Effect of desialylation on the biological properties of human plasminogen

M. Sharon STACK, Salvatore V. PIZZO and Mario GONZALEZ-GRONOW*

Departments of Pathology and Biochemistry, Duke University Medical Center, Durham, NC 27710, U.S.A.

There are two major isoenzymes of plasminogen (Pg) in human plasma, designated Pg1 and Pg2. Both Pg forms have an identical primary structure, but differ in their extent of glycosylation. Removal of the oligosaccharide chains alters the normal physiological function of the zymogen and decreases the circulation time of both Pg glycoforms. Recent studies in our laboratory demonstrated that Pg2, with one carbohydrate chain, binds to the surface of U937 monocytoid cells considerably better than Pg1, with two carbohydrate chains, indicating a major role for the carbohydrate chains as determinants for differential binding to the cell surface [Gonzalez-Gronow, Grenett, Fuller & Pizzo (1990) *Biochim. Biophys. Acta* 1039, 269–276]. In this report we provide evidence that removal of terminal sialic acid from the Thr³⁴⁵-linked oligosaccharide chain of Pg2 is accompanied by the appearance of spontaneous amidolytic and fibrinolytic activity in the single-chain zymogen. Kinetic data demonstrate that asialo-Pg hydrolyses peptide substrates approximately 10% as efficiently as Pm. In addition, the change in carbohydrate content also alters Pg binding to U937 cells. Asialo-Pg binds to U937 cells with a decreased capacity but with a greater affinity than native Pg. Furthermore, asialo-Pg does not compete with native Pg for cell binding. These studies directly demonstrate that the oligosaccharide chains contribute to the heterogeneity observed in the physicochemical and biological properties of Pg1 and Pg2.

INTRODUCTION

Plasminogen (Pg) is the plasma zymogen of the fibrinolytic enzyme plasmin (Pm). The active two-chain molecule Pm results from cleavage of the zymogen at the Arg⁵⁶⁰–Val⁵⁶¹ bond by a variety of activators (Robbins *et al.*, 1967). Two major isoenzymes of Pg are present in human plasma, designated Pg1 and Pg2, which have an identical primary structure but differ in their extent of glycosylation (Brockway & Castellino, 1972). Pg1 is glycosylated at both Asn²⁸⁸ and Thr³⁴⁵, whereas Pg2 contains only the Thr³⁴⁵-linked carbohydrate chain (Hayes & Castellino, 1979a–c). In addition, each major isoform of Pg can be resolved by isoelectric focusing (i.e.f.) into several subforms differing primarily in sialic acid content (Siefring & Castellino, 1974).

Many investigators have reported differences in the biological properties of the Pg glycoforms. For example, although the concentration of Pg2 in plasma is twice that of Pg1, clearance of Pg2 from the circulation is more rapid (Takada *et al.*, 1985; Gonzalez-Gronow *et al.*, 1990). Pg2 is also preferentially bound to the surface of U937 monocytoid cells, and surface-associated Pg2 is not displaced by Pg1 (Gonzalez-Gronow *et al.*, 1989). However, Pg1 is actually a better fibrinolytic enzyme, and the activation of Pg1 by either urokinase (u-PA) or streptokinase is enhanced to a greater degree than that of Pg2 in the presence of fibrin (Takada *et al.*, 1985). Since the primary structure of both Pg glycoforms appears to be identical (Sottrup-Jensen *et al.*, 1978; Powell & Castellino, 1983), differences in carbohydrate content have been offered as an explanation for the heterogeneity in biological properties.

Using recombinant Pg, we have directly demonstrated that the oligosaccharide chains influence the function of the Pg molecule (Gonzalez-Gronow *et al.*, 1990). Non-glycosylated recombinant Pg expressed in *Escherichia coli*, although properly folded, could not be activated and was cleared from the circulation 20 times

faster than native Pg. However, fully glycosylated recombinant Pg produced by COS cells had properties similar to those of native Pg. In this paper we demonstrate that desialylation of Pg alters the biological properties of the zymogen, providing further evidence for the influence of carbohydrate content on Pg function.

