The Effect of Partial Deglycosylation on the Structure of α1-Acid Glycoprotein

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Abstract. Changes in structure of α1-acid glycoprotein were followed after deglycosylation with neuraminidase, peptide N-glycohydrolase F or with a mixture of exoglycosidases Partially deglycosylated preparations of α1-acid glycoprotein free of sialic acids, one complete saccharide component, sialic acids and one saccharide component and sialic acids and some of the external saccharides were obtained The effect of these changes in saccharide components on the glycoprotein structure was studied by temperature perturbation difference spectroscopy, fluorescence spectroscopy, fourth-derivative of absorption spectra and spectra of CD

Partial deglycosylation resulted in transformation of the molecule to a more compact state in which phenylalanyl residues were even more buried, tyrosyl residues became more uniform and tryptophyl residues were less exposed The content of ordered secondary structures decreased The thermal stability of the molecule was not significantly affected Removal of one of the five saccharide components from the native molecule had apparently deeper effect than total desialylation of the glycoprotein

Key words: α1-Acid glycoprotein — Desialyization — Deglycosylation — Spectral characteristics

Abbreviations: α1-AG - α1-acid glycoprotein, α1-AG(-1 comp) α1-AG with one carbohydrate component missing, α1-AG(-Sia) completely desialyzed α1-AG, α1-AG(-Sia,-1 comp) completely desialyzed α1-AG with one carbohydrate component missing, α1-AG(-Sia,-Gal) - completely desialyzed α1-AG from which about half of galactose residues was removed, ΔA - differential molar absorbance, CD - circular dichroism, FDS - fourth-derivative spectroscopy, I - fluorescence

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intensity; $\lambda$ – wavelenght; PheNACEE – N-acetyl-L-phenylalanine ethyl ester; PN-Gase F – peptide N-glycohydrolase F; SDS – sodium dodecyl sulfate; $\Delta T$ – differential temperature; $\Theta$ – ellipticity; TPDS – temperature perturbation difference spectroscopy; TrpNACEE – N-acetyl-L-tryptophan ethyl ester; TyrNACEE – N-acetyl-L-tyrosine ethyl ester

Introduction

$\alpha_1$-Acid glycoprotein ($\alpha_1$-AG) is one of the best characterized glycoproteins of the human serum. Because of its high carbohydrate content (38% of the total weight) $\alpha_1$-AG has unusual stability and solubility. It is not precipitated by boiling or by some acids, usually used for precipitation of proteins. Carbohydrates protect the molecule against proteolytic enzymes and block antigenic determinants. $\alpha_1$-AG is a one chain molecule with 181 amino acids with possible variations at 21 sites. Five heterooligosaccharide units are N-glycosidically linked to asparagine residues in the N-terminal half of the molecule (Schwick et al. 1977). Saccharide components are of the lactosamine type, bi-, tri- or tetraantenary with L-fucose residue at the first glycosylation site and bi-, tri- and tetraantenary or tri- and tetraantenary with L-fucose residue from the second to the fifth glycosylation site (Fournet et al. 1978).

The biological function of $\alpha_1$-AG has not yet been established. It is only known to bind progesterone about fifteen times stronger than albumin. Partially desialyzed $\alpha_1$-AG has an affinity for vitamin B$_{12}$. Clinically, $\alpha_1$-AG is an acute phase protein. Together with haptoglobin it is an indicator of acute inflammation and some diseases (Schwick et al. 1977).

The three-dimensional structure of $\alpha_1$-AG, for the most part still unknown, is a rather compact one with a high number of buried residues (9 tyrosyls (Svobodová et al. 1977), 10 carboxyls (Karpenko and Kalous 1977) and 2 tryptophyls (Schmid et al. 1976)) in the native state. The native molecule was found to be an asymmetric one with an axial ratio 6 : 1. Desialyzation leads to change of the axial ratio to 4 : 1, a value which is much closer to the sphere (Kawahara et al. 1973). Data from CD spectra indicate the presence of 8% of $\alpha$-helix, 60% of $\beta$-structure and 10% of $\beta$-turn type II in the secondary structure of $\alpha_1$-AG (Kodiček et al. 1995).

