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Neoglycoproteins: Preparation of Noncovalent Glycoproteins through High-Affinity Protein- (Glycosyl) Ligand Complexes†

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ABSTRACT: This work was undertaken as part of a search for well-characterized glycoprotein models in which both the oligosaccharide structure, the number of oligosaccharide chains, and the precise location of these chains in the protein are known. On the basis of the fact that high-affinity ligand binding sites have been defined precisely for several proteins in terms of both number and relative location, the hypothesis to be tested was that if oligosaccharide chains were covalently attached to such high-affinity ligands, they would be specifically bound in the ligand sites of the appropriate protein, thus permitting the preparation of neoglycoproteins of precise predetermined oligosaccharide valency and topography. To test this hypothesis, pyridoxal 5′-phosphate was reductively (NaB^3H_4) aminated with the α-amino group of the asparagine oligosaccharide Man_6-$GlcNAc_2$-Asn from ovalbumin. When the resulting phosphopyridoxylated oligosaccharide (PG) was added to the apo form of aspartate aminotransferase (AAT; EC 2.6.1.1, the cytosolic enzyme from pig heart, consisting of two subunits and containing two coenzyme binding sites), a 2:1 (PG-AAT) complex was formed which could be characterized on the basis of tritium content, the absorbance and fluorescence of the pyridoxamine phosphate moiety of PG, and the concanavalin A binding properties acquired by AAT through the incorporation of the oligosaccharide. As expected from the established properties of the holoenzyme, the AAT-PG complex is stable in the absence of phosphate or vitamin B_6 derivatives and can be dialyzed for 24 h without any significant loss of PG. According to the three-dimensional model of AAT, the oligosaccharide chain of PG should be partially masked in the coenzyme binding pocket. This was confirmed by exposing the complex to α-mannosidase. Under conditions which gave complete removal of all five α-mannosyl residues in free PG, only one α-mannosyl residue was removed from the AAT-PG complex.

Synthetic glycoconjugates are important tools in biochemistry and cell biology (Stowell & Lee, 1980). For example, sugars covalently attached to proteins have been used as immunogens for eliciting antibodies specifically directed against carbohyrates (Goebel & Avery, 1929; Lonngren & Goldstein, 1978). Solid supports bearing glycosides have been prepared as column materials for affinity chromatography (Pazur, 1981) or as culture surfaces for studying cell adhesion to carbohydrate-derivatized matrices (Pless et al., 1983). A wide variety of neoglycoconjugates have been extensively employed as model compounds in studying sugar-lectin interactions ranging from the characterization of individual binding activities (Goldstein et al., 1977) to the investigations of cell surface receptor mediated uptake of macromolecules (Lee & Lee, 1982). However, synthetic glycoconjugates so far reported mainly consist of covalently bound saccharide moieties randomly dispersed over the surface of the matrix. These may not serve as accurate models for naturally occurring glycoproteins in which the carbohydrates are attached at specific locations (Sharon & Lis, 1982). Indeed, it has been postulated that the unique patterns of glycosylation in proteins may provide a basis for specificity in sugar-lectin interactions (Ashwell & Morell, 1974; Mencke & Wold, 1982). Therefore, it is desirable in a model system to be able to attach oligosaccharides of diverse structures to a matrix at defined locations and stoichiometry. One way to realize this is to conjugate an oligosaccharide to a protein via a ligand which possesses high affinity for specific loci on that protein. As an example of this approach for neoglycoprotein preparation, we have explored the binding of a glycosylated coenzyme (pyridox-

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amine 5′-phosphate)[1] to its apoenzyme (apoaspartate aminotransferase).[1] For this reaction PLP[1] was first coupled to the α-amino group of an asparagine oligosaccharide by reductive amination, and the phosphopyridoxylated product, PG, was subsequently reacted with the apo form of AAT to produce a neoglycoprotein.

The enzyme AAT contains two well-defined coenzyme binding sites; it is a dimeric protein and is composed of two identical subunits of M_r 46 000 which are related to each other by a pseudo 2-fold symmetry axis (Braunstein, 1973; Arnone et al., 1982). Since the location of the two coenzyme binding sites is known, stoichiometric saturation of AAT with PG automatically defines the resulting neoglycoprotein as one in which the two oligosaccharides are oriented in an anti relationship to each other. Previous work establishing that the coenzyme in the PMP form in the holoenzyme can be removed and replaced by a number of N-phosphopyridoxylated amines (Severin & Dixon, 1963; Relimpio et al., 1975; Misharin et al., 1979) provided the basis for undertaking the present work.