EXPERIMENTAL

Materials

Pancreatic trypsin inhibitor (PTI), thrombin and *Vibrio cholerae* neuraminidase (VC-neuraminidase) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The synthetic substrates D-Val-Leu-Lys-*p*-nitroanilide (VLK-pNA), D-Ile-Pro-Arg-*p*-nitroanilide (IPR-pNA) and D-Val-Leu-Arg-*p*-nitroanilide (VLR-pNA) were products of Helena Laboratories, Beaumont, TX, U.S.A. Human fibrinogen was purchased from KabiVitrum, Stockholm, Sweden, and was rendered Pg-free by chromatography on L-Lys-Sepharose. High *M_r* urokinase (urinary plasminogen activator; u-PA) was obtained from Calbiochem, San Diego, CA, U.S.A. Newcastle disease virus neuraminidase (NDV-neuraminidase) was generously provided by Dr. Francis Castellino, University of Notre Dame, Notre Dame, IN, U.S.A.

Protein preparation

Pg was purified from human plasma by affinity chromatography on L-Lys-Sepharose (Deutsch & Mertz, 1970) and separated into isoforms 1 and 2 by affinity chromatography on concanavalin A-Sepharose (Gonzalez-Gronow & Robbins, 1984). Affinity chromatography form 2, containing a single carbohydrate chain on Thr³⁴⁵, was used for all experiments. Pm was generated by incubating 100 µg of Pg with 100 µl of u-PA-Sepharose in 20 mM-Hepes, pH 7.4, at 25 °C for 1 h, followed by centrifugation (15000 *g*, 3 min) to remove the resin. Radioiodination was carried out by the method of Markwell (1982).

Abbreviations used: Pg, plasminogen; Pm, plasmin; u-PA, urinary plasminogen activator (urokinase); NDV-neuraminidase, Newcastle disease virus neuraminidase; VC-neuraminidase, *Vibrio cholerae* neuraminidase; PTI, pancreatic trypsin inhibitor; DCI, 3,4-dichloroisocoumarin; 6-AHA, 6-aminohexanoic acid; pNA, *p*-nitroanilide; i.e.f., isoelectric focusing electrophoresis.

* To whom correspondence should be addressed: Department of Pathology, Box 3712, Duke University Medical Center, Durham, NC 27710, U.S.A.

Radioactivity was measured in a LKB 1272 γ -radiation counter. Incorporation of ^{125}I was approx. 8×10^6 c.p.m./nmol of protein. All other chemicals were of reagent grade quality.

Desialylation of Pg

VC-neuraminidase and NDV-neuraminidase were incubated with 1 mM-3,4-dichloroisocoumarin (DCI) and 10 mM-*o*-phenanthroline for 30 min at room temperature and filtered through PTI-Sepharose to eliminate any contaminating proteolytic activity. Asialo-Pg was prepared by incubating Pg (5 mg/ml) in 0.1 M-sodium acetate/0.1 M-L-lysine/2 mM- CaCl_2 , pH 5.4, containing 100 units of VC-neuraminidase (Siefing & Castellino, 1974) or NDV-neuraminidase (Davidson & Castellino, 1991) for 10 h at 37 °C. The solution was then dialysed overnight against 20 mM-Hepes, pH 7.4, and Pg was repurified by chromatography on L-Lys-Sepharose. Aliquots of desialylated Pg (200 μg) were hydrolysed by incubating with 0.05 M- H_2SO_4 at 80 °C for 1 h. The sialic acid content of the hydrolysates was determined fluorometrically using the modified thiobarbituric acid assay (Hammond & Papermaster, 1976).