As the carbohydrate components of $\alpha_1$-AG are responsible for the unusual physico-chemical properties of this glycoprotein, the investigation of some properties of partially deglycosylated $\alpha_1$-AG was the aim of the present study. It was interesting to obtain information about the contribution of carbohydrate components to the spatial arrangement of the glycoprotein molecule. Also, the accessibility of some functional groups of the glycoprotein could be affected by the presence, composition and structure of the carbohydrate components. Partial deglycosylation (mainly desialyzation), incidentally, is an important part of the mechanism which determines the halflife of $\alpha_1$-AG in blood (Ashwell and Harford 1982).
Structure of $\alpha_1$-Acid Glycoprotein

Materials and Methods

$\alpha_1$-AG was isolated from Cohn's fraction VI of pooled normal human blood serum (Imuna, Šarišské Michalany, Slovakia) by the fractionation method according to Karpenko et al. (1968) The purity of the obtained preparation of the glycoprotein was checked by polyacrylamide gel electrophoresis in alkaline medium (Davis 1964, Ornstein 1964) as well as in the presence of sodium dodecyl sulfate (SDS) (Laemmli 1970) The concentrations of the native glycoprotein solutions were determined from absorbance at 280 nm ($A_{280} = 8.93$ (Schulze and Heremans 1966)) The value of 41,000 was chosen for the relative molecular weight of $\alpha_1$-AG (Karpenko et al. 1993)

The components of buffers were of analytical grade purity and were obtained from Lachema, Czech Republic. All other chemicals except enzymes were obtained from Sigma, USA. The origin of enzymes is specified below.

Desialylation of $\alpha_1$-AG

Sialic acids were removed from $\alpha_1$-AG with Clostridium perfringens neuraminidase (acylneuraminyldase, EC 3.2.1.18, Boehringer, Mannheim, FRG) $\alpha_1$-AG (25 mg) and 2.5 U of neuraminidase were dissolved in 3.6 ml of 0.1 mol dm$^{-3}$ acetate buffer pH 5.0 The mixture was incubated for 7 h at 37°C with a few drops of toluene. The resulting solution was used either for measurement of temperature perturbation difference spectra (TPDS) after proper dilution with 0.1 mol dm$^{-3}$ phosphate buffer, pH 7.4, and 3-day dialysis against the same buffer (3×5 l) or dialyzed 3 days against distilled water (3×5 l) and freeze-dried. Removal of sialic acids was controlled by determination of these acids by the method employing thiobarbituric acid (Spiro 1966 a). The homogeneity of the obtained preparation was checked by polyacrylamide gel electrophoresis in the presence of SDS (Laemmli 1970).

Hydrolysis with peptide N-glycohydrolase F (PNGase)

To release saccharide components from $\alpha_1$-AG, enzyme PNGase F from Flavobacterium meningosepticum (peptide N-glycohydrolase F, EC 3.2.2.18, Boehringer Mannheim, FRG) was employed (Tarentino et al. 1985) $\alpha_1$-AG (25 mg), 50 U of PNGase and 0.034 mg of disodium salt of ethylenediaminetetraacetic acid (EDTA) were dissolved in 3.6 ml of 0.1 mol dm$^{-3}$ phosphate buffer pH 7.4. The solution was incubated for 24 h at 37°C under toluene atmosphere. Then an aliquot (1.5 ml) of the reaction mixture was used directly for TPDS measurement. The remaining reaction mixture was dialyzed 3 days against distilled water (3×5 l) and freeze-dried. The sample of $\alpha_1$-AG was treated in the same way with PNGase F previously desialyzed with neuraminidase as described above. The degree of the removal of saccharide components was checked by determining neutral sugar content according to Dubois et al. (1956). The homogeneity of both preparations which should be deglycosylated to some extent was checked by electrophoresis in polyacrylamide gel.
in the presence of SDS (Laemmli 1970). The activity of the used PNGase preparation was assessed by splitting of saccharide components from trypsin inhibitor and using N-linked oligosaccharide profiling kit (N-linked oligosaccharide profiling kit).