Materials and Methods

Asparagine, cysteinesulfinic acid, PLP, PMP, methyl α-D-mannopyranoside (methyl mannoside), *p*-nitrophenyl α-D-mannopyranoside, ovalbumin (twice crystallized), porcine heart cytosolic aspartate aminotransferase (glutamic-oxalacetic transaminase), jack bean α-mannosidase, Sephadex G-25-80, and concanavalin A–Sepharose 4B were obtained from Sigma. Fetal bovine serum was purchased from GIBCO Laboratories. Cation exchanger AG-50W-X2 (H^+ form) was purchased from Bio-Rad. Tritium-labeled $NaBH_4$ (348 mCi/mmol) was obtained from New England Nuclear.

Asparagine oligosaccharide (fraction AC-D) from ovalbumin was isolated by cation-exchange chromatography according to Huang et al. (1970). PA was prepared by $NaBH_4$ treatment of a mixture of asparagine (25 μmol) and PLP (10 μmol) in 0.3 mL of a 2:1 mixture of dimethylformamide and methanol. The product, after purification by G-25 gel permeation chromatography (>90% yield), was found to be homogeneous by high voltage paper electrophoresis carried out at pH 9.5.

The concentration of AAT, expressed in terms of the number of PLP sites available for a given protein sample, was determined from its absorbance at 280 nm by using an apparent molar absorptivity of 92 000. This value was calculated from the absorbance of PLP at 390 nm observed in a solution of AAT in 0.1 M NaOH by using a molar absorptivity of 6550 for PLP.

In anticipation of the characterization of the neoglycoprotein, the commercially obtained AAT was first passed through a Con A–Sepharose column. The unbound PLP form of AAT was converted to the PMP form by incubation with cysteinesulfinate at pH 8 (Jenkins & D'Ari, 1966), and the apoenzyme of AAT was obtained by subsequent displacement of the coenzyme with phosphate at pH 5 following the methods of Scardi (1963).

The activity of α-mannosidase was determined with 10 mM *p*-nitrophenyl α-D-mannopyranoside in 0.02 M cacodylate buffer containing 1 mM zinc acetate at pH 5.6 by a procedure similar to that described by Li & Li (1972). Con A[1] affinity chromatography was performed at room temperature. The column was equilibrated in TEA[1] buffer (0.02 M TEA containing 100 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.02% NaN_3 at pH 7.8). Application of samples and elution of unbound material were carried out in the same buffer. The adsorbed material was recovered with methyl mannoside buffer (0.02 M TEA containing 100 mM methyl mannoside, 10 mM ethylenediaminetetraacetate, 100 mM NaCl, and 0.02% NaN_3 at pH 7.8).