Structural analysis of desialylated proteins

The integrity of the desialylated Pg forms was analysed by electrophoresis under reducing conditions on SDS/10%-polyacrylamide gels (Laemmli, 1970) using eight different preparations of desialylated Pg. *N*-Terminal sequence analysis of native and asialo-Pg was performed by automated Edman degradation in an Applied Biosystems 477A sequencer with on-line phenylhydantoin analysis using an Applied Biosystems 120A h.p.l.c. I.e.f. was carried out in a FMC Resolute HPM chamber system using Isogel agarose i.e.f. plates with a pH gradient of 5.5–8.5. Electrophoresis was performed at constant voltage (500 V) for 90 min at 10 °C using a Hepes/histidine buffer system. After focusing, the gels were fixed in 3.5% sulphosalicylic acid/10% acetic acid for 30 min and stained with Coomassie Blue R-250.

To assess the effect of desialylation on Pg secondary structure, c.d. spectra were recorded with a Jobin-Yvon Dichrograph Mark V interfaced with an Apple IIe computer. Duplicate measurements were carried out at 20 °C at 0.1 nm intervals. The instrument was calibrated with *d*-10-camphorsulphonic acid, $[\theta]_{192.5\text{ nm}}/[\theta]_{290.5\text{ nm}} = -2.00$ (Yang *et al.*, 1986). Samples of native and asialo-Pg were dissolved to a concentration of 0.1 mg/ml in 50 mM-sodium phosphate buffer, pH 7.4. Protein concentrations were determined based on the absorbance at 280 nm with a Shimadzu UV160 spectrophotometer using an M_r of 92000 and an $A_{1\text{ cm}}^{1\%}$ value of 16.7 (Castellino, 1981).

Activity assays

The amidolytic activity of Pm was determined in 20 mM-Hepes, pH 7.4, at 37 °C by monitoring the hydrolysis of the synthetic Pm substrate VLK-pNA (0.3 mM) at a wavelength of 405 nm. Substrate hydrolysis was quantified using a molar extinction coefficient of $8800\text{ M}^{-1}\cdot\text{cm}^{-1}$ for pNA at 405 nm (Erlanger *et al.*, 1961). All experiments were done in triplicate unless otherwise specified.

Fibrinolytic activity was determined using the fibrin plate method of Granelli-Piperno & Reich (1978). Briefly, 5 ml of fibrinogen stock solution (6 mg/ml) was added to 10 ml of 50 mM-sodium phosphate, pH 7.4, containing 2% low-melting-point agarose. After addition of 50 μl of thrombin (10 units/ml), the fibrin solution was poured into Petri dishes and allowed to solidify for 2 h at room temperature. Samples were added to 20 μl wells and the plates were incubated in a humid chamber for 18 h at 37 °C. Areas of fibrinolysis appear as clear zones against a white background.

Quantification of active sites

Exposure of active sites in native, asialo or u-PA-activated Pg was quantified using ^{125}I -PTI. Pg was dissolved in 0.1 M-sodium phosphate, pH 6.5, and incubated for 60 min at 37 °C with a 5-fold molar excess of ^{125}I -PTI (Gonzalez-Gronow *et al.*, 1978). The solution was passed over a column of L-Lys-Sepharose (0.5 cm $\times$ 2.5 cm) to remove excess ^{125}I -PTI and the radiolabelled complex was eluted directly into scintillation vials with 0.1 M-sodium phosphate/0.1 M-6-aminoheptanoic acid (6-AHA), pH 7.5.

Cells and ligand-binding analysis

U937 cells were grown in RPMI 1640 medium (Gibco Ltd.) containing 10% fetal calf serum. Prior to use in binding assays, the cells were washed three times in Hank's balanced salt solution and resuspended in Tyrode's buffer, pH 7.2, containing 20 mg of BSA/ml (Marguerie *et al.*, 1980). For binding assays, cells were incubated at a final concentration of 0.5×10^6 cells/ml with radiolabelled ligands. After incubation for 30 min at 22 °C, bound ligand was separated from free by layering triplicate 200 μl aliquots over 500 μl of Tyrode's buffer containing 20% sucrose in 1.5 ml conical polypropylene tubes. Following centrifugation for 5 min at 15000 *g* in an IEC Centra centrifuge, the tips containing the cells were cut off and counted for radioactivity in a γ -radiation counter. Bound ligand was calculated after subtraction of non-specific binding measured in the presence of 10 mM-6-AHA (Hajjar *et al.*, 1986). Scatchard plots were drawn based on linear regression analysis.

