard di- and trifucosylated glycans in Vf-3 and Vf-4 in the case of CDG-Ia AGP. Apparently, the glycans present in Vf-3 and Vf-4 did not contain α6-linked fucose, but only α3-linked residues, because of the absence of eluted materials close to (i.e. at −1.8 min) the positions of the corresponding non-fucosylated standards. Finally, it can be deduced from the various HPAEC-PAD profiles that in the patients the content of diantennary glycans of AGP was lower than in control AGP in agreement with the decreased reactivity with Con A in CAIE (cf. Fig. 3).

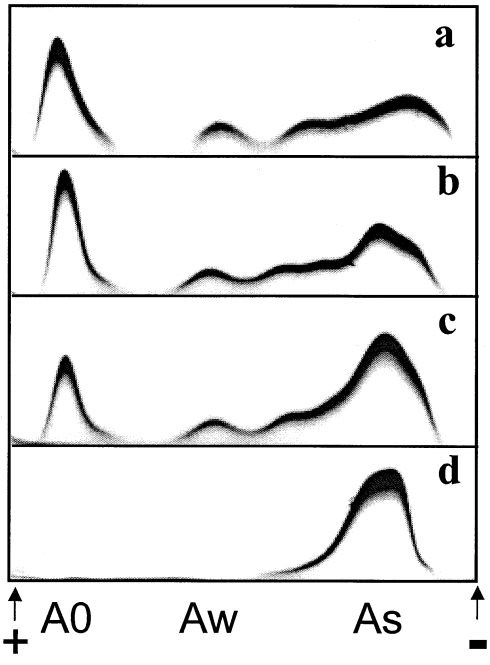


Fig. 4. CAIE with AAL of AGP. a: Control plasma. b: Patient dB.E. c: Patient R.M. d: Representative pattern for patients P.A., P.S., L.S., V.A., I.J., D.L. and S.L. A0, non-retarded AGP; Aw, weakly AAL-reactive AGP; As, strongly AAL-reactive AGP. All AGP glycoforms were recovered at A0 when AAL was omitted from the first dimension gel. See legend of Fig. 3 for further details.

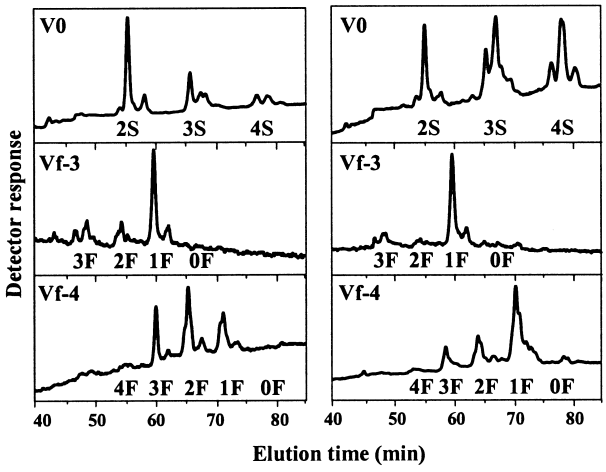


Fig. 5. HPAEC-PAD analysis of PNGase-F released glycans. The PNGase-F released glycans were subfractionated according to the scheme in Fig. 1 and subjected to HPAEC-PAD analysis on the PA-100 column. V0, non-retarded fraction on AAL-HiTrap column; Vf-3 and Vf-4, tri-, respectively, tetrasialylated AAL-HiTrap binding fraction as obtained by Mono-Q anion-exchange chromatography. Left panel: Patient P.A; right panel: control. 2S, 3S and 4S represent elution positions of standard di-, tri-, respectively, tetrasialylated complex type oligosaccharides; 0F, 1F, 2F, 3F and 4F represent standard non-, mono-, di-, tri-, respectively, tetra-α3-fucosylated complex type trisialylated (middle panels) or tetrasialylated (lower panels) oligosaccharides.