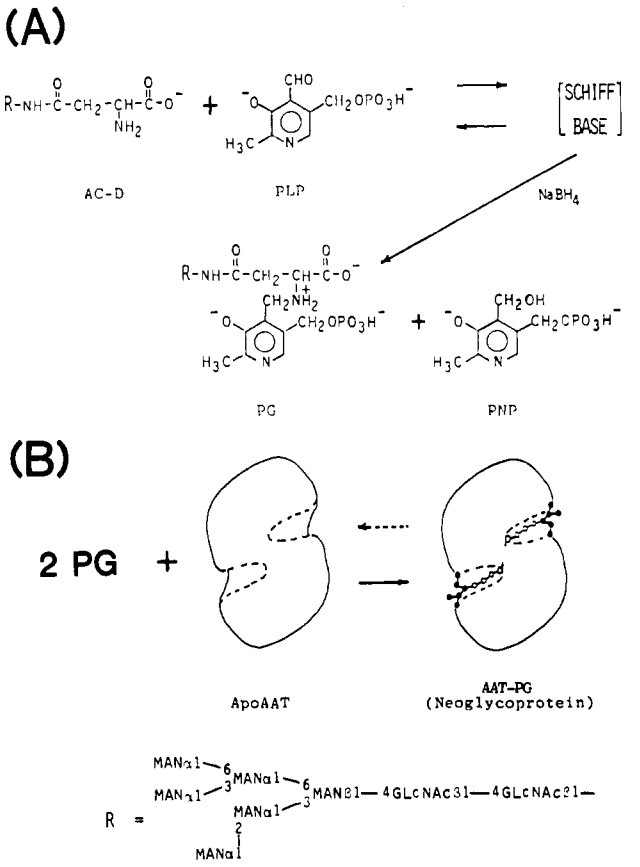

FIGURE 1: Reaction scheme for the preparation of neoglycoprotein AAT–PG. The diagrammatic representation of protein-bound PG with only one mannosyl residue in the nonreducing terminal protruding beyond the protein surface is suggested by experimental results (see text). It should be noted that according to recent data (Atkinson et al., 1981) only 85% of fraction AC-D prepared by the methods of Huang et al. (1970) has the asparagine oligosaccharide structure shown in this figure. The symbols and abbreviations used are (●) Man for mannosyl, (○) GlcNAc for *N*-acetylglucosaminyl and (p–◇) for the N-phosphopyridoxylated asparagine moiety.

Results

Phosphopyridoxylation of Glycopeptide. In a typical preparation of PG (reaction A, Figure 1), a mixture of asparagine oligosaccharide (2 μmol in asparagine), PLP (4.5 μmol as sodium salt), and K_2HPO_4 (70 μmol) in a volume of 0.5 mL of water was treated with solid $NaBH_4$ until the yellow color was completely bleached. After the mixture was allowed to stand at room temperature for 1 h, acetic acid (1 M) was added dropwise until the pH of the reaction mixture was 4. The solvent was evaporated in a gentle stream of nitrogen, and the residue, redissolved in water, was passed through a Sephadex G-25 column eluted with dilute acetic acid (0.02 M). The fractions that showed positive test for sugar with phenol–sulfuric acid (Dubois et al., 1956) as well as for absorption at 325 nm were pooled and lyophilized. Most of this material was found to adsorb onto a Con A affinity column in TEA buffer and was subsequently recovered by eluting the column with methyl mannoside buffer. The spectrum of the

[1] Abbreviations: AAT, aspartate aminotransferase; Con A, concanavalin A; PA, *N*-(phosphopyridoxyl)asparagine; PG, N-phosphopyridoxylated asparagine oligosaccharide (AC-D from ovalbumin); PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; PNP, pyridoxine 5′-phosphate; TEA, triethanolamine hydrochloride.

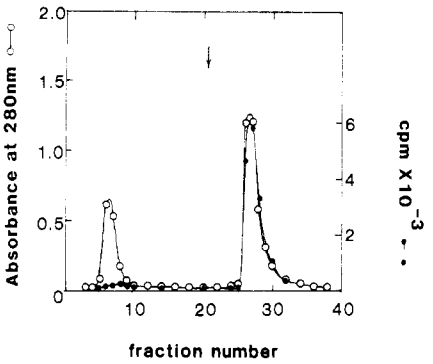

FIGURE 2: Elution profile of AAT–PG on Con A–Sepharose 4B. A preparation of apoAAT was reconstituted with PG as described in the text. The dialyzed sample in 3 mL was passed over a Con A–Sepharose 4B affinity collumn (1.5 × 6.5 cm) as described under Materials and Methods. The arrow indicates where elution with methyl mannoside buffer began.

Con A bound material after desalting was found to be very similar to that of PMP with two absorption bands centered at 327 and 251 nm (0.02 M sodium acetate, pH 5.6). Therefore, the molar absorptivity of PG was taken to be 8300 at 327 nm, and from the absorbance at this wavelength the yield of PG was typically 1.5 μmol (75%, based on starting glycopeptide). When tritiated PG was prepared, the commercial sample of NaB^3H_4 was dissolved in 0.05 M NaOH and added to the reaction mixture as this solution. In our hands the yield of PG in these cases was always lower (~40%). We presume that this may be due to both the dilution of the reaction mixture and the high initial pH.

The presence of a high mannose oligosaccharide in PG allows the use of Con A both as a tool for its purification and as a criterion for successful reductive amination. Therefore, the fact that the same spectral properties as PMP are exhibited by the material bound to Con A conforms its identity to be PG, having the structure indicated in Figure 1.