To determine the effect of removal of only the $\alpha 2,3$ -linked sialic acid on Pg binding to U937 cells, ^{125}I -Pg (2 μmol) was incubated with 10 units of NDV-neuraminidase. Aliquots were removed with time and added to 5×10^4 U937 cells at a final Pg concentration of 0.2 μM . Pg binding was determined as described above. In control experiments, U937 cells were incubated with NDV-neuraminidase and the binding of untreated ^{125}I -Pg was determined as described above.

RESULTS

Effect of desialylation on Pg structure

Treatment of Pg with either VC- or NDV-neuraminidase decreased the sialic acid content of the native zymogen (Table 1). Asialo-Pgs were analysed to determine the effect of desialylation on the protein structure. When subjected to SDS/PAGE analysis under reducing conditions (Fig. 1), a single protein component was present following desialylation with both neuraminidase preparations, indicating the absence of degraded Pg forms. *N*-Terminal sequence analysis was used to confirm the presence of a single polypeptide component with the sequence $\text{NH}_2\text{-EPL-DDYVNTQGA}$, analogous to that found in native Pg (Sottrup-Jensen *et al.*, 1978). The i.e.f. profile of asialo-Pg was altered

Table 1. Pg sialic acid content

Pg species	Sialic acid content (mol of sialic acid/mol of Pg)
Native Pg	1.9
Pg _{VC-N}	0.1
Pg _{NDV-N}	0.7

Pg samples were hydrolysed in 0.05 M- H_2SO_4 for 1 h at 80 °C. The sialic acid content was determined by the modified thiobarbituric acid method of Hammond & Papermaster (1976). Pg_{VC-N}, Pg treated with VC-neuraminidase; Pg_{NDV-N}, Pg treated with NDV-neuraminidase (see the Experimental section).

Fig. 1. SDS/PAGE of native Pg and asialo-Pg

Samples were electrophoresed on 10% gels under reducing conditions. Lane 1, native Pg (5 µg); lane 2, asialo-Pg (treated with NDV-neuraminidase; 10 µg); lane 3, native Pg (10 µg); lane 4, asialo-Pg (treated with VC-neuraminidase; 5 µg).

Fig. 2. Far-u.v. c.d. spectra of native Pg and asialo-Pg

Measurements were carried out at 20 °C at 0.1 nm intervals at a protein concentration of 0.1 mg/ml in 50 mM-sodium phosphate buffer, pH 7.4, as described in the Experimental section. —, Native Pg; ---, asialo-Pg (treated with VC-neuraminidase).

relative to that of native Pg (results not shown). The asialo-Pgs contain only the less acidic isoenzymes, consistent with the loss of negatively charged sialic acid. The far-u.v. c.d. spectra of native Pg and asialo-Pg (Fig. 2) indicate no large changes in secondary structure as a result of complete desialylation.

Effect of desialylation on Pg function

In the absence of any added activator protein, single-chain asialo-Pg was found to contain spontaneous amidolytic (Fig. 3) and fibrinolytic (Fig. 4) activities. The amidolytic activity of VC-neuraminidase-treated Pg (Pg_{VC-N}) was 2-fold greater than that of NDV-neuraminidase-treated Pg (Pg_{NDV-N}). Both asialo-Pg forms exhibited similar fibrinolytic activity (Pg_{NDV-N} , Fig. 4; Pg_{VC-N} , results not shown), as reflected by the diameter of the fibrinolysis zone. However, this experiment reflects the end point of an 18 h incubation, and cannot be used to assess quantitative differences in the initial rate of fibrinolysis. Because these results indicated that the accessibility of the Pg active site to both peptide and macromolecular substrates was altered as a consequence of desialylation, active-site titration was performed using ^{125}I -PTI (Table 2). In both of the asialo-Pg species, approx. 80–90% of