Deglycosylation with a mixture of exoglycosidases

α1-AG (50 mg) was dissolved in 2.5 ml of 0.05 mol.dm\(^{-3}\) acetate buffer (pH 5.0) and 2.5 U of Clostridium perfringens neuraminidase (Boehringer, Mannheim, FRG), 12 mg of emulsin (mixture of exoglycosidases (Spiro 1966b), Serva, Heidelberg, FRG) and 0.5 ml of toluene were added. The mixture was incubated either for 60 h or for 120 h at 37°C, then dialyzed for 3 days (3×5 l) and freeze-dried. The degree of deglycosylation was followed by determining neutral sugar content according to Dubois et al. (1956). The homogeneity of the obtained preparation was examined by polyacrylamide gel electrophoresis in the presence of SDS (Laemmli 1970).

The activity of emulsin preparation was checked with respect to the most important enzyme (for use in our case) by determining β-galactosidase activity with o-nitrophenyl β-D-galactopyranoside (Hu et al. 1959; Spiro 1962).

TPD-spectrometry

The spectra were recorded with a Cecil 8000 spectrophotometer equipped with R 0035 software and CE 245 temperature regulation block with a precision of ±0.3°C. Spectra were registered in the wavelength region of 240–320 nm at the registration rate of 300 nm/min. Quartz cells with 1 cm optical path were used. The concentrations of the samples in 0.1 mol.dm\(^{-3}\) phosphate buffer, pH 7.4, ranged between 4.1 \(\times\) 10\(^{-5}\) and 7.4 \(\times\) 10\(^{-5}\) mol.dm\(^{-3}\). The reference sample was heated from 25°C to 75°C in 5°C steps. Differential molar absorbances ΔA were determined at wavelengths of 255, 264, 273, 285, 294 and 300 nm. These wavelengths were chosen according to previously determined maxima of the fourth-derivative of absorption spectra and experimentally found values of TPDs maxima of α1-AG.

Values of ΔA at the constant wavelength were plotted as a function of ΔT and the curves were fitted using Origin polynomial.

Fluorescence spectrometry

A Perkin-Elmer LS 50B luminescent spectrometer was used to measure fluorescence spectra. Quartz cuvettes with 1 cm optical path were used. The sample concentration in 0.1 mol.dm\(^{-3}\) phosphate buffer, pH 7.4, was 0.125 mg/ml. This value was confirmed spectrophotometrically using absorbance at 278 nm. Excitation wavelength was 286 nm (experimentally determined maximum of excitation spectrum for emission wavelength 350 nm), recording speed 50 nm/min and slit width 2.5 nm. The temperature of the sample was increased at 10°C steps (from 25°C to 55°C) and at 5°C steps (from 55°C to 80°C). Then, the temperature was decreased at the same steps back to 25°C. To achieve temperature equilibrium, the samples were incubated at each temperature for 20 min. Wavelengths of emission maxima and intensities of these maxima in relative units were determined for the individual spectra. The temperature dependences of these values are presented in graphs.
Fourth-derivative spectrometry (FD-spectrometry)

Measurements of absorption spectra and their consequential derivatives were carried out with a Cecil 8000 spectrophotometer equipped with R 0035 software in the wavelength region of 250–300 nm, in quartz cuvettes with 1 cm optical path. Samples were prepared in the concentration range of $4.1 \cdot 10^{-5} - 7.4 \cdot 10^{-5}$ mol dm$^{-3}$ and the molar absorbance was calculated. Absorption spectra were also measured for model substances: N-acetyl-L-tyrosine ethyl ester (TyrNAcEE), N-acetyl-L-tryptophan ethyl ester (TrpNAcEE) and N-acetyl-L-phenylalanine ethyl ester (PheNAcEE). All spectra were taken at 25°C. FD-spectra were interpreted according to Padros et al. (1984).