### 3.3. Activity of plasma α3-fucosyltransferase

The plasma α3-fucosyltransferase activity was higher (4.1 ± 0.4 pmol/min/ml) in the five patients with strongly AAL-reactive AGP, than in control plasma (2.5 ± 0.9 pmol/min/ml). The enzyme activity was normal (1.9 pmol/min/ml) in the plasma of patient dB.E. were the AAL reactivity of AGP was only slightly higher than in control plasma (see Fig. 4b).

## 4. Discussion

The results of this study clearly show that in patients with CDG-Ia the fucosylation of AGP is increased, albeit to different extents. This is a remarkable finding because GDP-*L*-fucose is the donor for the fucosylation reaction, but the availability of GDP-*D*-mannose, its major biosynthetic precursor, is decreased in CDG-Ia patients [1,2]. The increase in fucosylation was not restricted to AGP, since it was also found for two other plasma acute-phase proteins synthesized by the liver, $\alpha_1$-anti-chymotrypsin and $\alpha_1$-protease inhibitor. The HPAEC-PAD analyses (Fig. 5) demonstrated that the increase in fucosylation of AGP involved a substitution of the lactosaminyl branches of tri- and tetrasialylated glycans with L-fucose in an α3-linkage, also known as the Lewis$^x$ blood group determinant. This results in an increase in mono- as

well as di- and trifucosylated tri- and tetrasialylated glycans, and consequently in an increase in sialyl Lewis$^x$ determinants on AGP.

An increased expression of sialyl Lewis$^x$ determinants on AGP has been shown to indicate the occurrence of a hepatic inflammatory response [12,17], which is of a chronic type when the extent of branching of the glycans of AGP is also increased [17]. Remarkably, this study shows that corresponding type of changes in glycosylation of AGP occur in the CDG-Ia patients. This suggests that the strong increase in fucosylation observed in the majority of the CDG-Ia patients is induced by a chronic hepatic inflammatory response. The occurrence of such a response is also indicated by the elevated activity of plasma α3-fucosyltransferase, a marker for increased hepatic fucosylation, which generally coincides with acute and chronic hepatic inflammatory responses [9,17]. Consequently, these patients seem to occur in a continuous state of inflammation. Normally, this will result also in a decrease in the plasma concentration of albumin. Indeed, hypoalbuminaemia is observed in these patients, which as yet was not totally explainable by circulatory losses associated with, for example, nephritic syndrome or diarrhea in these patients [1]. It is tempting to speculate that the high expression of sialyl Lewis$^x$ groups on AGP is helpful in the defense against complications of CDG. Such decorated AGP molecules have been shown to be able to interact with E-selectin and thus might counteract excessive selectin-mediated attraction of leukocytes into inflamed tissues [17,18].

It can be considered that the CDG-induced increase in the extent of branching of the glycans in itself will have induced an increase in α3-fucosylation, because the tri- and tetraantennary glycans on AGP can be regarded to be better acceptors for α3-fucosyltransferase than di-antennary glycans. However, in two out of eight patients, only a low or moderate increase in fucosylation of AGP was observed (see Fig. 4b,c), despite the fact that these patients (dB.E. and R.M.) belonged to the group with a high content of tri- and tetraantennary glycans according to CAIE with Con A (cf. relative content of C0 in Fig. 3a–c).

Finally, we cannot exclude that in CDG-Ia patients an increased availability of GDP-L-fucose has contributed to the increased fucosylation. Two biosynthetic pathways for GDP-L-fucose are known: (i) the more common route starting from GDP-D-mannose and (ii) a slowly occurring direct formation from L-fucose-1-phosphate [3–7]. It is not known whether the latter route is normally suppressed, e.g. by feed-back inhibition of GDP-L-fucose formed via the GDP-D-mannose route or by inhibition through GDP-Man. But if so, this cannot occur in the liver of CDG-Ia patients and consequently will result in a higher production along this route. Alternatively, the low availability of GDP-D-mannose may have changed the kinetics of the GDP-*D*-mannose-dependent route in an unexpected direction, i.e. a higher production of GDP-L-fucose, as has been detected in homogenates of *Perinereis cultrifera* oocytes [5]. Studies are in progress to delineate the synthesis route of GDP-fucose in fibroblasts of CDG-Ia patients.

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