Fig. 3. Amidolytic activity of native and desialylated Pg forms

Pg samples (0.3 µM) were incubated at 37 °C in 20 mM-Hepes, pH 7.4, containing the chromogenic plasmin substrate VLK-pNA (0.3 mM). ▲, Native Pg; ●, asialo-Pg (treated with NDV-neuraminidase); ●, asialo-Pg (treated with VC-neuraminidase).

Fig. 4. Fibrinolytic activity of native Pg and asialo-Pg

Samples were added to 20 µl wells in fibrin-agar plates and incubated in a humid chamber for 18 h at 37 °C as described in the Experimental section. Well 1, asialo-Pg (NDV-neuraminidase-treated; 10 µg); well 2, asialo-Pg (10 µg) + u-PA (5 units); well 3, u-PA (5 units); well 4, native Pg (10 µg); well 5, native Pg (10 µg) + u-PA (5 units); well 6, 20 mM-Hepes, pH 7.4.

the active sites are accessible. However, comparison of the amidolytic activity of asialo-Pgs relative to that of native Pg fully activated with u-PA demonstrated that asialo-Pg hydrolyses peptide substrates only 5–10% as efficiently as Pm (Table 2). Desialylation did not dramatically alter the catalytic efficiency of the fully activated enzyme, as evidenced by only a 2-fold decrease in the k_{cat}/K_m of asialo-Pm relative to native Pm (252 min⁻¹·mM⁻¹ and 573 min⁻¹·mM⁻¹ respectively). The proteolytically active zymogen did not hydrolyse VLR-pNA or IPR-pNA, nor was it able to induce spontaneous activity in native Pg species (results not shown), indicating that the substrate specificity of the active site was not altered.

Effect of desialylation on cell binding

In order to characterize further the functional significance of changes in the Pg molecule induced by desialylation, the cell-

Fig. 5. Binding of Pg and asialo-Pg to U937 cells

Increasing concentrations of ^{125}I -Pg ($\circ$) or ^{125}I -asialo-Pg (treated with VC-neuraminidase; $\bullet$) was added to 1×10^5 cells. Molecules of ligand bound were calculated after subtraction of non-specific binding measured in the presence of 10 mM-6-AHA (results not shown) as described in the Experimental section. Inset: Scatchard plot of the binding data.

Fig. 6. Inhibition of ^{125}I -Pg binding to U937 cells

^{125}I -Pg ($0.2 \mu\text{M}$) was incubated with 1×10^5 cells at 22°C for 30 min in the presence of unlabelled Pg ($\bullet$) or asialo-Pg ($\circ$). Bound Pg was determined as described in the Experimental section.

binding properties of asialo-Pg were determined using U937 cells. Comparison of the binding isotherms obtained with native and asialo-Pg (Fig. 5) demonstrated that U937 cells bind asialo-Pg with a decreased capacity (120×10^6 molecules/cell and 30×10^6 molecules/cell for native Pg and asialo-Pg respectively). However, the affinity of asialo-Pg for U937 cells (K_d $0.8 \mu\text{M}$) was higher than that of native Pg (K_d $2 \mu\text{M}$). Asialo-Pg does not compete with native Pg for cellular binding sites (Fig. 6), as indicated by the inability of asialo-Pg to displace U937-cell-bound native Pg. Incubation of ^{125}I -Pg with NDV-neuraminidase

Table 2. Active-site titration and amidolytic activity of Pg

Active-site titration was performed using ^{125}I -PTI as described in the Experimental section. Amidolytic activity was determined in 20 mM-Hepes, pH 7.4, at 37°C using the synthetic Pm substrate, VLK-pNA, as described in the Experimental section. Values shown represent the mean of eight experiments. Pg_{VC-N}, Pg treated with VC-neuraminidase; Pg_{NDV-N}, Pg treated with NDV-neuraminidase; Pg + u-PA, Pg fully activated to Pm with u-PA.