CD-spectrometry

CD-spectra were measured with Jobin-Yvon Autodiograph MARK V, equipped with Dichrosoft Version A software. Quartz cuvettes JASCO with 1 mm optical path were used. The cell space was heated by external water bath. To ensure temperature and conformational equilibrium the samples were kept at each temperature for 20 min before the spectrum was measured. Sensitivity was set at $5 \cdot 10^{-6}$ Each measurement was the result of 3 repeated records. The concentration of all samples in 0.1 mol dm$^{-3}$ phosphate buffer, pH 7.4, was 0.25 mg/ml and this value was checked spectrophotometrically using absorbance at 278 nm. The shares of the individual ordered secondary structures were calculated using the program developed by Kodíček et al. (1995).

Results

$\alpha_1$-AG isolated from Cohn’s fraction VI was found to be homogeneous on polyacrylamide gel electrophoresis in alkaline medium (Davis 1964; Ornstein 1964) as well as in the presence of SDS (Laemmli 1970). From 6 g of Cohn’s fraction VI about 0.3 g of pure freeze-dried $\alpha_1$-AG was obtained. This $\alpha_1$-AG had exactly the same electrophoretic properties as $\alpha_1$-AG isolated directly from normal pooled human serum.

Desialyization of $\alpha_1$-AG

Treatment of $\alpha_1$-AG with Clostridium perfringens neuraminidase under conditions as described under Materials and Methods resulted in complete removal of sialic acid residues from the molecule. The original content of sialic acids ($12.5 \pm 0.2\%$, $n = 5$) decreased below detection limits of the method used (Spiro 1966a). The relative molecular weight of desialyzed $\alpha_1$-AG ($\alpha_1$-AG(-Sia)) was calculated to be 35,875 (based on the value of 41,000 for native $\alpha_1$-AG), and the extinction coefficient was found to be $A_{278.1\%} = 10.05$. The preparation of $\alpha_1$-AG(-Sia) was homogeneous on polyacrylamide gel electrophoresis in the presence of SDS (Laemmli 1970).
Deglycosylation with PNGase F

The action of PNGase F on native α₁-AG or desialyzed α₁-AG (α₁-AG(-Sia)) resulted in a ~ 20% decrease (22%) of the neutral sugar content of both α₁-AG forms. The original value of 21.1 ± 0.3%, n = 5 decreased to 16.5 ± 0.3%, n = 5, irrespective of the incubation period with the enzyme (24 or 72 h). As the native molecule contains 5 saccharide components with about the same content of neutral sugar, it can be concluded that PNGase F, under the conditions used, removed one of the five saccharide components from both α₁-AG and α₁-AG(-Sia). Preparations of α₁-AG and α₁-AG(-Sia) with one saccharide component missing (α₁-AG(-1 comp.) and α₁-AG(-Sia,-1 comp.)) were homogeneous on electrophoresis in polyacrylamide gel in the presence of SDS (Laemmli 1970). The relative molecular weight of α₁-AG(-1 comp.) and α₁-AG(-Sia,-1 comp.) were calculated to be 37,310 and 32,800, respectively. The corresponding extinction coefficients were A_{278,1%} = 9.73 and A_{278,1%} = 10.72, respectively.

Desialyization and deglycosylation with a mixture of exoglycosidases

The combined treatment of α₁-AG with neuraminidase and a mixture of exoglycosidases (emulsin) resulted in complete removal of sialic acids and release of some additional external saccharides from saccharide components of α₁-AG. Prolongation of the incubation period with enzymes from 60 h to 120 h had an only slight effect on the neutral sugar content (Dubois et al. 1956). After 120 h incubation the neutral sugar content decreased from 21.1 ± 0.3%, n = 5 to 15.5 ± 0.3%, n = 5. This decrease (~ 27%) of the neutral sugar content corresponds to the removal of about 2 (1.89) D-galactose residues (in addition to the release of all sialic acids) from each of the five saccharide components assuming that all saccharide components are tetraantenary. Roughly, about half of the D-galactose residues was removed from saccharide components regardless of how antenary they are. This preparation will be termed α₁-AG(-Sia,-Gal). Polyacrylamide gel electrophoresis in the presence of SDS (Laemmli 1970) revealed small admixtures of proteins with relative molecular weights differing significantly from that of α₁-AG. The corresponding zones apparently belong to exoglycosidases used for deglycosylation. The calculated relative molecular weight of α₁-AG(-Sia,-Gal) was 31,980 and the extinction coefficient A_{278,1%} = 10.89.

