\(\alpha_1\)-Acid glycoprotein binds human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein via N-linked glycans

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In the present study, we demonstrate a specific low-affinity interaction between recombinant precursor gp160 (rgp160) or surface unit gp120 (rgp120) of human immunodeficiency virus type 1 (HIV-1) and \(\alpha_1\)-acid glycoprotein (AGP), a human glycoprotein displaying complex type N-glycans. Binding of rgp160/rgp120 to agarose-coupled AGP was dose-dependent, saturable, calcium-, pH- and temperature-dependent. Binding was inhibited by soluble AGP, asialo-AGP, fetuin, \(\beta\)-D-GlcNAC-\(\beta\)-BSA, \(\alpha\)-D-Man\(\beta\)-BSA, mannan, complex-type asialo-agalacto-tetraantenary precursor oligosaccharide from human AGP and oligomannose 9 from porcine thyroglobulin; fully deglycosylated AGP was not inhibitory. The three AGP glycoforms separated on immobilized ConA bound rgp160 to the same extent as did unfractionated AGP. These findings extend our previous results on the carbohydrate-binding properties of HIV-1 envelope glycoprotein (Env) in that they demonstrate the involvement of AGP glycan moieties in the binding to rgp160/rgp120. Preincubation of rgp160 with AGP or mannan significantly reduced its binding to monocyte-derived macrophages (MDM), suggesting that AGP may play a role in preventing binding of soluble or virus-bound Env glycoprotein to CD4+ monocytic cells.

Keywords: Env glycoproteins, HIV-1, \(\alpha_1\)-acid glycoprotein, N-linked glycans

Introduction

The envelope glycoprotein (Env) of human immunodeficiency virus type 1 (HIV-1) is synthesized as a 160 kDa precursor (gp160), which is subsequently cleaved into outer-membrane gp120 and transmembrane gp41 [1, 2]; gp160 is heavily glycosylated, and N-linked glycans represent about 50% of its molar mass [3–5]. The role of glycans in the pathophysiology of HIV-1 infection is currently under investigation [6–8]. We have recently shown that gp160 and processed gp120 display specific carbohydrate-binding properties [9, 10]: they recognize N-acetyl-glucosamine residues of oligosaccharicidic structures, oligomannose glycans and the mannosyl core of complex type N-linked glycans such as, for example, those presented by mannan, thyroglobulin, fetuin or asialofetuin.

We hypothesized that these carbohydrate-binding properties may be involved in biological interactions with a human serum glycoprotein such as human \(\alpha_1\)-acid glycoprotein (AGP or orosomucoid). AGP is characterized by a high carbohydrate content (42%) [11], with five N-linked bi-, tri- or tetra-antennary glycans per molecule [12, 13]. It can be separated into nonreactive, weakly reactive and reactive fractions by concanavalin A (Con A)-Sepharose chromatography [14]. The ability of Con A, a mannos-e-specific lectin, to bind glycopeptides containing two antennae linked to the core pentasaccharide has been described [15]. Ogata et al. [16] found that the presence of at least two \(\alpha\)-mannosyl residues with free hydroxyl groups at C-3, 4 and 6 is required for oligosaccharides to be retained by Con A. AGP is an acute-phase reactant, and as such its plasma level increases two- to four-fold in response to infection and inflammation [17]; an increase in the proportion of Con A-unreactive fraction is observed during pregnancy and liver damage, and Con A-reactive variants increase during acute inflammatory disorders [18]. Although the physical and chemical properties of AGP have been extensively described, its biological role remains poorly understood. It binds to various substances [19] and it appears to have a modulatory activity on the immune response [20]. Con A-reactive AGP has been reported to bind to the Man/GlcNAc specific macrophage lectin [21]; this might imply that each AGP variant has a different immunoregulatory property.

The aim of this study was to investigate whether AGP could bind to gp160/120 and possibly interfere with the binding of Env glycoprotein to CD4+ cells.