Pg species	Active-site titration (mol of active site/ mol of Pg)	Amidolytic activity (mol of pNA released/ min per mol of enzyme)
Native Pg	0.20	0
Pg _{VC-N}	0.80	1.4
Pg _{NDV-N}	0.73	0.7
Pg + u-PA	0.90	14.3

Fig. 7. Effect of desialylation on Pg binding to U937 cells

^{125}I -Pg ($0.2 \mu\text{M}$) was incubated with 5×10^4 cells in the presence of NDV-neuraminidase (10 units). Bound Pg was determined as described in the Experimental section.

for increasing periods of time caused a decrease in Pg binding to U937 cells, demonstrating that removal of only the $\alpha 2,3$ -linked sialic acid residue is sufficient to alter the Pg-U937-cell interaction (Fig. 7). Treatment of U937 cells with NDV-neuraminidase had no effect on the binding of untreated ^{125}I -Pg (results not shown).

DISCUSSION

Although the structure and biosynthesis of Pg have been extensively studied, the mechanism by which glycosylation alters Pg structure and function remains unclear. Both Pg forms have an identical primary structure (Sottrup-Jensen *et al.*, 1978), but differ in their extent of glycosylation (Hayes & Castellino, 1979a-c). Previous studies have demonstrated that secondary glycosylation is essential for the secretion of Pg1 (Powell *et al.*, 1981). Removal of the oligosaccharide chains alters the normal physiological function of the zymogen and decreases the circulation time of both Pg glycoforms (Gonzalez-Gronow *et al.*, 1990). Pg2, with one carbohydrate chain, binds to U937 cells considerably better than does Pg1, and at equilibrium demonstrates approx. 10-fold greater binding (Gonzalez-Gronow *et al.*, 1989). A similar observation has been reported with primary cultures of rat hepatocytes and rat C6 glioma cells (Hall *et al.*, 1990). Both studies indicate that a major role for the carbohydrate chain(s) is as a determinant of differential binding to cellular Pg-binding sites.

Many of the proteins involved in both coagulation and fibrinolysis are present as differentially glycosylated isoforms, suggesting a role for carbohydrate microheterogeneity in the regulation of biological activity (for a review of the effects of oligosaccharide content on glycoprotein function, see Rademacher *et al.*, 1988). The present study demonstrates that individual sugar components of the carbohydrate chain can influence the functional properties of Pg. Pg isoform 2 contains two sialic acid residues on the Thr³⁴⁵ carbohydrate chain; one which is linked in the α 2,3 configuration to a galactose residue and one which is attached to *N*-acetylgalactosamine in an α 2,6 linkage (Hayes & Castellino, 1979*a-c*). Treatment of Pg with NDV-neuraminidase removes only the α 2,3-linked sialic acid (Ada *et al.*, 1961), whereas VC-neuraminidase removes both the α 2,3 and the α 2,6 sialic acid residues (Davidson *et al.*, 1990). Removal of one or both of the terminal sialic acid residues from Pg had no effect on either the primary or the secondary structure of the protein. However, the i.e.f. profile exhibited a decrease in the number of acidic isoforms, consistent with the loss of negatively charged sialic acid. Similar results were reported by Siefring & Castellino (1974) using rabbit Pg.

In addition to altering the i.e.f. profile, desialylation of Pg is accompanied by the appearance of spontaneous amidolytic and fibrinolytic activity in the single-chain zymogen. Since the neuraminidases used in this study were pretreated with a variety of proteinase inhibitors prior to incubation with Pg, the observed activities are unlikely to result from the presence of contaminating Pg activators in the neuraminidase preparation. In addition, the desialylation reaction is performed at pH 5, which is well below the pH optimum for known Pg activators. Furthermore, both SDS/PAGE and *N*-terminal sequence analysis indicated the absence of any additional protein species (such as two-chain Pm) in the asialo-Pg preparation.