TPD-spectroscopy

TPDS were measured for model substances: TrpNAcEE, TyrNAcEE and PheNAcEE and for glycoprotein samples: α₁-AG, α₁-AG(-Sia), α₁-AG(-1 comp.), α₁-AG(-Sia,-1 comp.) and α₁-AG(-Sia,-Gal). A linear dependence of ΔAA on ΔT was found for all model substances at the wavelengths of their absorption maxima.

At wavelengths of 255 and 264 nm (the region of phenylalanine absorbance) the spectra of all examined samples were rather similar. Apparently, the environment of the phenylalanyl residues was not drastically altered as a result of deglycosylation. The temperature of the extreme in the temperature dependence of ΔAA in
the 250–264 nm region is always higher for partially deglycosylated preparations of α₁-AG than that for the native molecule. This finding indicates that the phenylalanyl chromophores of partially deglycosylated preparations are even more buried in hydrophobic regions. The largest differences were found at wavelengths 294 and 300 nm (the region of tryptophan absorbance) (Figs. 1 and 2). There was an obvious difference between the spectra of α₁-AG(-1 comp.) and α₁-AG(-Sia,-1 comp.) on one hand and α₁-AG, α₁-AG(-Sia) and α₁-AG(-Sia,-Gal) on the other one. Apparently, the environment of tryptophyl residues is affected by removal of one of the five saccharide components from the glycoprotein molecule. Also, desialyzation had a pronounced effect on tryptophyl chromophores. For α₁-AG(-Sia) some estimations were possible. At the wavelength of 294 nm, the approximate numbers of exposed tryptophyl residues could be calculated according to Nicola and Leach (1976). In α₁-AG(-Sia) the numbers of exposed tryptophyl residues decreased from 1.5–2.5 (20–40°C) for native α₁-AG to 0.5–1.5 (20–40°C).
Figure 2. Temperature perturbation difference spectra (TPDS) Dependence of \( \Delta A \) on \( \Delta T \) at 300 nm (+), \( \alpha_1\text{-AG,}(x), \alpha_1\text{-AG(-1 comp ), (■), \alpha_1\text{-AG(-Sia), (○), \alpha_1\text{-AG(-Sia,-1 comp })},(∗), \alpha_1\text{-AG(-Sia,-Gal)} \)

**Fluorescence spectroscopy**

Fluorescence spectra were recorded for samples of \( \alpha_1\text{-AG,} \alpha_1\text{-AG(-1 comp ),} \alpha_1\text{-AG(-Sia,-1 comp) and} \alpha_1\text{-AG(-Sia,-Gal). The fluorescence intensity at any temperature was found to be highest for} \alpha_1\text{-AG and decreased in the order:} \alpha_1\text{-AG(-1 comp ),} \alpha_1\text{-AG(-Sia,-1 comp) and} \alpha_1\text{-AG(-Sia,-Gal) (Fig. 3). The emission maxima showed a red shift with the increasing temperature (Fig. 4). The fluorescence intensity decreased with the increasing temperature, and this phenomenon was fully reversible (Fig. 5). Samples of} \alpha_1\text{-AG(-1 comp) and} \alpha_1\text{-AG(-Sia,-1 comp) behaved similarly as far as the temperature dependence of fluorescence intensity is concerned. The same phenomenon was observed on TPDS.**

**FD-spectra**

The fourth-derivative of absorption spectra of the model substance – PheNAcEE showed maxima at 258, 263 and 267 nm. In spectra of native \( \alpha_1\text{-AG} \) or its deglycosylated preparations (Figs. 6, 7), these maxima were shifted towards shorter wavelengths (255, 257 and 260 nm, respectively). Very close wavelengths of maxima were found for the native and all the partially deglycosylated preparations. Apparently,
the changes in the glycoprotein molecule induced by partial deglycosylation do not significantly affect phenylalanyl chromophores. The shift of the maxima towards shorter wavelengths suggests a nonpolar environment of phenylalanyl residues in this glycoprotein.