Preparation of Neoglycoprotein. For the preparation of the AAT–PG complex (reaction B, Figure 1), apoAAT (10^{-5} M) was incubated with 1.2 equiv of PG in 0.02 M TEA at pH 8 for 30 h at room temperature. The excess PG, the displaced phosphate, and any underivatized oligosaccharides were removed by dialysis against 5 × 1 L TEA buffer at 4 °C over a 24-h period. In general, this was sufficient to bring the free PG concentration in the dialysate down to undetectable levels. Protein not containing bound PG was eliminated on a Con A affinity column. A typical elution profile is shown in Figure 2.

The following facts suggest that the binding of PG occurs stoichiometrically at the active site. First, in a pilot experiment apoAAT was mixed with phosphopyridoxylated asparagine oligosaccharides, PG′, from an unfractionated sample obtained from exhaustive Pronase digestion of ovalbumin. Fluorescence due either to tryptophan residues of the protein or to free PG′ chromophore was quenched in a time-dependent manner analogous to that observed for the binding of PMP to apoAAT (Table I). Second, when the same apoAAT preparation was reconstituted separately with PMP and PG, a common absorption spectrum was obtained which was characterized by a band at 335 nm with an intensity 0.10 ± 0.01 that of the protein band at 280 nm. Third, when tritiated PG was used in the reconstitution, the Con A binding complex was found to have a stoichiometry of 2.0 ± 0.1 PG bound per dimer.

Stability of the Neoglycoprotein. Because of the noncovalent nature of the AAT–PG neoglycoprotein, it is necessary to establish if it is sufficiently stable to be used in both in vitro and in vivo cellular binding and uptake studies. To this end the dissociation of AAT–PG was assayed in a buffer system commonly used in such studies along with other reference buffers. The time profiles for AAT–PG dissociation in the various buffer systems are given in Figure 3 and show that the complex is sufficiently stable for in vitro cellular binding studies, if the proper buffer can be used. The use of the complex for in vitro cellular uptake will be limited by the cellular phosphate, PLP, and PMP concentrations and be restricted to short-time experiments. Similarly, the in vivo half-life of the complex will probably be too short to make the complex useful in any study other than fast clearance processes. As expected from the fact that extensive dialysis was used to produce the complex, AAT–PG is stable in TEA, and also as expected, PG can be displaced from the complex by PLP or by phosphate alone. The dissociation rate of AAT–PG in the buffer for culturing macrophages probably also reflects the presence of phosphorylated compounds in serum. The release of PG in pH 5.6 cacodylate buffer may be accelerated by a combination of low pH and the anion competition with substrate binding described previously (Jenkins, 1980; Harruff & Jenkins, 1978). This low pH buffer was included as a control for the α-mannosidase experiment reported below in which pH 5.6 represents a compromise between optimal AAT–PG stability and α-mannosidase (pH optimum 4.5) activity.

Extent of Exposure in the Nonreducing End of the Oligosaccharide in the Neoglycoprotein. The results from X-ray

Table I: Changes in Fluorescence Intensity during Ligand Binding to ApoAAT

ligand	fluorescence intensity change: excited at 280 nm, observed at 340 nm	fluorescence intensity change: excited at 330 nm, observed at 390 nm
PMP[a]	100% at 0 min	100% at 0 min
	75% at 25 min	78% at 15 min
	63% at 65 min	47% at 75 min
PG′[b]	100% at 0 min	100% at 0 min
	73% at 20 min	52% at 120 min
	61% at 120 min	

[a] Data from Churchich (1976). [b] Protein concentration was 3 μM, and PG′ (phosphopyridoxylated asparagine–oligosaccharide mixture) was 2.8 μM in 0.02 M TEA, pH 8.