Previous studies have demonstrated that serine proteinase zymogens, including trypsinogen, chymotrypsinogen and Pg, can hydrolyse ester and amide substrates with a mechanism identical to that of the corresponding active enzyme, although at a greatly reduced rate (Gertler *et al.*, 1974; Kerr *et al.*, 1975; Wohl *et al.*, 1977). A more recent study by Wohl (1984) provided evidence that the potential active site in Pg is much less distorted than that in other serine proteinase zymogens, and may simply be blocked by a small ligand in the substrate-binding pocket. Our data demonstrate that removal of sialic acid(s) from the Thr³⁴⁵-linked oligosaccharide chain results in both amidolytic and fibrinolytic activity without zymogen activation (cleavage of the internal Arg⁵⁶⁰-Val⁵⁶¹ bond) in both Pg2 and Pg1 (results not shown), suggesting that the sialic acid residue(s) may block access to the substrate-binding pocket in the native zymogen. This hypothesis is supported by the data of Table 2, which demonstrate that desialylation results in increased accessibility of the zymogen active site. Active-site titration, amidolytic activity and fibrinolytic activity data demonstrate that removal of only the α 2,3 sialic acid is required for active site exposure, indicating that the α 2,3 sialic acid may block access to the substrate-binding pocket. However, since the activity of asialo-Pg is only approx. 10% that of Pm, cleavage of the Arg⁵⁶⁰-Val⁵⁶¹ bond may be necessary to generate a fully functional substrate-binding pocket, thereby increasing the catalytic efficiency of the enzyme.

We have previously reported that asialo-Pg is removed from the circulation more rapidly than native Pg (Gonzalez-Gronow *et al.*, 1990). This study demonstrates that changes in carbohydrate content also affect Pg binding to cellular binding sites. Asialo-Pg binds to U937 monocytoid cells with a higher affinity than does native Pg, but with a decreased capacity. Furthermore, U937-cell-associated Pg is not displaced by asialo-Pg. These data suggest an alteration in the mechanism of asialo-Pg recognition

by U937 cells which may result from changes in protein tertiary structure following desialylation. The decrease in the binding affinity of Pg to U937 cells following progressive removal of the α 2,3-linked sialic acid residue indicates that the presence of this residue is necessary for proper recognition of the Pg molecule by U937 cellular binding sites. A similar phenomenon has been reported for human chorionic gonadotropin, wherein the presence of the α 2,3 sialic acid residues affected both binding of the hormone to its target cells as well as expression of hormonal activity (Amano *et al.*, 1989).

In summary, we have demonstrated that desialylation of Pg alters the kinetic and cell-binding activities of the zymogen without changing the primary or secondary structure of the polypeptide chain. Our data and that of others indicate that minor alterations in carbohydrate content may represent a fine regulatory mechanism for localized changes in the activity of glycoproteins. This is particularly interesting in view of the fact that neuraminidases are produced by a variety of cells involved in fibrinolysis, including platelets, macrophages and leukocytes (Marchesini *et al.*, 1984; Pilatte *et al.*, 1987).