The maxima at 282 and 284 nm correspond to tyrosine residues. Parameter R introduced by Padros et al. (1984) could be calculated; its increase points to an increased heterogeneity of tyrosyl residues or an increase of the strength of hydrogen bonds (Padros et al. 1984). The values for native and partially deglycosylated preparations of α1-AG are given in Table 1. The values for all desialyzed prepa-
Figure 4. Fluorescence spectroscopy Temperature dependence of the emission maximum wavelength 1 (+), α1-AG, 2 (x), α1-AG(-1 comp ), 3 (●), α1-AG(-Sia,-1 comp ), 4 (●), α1-AG(-Sia,-Gal).

Rations are much lower than those for preparations in which the majority of sialic acids was retained. Desialyzation apparently decreased the heterogeneity of tyrosyl residues.

Maxima corresponding to tryptophan (296 and 298 nm) are very small, and no analysis according to Dunách et al. (1983) could therefore be done.

CD-spectroscopy

CD-spectra of all examined samples had a negative maximum at 218 nm which decreased after all types of deglycosylation attempts (Fig. 8). Curves were fitted to the measured spectra with the use of CD-spectra for the basic types of secondary structures and the equation of Levitt and Chothia (1976). The calculated contents of secondary structures in the native and modified preparations of α1-AG (see Materials and Methods) are summarized in Table 2.

Although the values cannot be taken as absolute ones, it is apparent that in partially deglycosylated preparations the shares of α-helix and β-structure decreased and that of β-turn or aperiodic structures proportionally increased. With the increasing temperature (from 25 to 80°C) CD-spectra of native as well as partially deglycosylated preparations of α1-AG changed only slightly, and after cooling...
Figure 5. Fluorescence emission spectra of $\alpha_1$-AG(-$\text{Sia}$,-1 comp) at different temperatures Increasing temperature (1) 25°C, (2) 50°C, (3) 75°C Decreasing temperature (1) 25°C, (2) 50°C, (3) 75°C

Table 2. The content of ordered secondary structures in native and partially deglycosylated $\alpha_1$-AG preparations

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$-helix</th>
<th>$\beta$-structure</th>
<th>$\beta$-turn</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$-AG</td>
<td>7%</td>
<td>58%</td>
<td>12%</td>
</tr>
<tr>
<td>$\alpha_1$-AG(-1 comp)</td>
<td>5%</td>
<td>23%</td>
<td>22%</td>
</tr>
<tr>
<td>$\alpha_1$-AG($\text{Sia}$)</td>
<td>4%</td>
<td>20%</td>
<td>21%</td>
</tr>
<tr>
<td>$\alpha_1$-AG(-$\text{Sia}$,-1 comp)</td>
<td>4%</td>
<td>4%</td>
<td>27%</td>
</tr>
<tr>
<td>$\alpha_1$-AG(-$\text{Sia}$,-Gal)</td>
<td>5%</td>
<td>12%</td>
<td>23%</td>
</tr>
</tbody>
</table>

down the CD-spectra became indistinguishable from those recorded before the heating. Slight changes in secondary structures induced by an increase of temperature were reversible.

Discussion

According to the structure of its saccharide components, $\alpha_1$-AG is a typical serum glycoprotein with a relatively very high content of these components. Therefore
it seemed to be a convenient model for the study of influence of saccharide components on physico-chemical properties and, perhaps, biological properties of this substance. It was apparently easily possible to remove sialic acids from the glycoprotein and to study some of its physico-chemical properties (Kawahara et al. 1973). Unfortunately, the so-called desialyzed α1-AG was not checked for sialic acid content (Kawahara et al. 1973). Partially deglycosylated α1-AG was also studied with respect to its optical rotation. However, no significant influence of saccharide components on this property of α1-AG was found (Schmid and Kamiyama 1963). When PNGase F was discovered (Tarentino et al. 1985) (an enzyme which can release whole N-glycosidically bound saccharide components from a glycoprotein) it was tested for the ability to release the saccharide components also from α1-AG. Since that time it has been recommended to denature α1-AG before PNGase F treatment to achieve optimum deglycosylation (Tarentino et al. 1985).