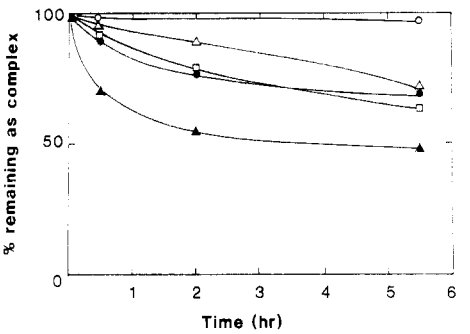

FIGURE 3: Rate of dissociation of AAT–PG in five different buffers. Small aliquots from stock solutions (2 × 10^{-5} M) of AAT–PG in 0.02 M TEA, pH 7.8, were diluted to about 10^{-7} M AAT–PG in 1.0 mL of the following buffers: 0.02 M TEA, pH 7.8 (○); 0.02 M TEA + 0.5 mM PLP (□); 0.01 M potassium phosphate, pH 6.9 (▲); 0.02 M cacodylate containing 1 mM zinc acetate, pH 5.6 (●); macrophage culturing buffer, containing 20% fetal calf serum (Stahl et al., 1978) (△). At different times the dissociated ligands were separated from the protein by ultrafiltration of the incubation mixtures through Amicon YMT membranes. The ratios of radioactivity retained by the membrane to that originally present in the incubation mixture represent the fractions remaining as AAT–PG complex. All incubations were conducted at 25 °C.

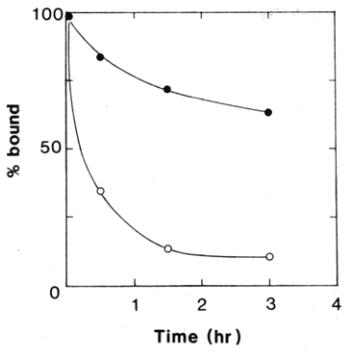

FIGURE 4: Susceptibilities of free PG and AAT–PG to α-mannosidase digestion. Con A bound AAT–PG was prepared as described in the text. It was then further dialyzed for 4 h at 4 °C against 500 mL of 0.02 M cacodylate buffer containing 1 mM zinc acetate, pH 5.6. The sample was divided into two equal portions (2.3×10^{-5} M PG). One (○) was heat denatured so that PG was free from the complex while AAT–PG in the other (●) was left intact. The two samples were treated at 26.5 °C with 35 units of jack bean α-mannosidase. At the desired times, aliquots from each incubation were removed, adjusted to pH 8 to stop the digestion, and heated over boiling water to release all protein-bound PG. After removal of precipitated material by centrifugation, the mixtures of modified PG were allowed to pass through small (2 mL) Con A–Sepharose 4B columns. For each time point the ratio of radioactivity eluted with the methyl mannoside buffer to total radioactivity recovered reflects the fraction of Con A binding PG derivatives.

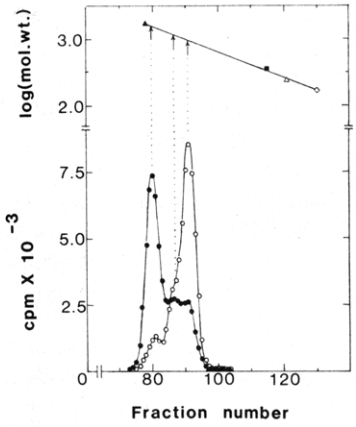

FIGURE 5: Fractionation of α-mannosidase-modified PG by G-25 gel filtration chromatography. Free PG (○) and AAT-PG (●) of Figure 4 were treated with 35 units of α-mannosidase for 3 h. The proteins were heat denatured and removed by centrifugation. Aliquots of the supernatant (0.5 mL) were passed through a Sephadex G-25-80 column (1.5 × 166 cm) eluted with 0.2 M NH_4HCO_3, pH 7.7. Fractions collected were about 2 mL. Lower graph: elution profile. Upper graph: relationship between the log of the molecular weight and the elution position standardized with intact PG (▲) (M_r 1741), PA (■) (M_r 363), PNP (△) (M_r 248), and methyl mannoside (◇) (M_r 180).