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REFERENCES

- Ada, G. L., French, E. L. & Lind, P. E. (1961) *J. Gen. Microbiol.* **24**, 409-412
- Amano, J., Sato, S., Nishimura, R., Mochizuke, M. & Kobata, A. (1989) *J. Biochem. (Tokyo)* **105**, 339-340
- Brockway, W. J. & Castellino, F. J. (1972) *Arch. Biochem. Biophys.* **151**, 194-195
- Castellino, F. J. (1981) *Chem. Rev.* **81**, 431-436
- Davidson, D. J. & Castellino, F. J. (1991) *Biochemistry* **30**, 625-633
- Davidson, D. J., Fraser, M. J. & Castellino, F. J. (1990) *Biochemistry* **29**, 5584-5590
- Deutsch, D. & Mertz, E. T. (1970) *Science* **170**, 1095-1096
- Erlanger, B. R., Kokowsky, N. & Cohen, W. (1961) *Arch. Biochem. Biophys.* **95**, 271-278
- Gertler, A., Walsh, K. A. & Neurath, H. (1974) *Biochemistry* **13**, 1302-1310
- Gonzalez-Gronow, M. & Robbins, K. C. (1984) *Biochemistry* **23**, 190-196
- Gonzalez-Gronow, M., Siefring, G. & Castellino, F. J. (1978) *J. Biol. Chem.* **253**, 1090-1095
- Gonzalez-Gronow, M., Edelberg, J. M. & Pizzo, S. V. (1989) *Biochemistry* **28**, 2374-2377
- Gonzalez-Gronow, M., Grenett, H. E., Fuller, G. M. & Pizzo, S. V. (1990) *Biochim. Biophys. Acta* **1039**, 269-276
- Graneli-Piperno, A. & Reich, E. (1978) *J. Exp. Med.* **148**, 223-234
- Hajjar, K. A., Harpel, P. C., Jaffe, E. A. & Nachman, R. L. (1986) *J. Biol. Chem.* **261**, 11656-11662
- Hall, S. W., Van den Berg, S. R. & Gonias, S. L. (1990) *J. Cell. Biochem.* **43**, 213-227
- Hammond, K. S. & Papermaster, D. S. (1976) *Anal. Biochem.* **74**, 292-297
- Hayes, M. & Castellino, F. J. (1979*a*) *J. Biol. Chem.* **254**, 8768-8771
- Hayes, M. & Castellino, F. J. (1979*b*) *J. Biol. Chem.* **254**, 8772-8776
- Hayes, M. & Castellino, F. J. (1979*c*) *J. Biol. Chem.* **254**, 8777-8781
- Kerr, M. A., Walsh, K. A. & Neurath, H. (1975) *Biochemistry* **14**, 5088-5094
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685
- Marchesini, S., Cestaro, B., Lombardo, A., Sciorelli, G. & Preti, A. (1984) *Biochem. Int.* **8**, 151-158
- Marguerie, G. A., Edgington, T. S. & Plow, E. F. (1980) *J. Biol. Chem.* **255**, 154-161
- Markwell, M. A. K. (1982) *Anal. Biochem.* **125**, 427-432

- Pilatte, Y., Bignon, J. & Lambre, C. R. (1987) *Biochim. Biophys. Acta* **923**, 150–155
- Powell, J. R. & Castellino, F. J. (1983) *Biochemistry* **22**, 923–927
- Powell, R., Brefthaver, R. K. & Castellino, F. J. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6836–6839
- Rademacher, T. W., Parekh, R. B. & Dwek, R. A. (1988) *Annu. Rev. Biochem.* **57**, 785–838
- Robbins, K. C., Summaria, L., Hsieh, B. & Shah, R. J. (1967) *J. Biol. Chem.* **242**, 2333–2342
- Siefring, G. E. & Castellino, F. J. (1974) *J. Biol. Chem.* **249**, 7742–7746
- Sottrup-Jensen, L., Claeys, H., Zajdel, M., Petersen, T. E. & Magnusson, S. (1978) in *Fibrinolysis and Thrombolysis* (Davidson, J. F., Rowan, R. M., Samama, M. M. & Desnoyers, P. C., eds.), vol. 3, pp. 199–209, Raven Press, New York
- Takada, Y., Makino, Y. & Takada, A. (1985) *Thromb. Res.* **39**, 289–296
- Wohl, R. C. (1984) *Biochemistry* **23**, 3799–3804
- Wohl, R. C., Arzadon, L., Summaria, L. & Robbins, K. C. (1977) *J. Biol. Chem.* **252**, 1142–1147
- Yang, J. T., Wu, C. C. & Martinez, H. M. (1986) *Methods Enzymol.* **130**, 208–227

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