Materials and Methods

Recombinant gp160 and gp120 Soluble recombinant gp160 (rgp160) of HIV-1(LAI) [22] purified to 90% homogeneity (a gift from PASTEUR MERIEUX Sérum et Vaccins, Lyon, France) was produced by BHK-21 cells infected with recombinant gp160 vaccinia virus as described [1]. This rgp160 has the same characteristics as rgp120 regarding the binding to
CD4 [6]. Because of this, in some experiments only rgp160 was used. Soluble rgp120, > 90% pure, was a gift from the MRC AIDS Directed Programme (South Mimms, UK).

Radiolabelling was performed by the iodogen method as described [6]. Iodinated glycoproteins were separated from Na\(^{125}\)I by filtration through a Sephadex G-25 (PD10) Column (Pharmacia, Uppsala, Sweden). Specific activity was 1.1 MBq \(\mu\)g\(^{-1}\).

Homogeneity of the preparation was assessed by SDSPAGE (4–20%). It was also verified that the labelled glycoproteins were still immunoreactive: HIV-positive human antiserum (2 \(\mu\)l diluted 1:10 or 1:100) were dotted on to nitrocellulose filters; after saturation with 2 \(\mu\)l of phosphate-buffered saline (PBS), 5% bovine serum albumin (BSA, Sigma Chemical Co, St Louis, MO), for 1 h at 37°C to prevent non-specific binding, and washing with PBS-0.5% BSA, 0.02% Tween 20 (Sigma), the strips were incubated for 1 h at 37°C with \([^{125}\text{I}]\text{rgp160 or }^{[125}\text{I}]\text{rgp120, washed twice with PBS-0.5% BSA, Tween 0.02%, and then autoradiographed.}

Binding of rgp160/rgp120 to \(\alpha\)-acid glycoprotein-agarose (AGP-agarose) AGP coupling to cyanogen bromide-activated Sepharose-4B (Pharmacia), 6 mg of AGP per ml of gel, was carried out as recommended by the manufacturer. The binding of \([^{125}\text{I}]\text{rgp160 or }^{[125}\text{I}]\text{rgp120 to AGP-agarose was determined as follows:} 10 \(\mu\)l of packed affinity matrix were suspended in 500 \(\mu\)l of buffer, 0.02 M Tris, 0.15 M NaCl, 0.01 M CaCl\(_2\), 0.05% BSA, pH 7.4 (Tris-Ca-BSA), centrifuged and washed twice in 500 \(\mu\)l of buffer. Incubation of the matrix with various concentrations of radiolabelled glycoproteins (0.1–5 \(\times\) \(10^{-10}\) m) was for 1 h at 37°C, i.e. under equilibrium conditions since longer incubations did not increase the binding; the matrix was then washed twice in 500 \(\mu\)l buffer and solid phase-bound radioactivity was counted in a \(\gamma\) counter (LKB).

As a negative control, rgp120/160 was incubated with non glycoprotein-derivatized CNBr-activated Sepharose. Results were expressed as mean values of duplicates.

The physicochemical characteristics of the interactions were analysed by performing the assays under different conditions: pH from 6.4 to 7.8 (experiments outside this range were not performed in order to avoid rgp160/rgp120 precipitation or denaturation); buffer without or with different CaCl\(_2\) concentrations, or with 7.5 mM MgCl\(_2\) or 7.5 mM EDTA (dissodium Salt), both from Sigma; different temperatures (4, 20 and 37°C).