crystallographic studies on native AAT indicate that the PLP/PMP binding site is located in a groove within the protein matrix (Arnone et al., 1982). If PG is bound at the same site, one would predict that part of the oligosaccharide chain is masked by the polypeptide. To test this prediction, an experiment was designed to determine the degree to which apoAAT can protect the oligosaccharide in PG from digestion by α-mannosidase. Two samples, one containing free PG and the other containing AAT–PG complex, were digested with α-mannosidase under identical conditions, and the progress of the digestion was followed by assaying the change in Con A binding ability of PG. The results presented in Figure 4 show that free PG lost 90% of its Con A binding material in 3 h while complexed PG only lost 40% during the same time. When aliquots of unfractionated 3-h digestion mixtures of free PG and AAT–PG were analyzed on a G-25 column, the elution profiles presented in Figure 5 were obtained. The component in fractions 74–84 (peak at 80) is consistent with the elution position of a PG derivative (M_r 1578) having one less mannosyl unit than the starting material (M_r 1741); it should be expected to bind tightly to Con A (Baenziger & Fiete, 1979). This component accounts for 12% in free PG, and 63% in AAT–PG, of all the radioactivity passed through the G-25 column (fractions 74–96). The component with a peak at fraction 91 corresponds to a PG derivative having lost all five α-mannosyl units (M_r 930) as the result of exhaustive digestion by α-mannosidase. Another minor component eluted at about fraction 87 can be assigned to a PG derivative with only one α-mannosyl residue remaining. These latter two components account for the remaining 88% in free PG, and 37% in AAT-PG. Assuming that both of these components are eluted from the Con A column in TEA buffer, the agreement with the 3-h data points in Figure 4 (90% and 40% loss in Con A binding, respectively) is excellent. We conclude that under conditions in which 90% of all α-mannosyl units in free PG were hydrolyzed, only one α-mannosyl unit in AAT–PG is cleaved and that the remainder of the high mannose structure was protected by the polypeptide of apoAAT. Implicit in this conclusion is the assumption that the 40% that was completely digested in the AAT–PG complex represents free PG dissociated from the complex. This assumption is supported by two observations; first, the dissociation rate (cacodylate buffer in Figure 3) was virtually identical with the digestion rate for AAT–PG (figure 4), and second, at 3 h the reaction mixture of the sample containing AAT–PG, whether heat denatured or not, gave the same Con A elution profiles (data not shown). The latter observation shows that while the AAT–PG complex with one mannose removed is no longer a substrate for α-mannosidase, it still binds to Con A. We can visualize a model in which several mannosyl residues in AAT–PG are exposed to the degree that those atoms necessary for binding to Con A can interact in its shallow cavity (Hardman, 1979) while only one mannosyl residue extends far enough beyond the surface of the complex to interact with the active site of α-mannosidase. This model is graphically illustrated in Figure 1. It emphasizes the need to qualify the concept of exposure in terms of the specificity determinant of binding for a particular kind of biological activity. By the criterion of α-mannosidase activity, AAT–PG has only one "exposed" mannosyl unit, while by the criterion of Con A binding, it has at least two to three (Baenziger & Fiete, 1979).

Discussion

The three-dimensional structure of AAT is constructed with a compact and well-ordered large domain and a loosely packed but more mobile small domain (Arnone et al., 1982). The binding site for the coenzyme is contained in the large domain while the binding site for the substrate amino acid is located in the small domain and immediately adjacent to the coenzyme pocket. Provided that the coenzyme portion of PG occupies the normal coenzyme position in the large domain, its oligosaccharide portion should interact primarily with the small domain, the loose structure of which should facilitate accommodation of the bulky sugar moiety. However, we note that the curves for the dissociation of PG presented in Figures 3 and 4 are suggestive of the presence of anticooperativity. Although the phenomenon of anticooperativity has never been clearly demonstrated for AAT in solution, functional assymmetry between the two subunits induced by crystallization has been well documented (Arnone et al., 1982; Kirsten et al., 1983). Thus, it is not unreasonable that the binding of the

first PG with its bulky oligosaccharide causes enough perturbation in the three-dimensional structure of the dimer to significantly decrease the affinity for the second PG. Further studies will be required to unequivocally establish whether anticooperative binding may indeed be in effect for the saturation of AAT with PG.