In our experiments we easily prepared completely desialyzed α1-AG. However, even prolonged treatment of native or desialyzed α1-AG with PNGase F resulted in the removal of only one of the five saccharide components of α1-AG. Of course, it could not be specified which of the five saccharide components was released. Denaturation before the treatment would make no sense for our purposes. Surprisingly,

**Figure 6.** The fourth-derivative of α1-AG absorption spectrum (FD-spectrum).
deglycosylation of desialyzed α₁-AG with a mixture of exoglycosidases was also very limited and stopped after the supposed removal of about half of D-galactose residues from the nonreducing terminus of the antennas.

Obviously, our conclusions concerning the structure of partially deglycosylated preparations are somewhat speculative because we rely on the specificity of the enzymes used and the knowledge of the structures of the saccharide components. However, a direct detailed analysis would be complicated by the microheterogeneity of the saccharide components of α₁-AG.

All data obtained by spectral methods indicate that any kind of deglycosylation attainable without prior denaturation of the glycoprotein resulted in the transformation of the molecule to a more compact state apparently by an increase of hydrophobic interactions. According to CD-spectra this transformation is accompanied by a decrease of the content of α-helix and β-structure and an increase of the content of β-turn and aperiodic structures.

The trends to form more compact structures after deglycosylation do not affect significantly phenylalanyl residues of the α₁-AG molecule. This is clearly shown by fourth-derivative absorption spectra as well as by TPDS. These residues are apparently located in hydrophobic regions already in the native molecule of the glycoprotein. Transformation to a more compact molecule is also accompanied by
Figure 8. Circular dichroic (CD) spectra at 25°C (1), α1-AG, (2), α1-AG(-1 comp ), (3), α1-AG(-Sia,-1 comp ), (4), α1-AG(-Sia,-Gal), (5), α1-AG(-Sia)

a decrease of the heterogeneity of tyrosyl residues. This can be derived from the decrease of parameter R of FD-spectra.

The idea about the transformation to a more compact state as a result of partial deglycosylation is also supported by fluorescence measurements. The observed decrease of fluorescence intensity of partially deglycosylated preparations suggests the sinking of tryptophyl residues into internal regions of the molecule. A lower number of exposed tryptophyl residues compared with the native molecule was found also by calculation from TPDS at 294 nm for the α1 AG(-Sia) sample.

Taking into account the temperature dependence of the spectra, it can be concluded that the changes in the saccharide components attainable without prior denaturation have no significant effect on the thermal stability of the α1-AG molecule within the examined temperature interval (25-80°C). The changes in fluorescence and CD-spectra were fully reversible. Moreover, the CD-spectrum only slightly changed with the increasing temperature.

From TPDS a really surprising conclusion can be derived. Apparently, removal of one of the five saccharide components, obviously together with its sialic acids, has a more pronounced influence on α1-AG structure than total desialyzation of all saccharide components.
The effect of partial deglycosylation of $\alpha_1$-AG on its biological functional properties could not be determined because there are no biological characteristics which can be easily measured. As already mentioned in Introduction, the biological function of $\alpha_1$-AG has not yet been established. However, it is a well-known fact that desialylation of this glycoprotein results in the deblocking of antigenic determinants (Schwick et al. 1977). Sialic acid-deficient $\alpha_1$-AG has been found in the sera or urine of patients with Hodgkin’s disease, psoriatic arthritis, diabetes, neoplastic disease, and chronic myeloid leukemia (Schmid et al. 1964). $\alpha_1$-AG with altered saccharide components was also found in human bile (Abei et al. 1994).

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