Carbohydrate specificity of rgp160/rgp120 binding to AGP-agarose To determine the carbohydrate specificity of the interaction, radiolabelled glycoproteins (1–3 \(\times\) \(10^{-10}\) m) were preincubated in Tris-Ca\(^{2+}\)-BSA for 45 min at 37°C with the following carbohydrates, carbohydrate derivatives, or glycoproteins (all from Sigma): d-galactose, N-acetyl-d-glucosamine, d-mannose, methyl \(\alpha\)-d-galactopyranoside were tested at mM concentrations; d-mannan and \(\beta\)-d-glucan at 2 mg ml\(^{-1}\); AGP and fetuin in the \(\mu\)M range. BSA (30 \(\mu\)M) was used as a control. Synthetic neoglycoproteins were tested up to 30 \(\mu\)M. The sugar/BSA substitution ratio was 17 for \(\beta\)-d-Gal-BSA and 47 for \(\beta\)-d-GlcNAc-BSA [9] (gifts from D. Bladier), 20 for \(\alpha\)-d-Man-BSA and \(\beta\)-d-Lac-BSA (gifts from M. Monsigny), and 15 for melibiosyl-BSA (Sigma). Chitotriose (E.Y. Labs Inc., USA.) was also used up to 30 \(\mu\)M. In some experiments, 0–30 \(\mu\)M concentrations of the following compounds (Oxford Glycosystems, UK) were used: complex-type asialo-agalactotetraantennary oligosaccharide prepared by exo-glycosidase digestion of human AGP; oligomannose 9 from porcine thyroglobulin; fucose-substituted complex-type asialo-agalacto biantennary core from porcine thyroglobulin and complex-type asialo-galactosylated triantennary oligosaccharide from bovine fetuin.

AGP and mannann (Sigma) (both at 20 mg ml\(^{-1}\)) were used in experiments devised to assess reversion of rgp160 or rgp120 binding to the matrix: 100 \(\mu\)l of \([^{125}\text{I}]\text{rgp160/rgp120 (2 and 7 } \times\) \(10^{-10}\) m, respectively) were incubated for 1 h at 37°C with 200 \(\mu\)l of the matrix in Tris-Ca\(^{2+}\)-BSA. Unbound rgp160/rgp120 was washed out with buffer until no significant radioactivity was detected; matrix-bound rgp160/rgp120 was incubated with 500 \(\mu\)l of AGP or mannann diluted in the same buffer at 37°C for 2–20 h. The supernatants were characterized by SDS-PAGE (4–20%).

Deglycosylation of AGP Two glycosidases were used: sialidase (EC 3.2.1.18) (Behringwerke, Marburg, Germany) and endo F-N-glycanase F (Boehringer Mannheim, Mannheim, Germany). AGP (10 mg ml\(^{-1}\) in PBS pH 7.4) was desialylated by adding 0.3 U of sialidase (300 \(\mu\)l in 0.05 M NaHCO\(_3\), 0.15 M NaCl, 0.009 M CaCl\(_2\), pH 5.5) for 2 h at 37°C. Free sialic acid (36 \(\mu\)g per mg AGP) was quantified by the thiobarbituric acid reaction [23]. AGP was then applied to a PD10 column (Pharmacia Fine Chemicals) in PBS pH 7.4 in order to remove free sialic acid.

Oligosaccharides were removed under nondenaturing conditions by addition of 3 U of endoglycosidase F/N glycosidase F to 5 mg of AGP in PBS (500 \(\mu\)l). The samples were incubated for 24 h at 37°C, and then submitted to gel filtration on a PD10 column in PBS.

Intact, desialylated or endoglycosidase-treated AGP preparations were analysed by SDS-PAGE (4–20%) under reducing conditions in a Tris buffer system according to Laemmli [24]. The effect of glycosidase treatment of AGP on \([^{125}\text{I}]\text{rgp160 interaction with AGP-agarose was evaluated. As a control, it was verified that the boiled enzymes incubated without AGP-derived products under the same conditions had no effect on rgp160 interaction with the matrix.}

In other experiments, aliquots (2 \(\mu\)g of each) of intact, desialylated or endoglycosidase-treated AGP were dotted on to nitrocellulose strips. After 30 min at 20°C, the strips were saturated for 1 h at 37°C with Tris-Ca\(^{2+}\), 5% BSA, pH 7.4, to prevent non-specific protein binding. Excess BSA was washed out with Tris-Ca\(^{2+}\), 0.5% BSA, 0.02% Tween 20, pH 7.4, and strips were incubated with \([^{125}\text{I}]\text{rgp160 (1 } \times\) \(10^{-10}\) m) for 1 h at...