Although the fact that the oligosaccharides in AAT–PG are noncovalently attached undoubtedly imposes some limitations on the applicability of this type of neoglycoprotein, especially for in vivo studies, this mode of oligosaccharide attachment also has some very attractive and advantageous features. Thus, in most work involving glycoproteins it is desirable to be able to separate the oligosaccharide from the protein for analysis or for control experiments. A neoglycoprotein containing covalently bound carbohydrate requires chemical or enzymatic treatments of limited specificity and applicability and often of excessive harshness to liberate the oligosaccharide units, while in the case of the noncovalent ones, like AAT–PG, the oligosaccharide can be removed specifically and completely by displacement with unmodified ligand or by heat denaturation of the protein. Another attractive feature of a noncovalent oligosaccharide–protein complex is that all the chemical manipulations involved in the derivatization of the oligosaccharide to the ligand are performed in the absence of the protein. The actual formation of the neoglycoprotein through the binding of the glycosylated ligand to the apoprotein must ideally be carried out under gentle, nondenaturing conditions preserving the "native" protein structure required for proper binding to take place. Parallel formation of natural holoenzyme with unmodified ligand again provides ideal reference complexes for control experiments. A third advantage is realized whenever the ligand-binding site is located in the interior of the protein as it is in AAT to the extent that the chemical linkage group involved in attaching the oligosaccharide to the protein becomes masked in the neoglycoprotein complex. Direct chemical glycosylation of the protein generally leaves an exposed organic oligosaccharide–protein linkage group which can participate in subsequent neoglycoprotein interactions. Such Participation has been observed in a galactosyl–bovine serum albumin in which an unsaturated group at the γ-position to the anomeric carbon enhances the binding of the neoglycoprotein to the hepatic galactose/*N*-acetylgalactosamine–lectin (Lee et al., 1982). This kind of interaction is not unprecedented, since in some plant lectins, a "hydrophobic site" has been found in addition to the sugar-specific site (Roberts & Goldstein, 1982; Edelman & Wang, 1978).

A partially buried oligosaccharide such as that in AAT–PG also provides a unique opportunity to investigate the effects of intramolecular sugar–protein interaction that may modulate the reactivities of an oligosaccharide toward an enzyme or other reagents. Perhaps because carbohydrates have been implicated in mediating a large variety of cell surface communication processes (Gahmberg, 1981), it has become popular to depict oligosaccharides attached on cell surface components as flexible structures protruding into the solvent matrix. Although evidence supporting this representation can also be found for glycoproteins in solution (Dill & Allerhand, 1979; Berman et al., 1981; Torchia et al., 1981), it may not be true for all glycosyl units attached to polypeptides. In three glycoproteins of known three-dimensional structures [human IgG (Deisenhofer, 1981); influenza hemagglutinin membrane glycoprotein (Wilson et al., 1981); influenza membrane neuraminidase (Varghese et al., 1983)], some glycosylated sites are located along domain interfaces, and many residues of the oligosaccharides are interacting with side chains of the polypeptide. Given the present understanding of the mechanism of glycoprotein biosynthesis (Bergman & Kuehl, 1982) in which a preassembled glycosyl unit is transferred en bloc to the growing polypeptide chain followed by further processing of the glycosyl unit subsequent to the folding of the polypeptide, it seems clear that sugar–protein interactions in fact could influence the reactivities of an oligosaccharide toward the processing enzymes themselves. This possibility can be investigated by testing the processing enzymes in reactions with a series of AAT–PG complexes in which the same oligosaccharide is exposed to different degrees by interposing a spacer arm of variable lengths between the asparaginyl and the phosphopyridoxyl portions of PG.

In conclusion, the preparation of neoglycoproteins through noncovalent complex formation between a glycosylated ligand and its apoprotein is a workable approach. This should be a general concept and not restricted to the use of PLP/PMP-dependent enzymes. Another system under consideration in this labortory is one involving the binding of glycosylated biotin to avidin. Avidin complexes of hormone–biotin conjugates have been prepared, and these have been found to retain the biological activites of the underivatized hormones (Hofmann et al., 1977; Lavielle et al., 1983; Manz et al., 1983). Depending on the ligand and the protein chosen, different modes of oligosaccharide attachment and stoichiometry of incorporation can be obtained. This together with the mild condition under which the neoglycoprotein is prepared and the ease of subsequent removal of the oligosaccharide for analysis makes noncovalent carbohydrate–protein complexes potentially useful tools as model systems for studying various aspects of glycoprotein biochemistry.

Acknowledgments

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Registry No. PLP, 54-47-7; Man_6-$GlcNAc_2$-Asn, 39114-02-8; PG, 90220-07-8; L-asparagine, 7006-34-0; PA, 14942-20-2.

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