The Effect of Partial Deglycosylation on the Structure of α_1-Acid Glycoprotein

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Abstract. Changes in structure of α_1-acid glycoprotein were followed after deglycosylation with neuraminidase, peptide N-glycohydrolase F or with a mixture of exoglycosidases Partially deglycosylated preparations of α_1-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 desialyzation of the glycoprotein

Key words: α_1-Acid glycoprotein — Desialyzation — Deglycosylation — Spectral characteristics

Abbreviations: α_1-AG – α_1-acid glycoprotein, α_1-AG(-1 comp) α_1-AG with one carbohydrate component missing, α_1-AG(-Sia) completely desialyzed α_1-AG, α_1-AG(-Sia,-1 comp) completely desialyzed α_1-AG with one carbohydrate component missing, α_1-AG(-Sia,-Gal) – completely desialyzed α_1-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; λ – wavelenght; PheNAcEE – N-acetyl-L-phenylalanine ethyl ester; PNGase F – peptide N-glycohydrolase F; SDS – sodium dodecyl sulfate; ΔT – differential temperature; Θ – ellipticity; TPDS – temperature perturbation difference spectroscopy; TrpNAcEE – N-acetyl-L-tryptophan ethyl ester; TyrNAcEE – N-acetyl-L-tyrosine ethyl ester

Introduction

α_1-Acid glycoprotein (α_1-AG) is one of the best characterized glycoproteins of the human serum. Because of its high carbohydrate content (38% of the total weight) α_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. α_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 α_1-AG has not yet been established. It is only known to bind progesterone about fifteen times stronger than albumin. Partially desialyzed α_1-AG has an affinity for vitamin B_{12}. Clinically, α_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 α_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 α-helix, 60% of β-structure and 10% of β-turn type II in the secondary structure of α_1-AG (Kodíček et al. 1995).

As the carbohydrate components of α_1-AG are responsible for the unusual physico-chemical properties of this glycoprotein, the investigation of some properties of partially deglycosylated α_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 α_1-AG in blood (Ashwell and Harford 1982).

Materials and Methods

α_1-AG was isolated from Cohn's fraction VI of pooled normal human blood serum (Imuna, Šarišske Michaľany, 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 ($A_{278\ 1\%}$= 8 93 (Schulze and Heremans 1966)) The value of 41,000 was chosen for the relative molecular weight of α_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, U S A The origin of enzymes is specified below

Desialyzation of α_1-AG

Sialic acids were removed from α_1-AG with *Clostridium perfringens* neuraminidase (acylneuraminyl hydrolase, EC 3 2 1 18, Boehringer, Mannheim, FRG) α_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 α_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) α_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 α_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

α_1-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 $4.1 \cdot 10^{-5}$ and $7.4 \cdot 10^{-5}$ 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 α_1-AG.

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

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

α_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 α_1-AG was obtained. This α_1-AG had exactly the same electrophoretic properties as α_1-AG isolated directly from normal pooled human serum.

Desialyzation of α_1-AG

Treatment of α_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 α_1-AG (α_1-AG(-Sia)) was calculated to be 35,875 (based on the value of 41,000 for native α_1-AG), and the extinction coefficient was found to be $A_{278,1\%} = 10.05$. The preparation of α_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 α_1-AG or desialyzed α_1-AG (α_1-AG(-Sia)) resulted in a $\sim$ 20% decrease (22%) of the neutral sugar content of both α_1-AG forms. The original value of 21.1 $\pm$ 0.3%, $n = 5$ decreased to 16.5 $\pm$ 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 α_1-AG and α_1-AG(-Sia). Preparations of α_1-AG and α_1-AG(-Sia) with one saccharide component missing (α_1-AG(-1 comp.) and α_1-AG(-Sia,-1 comp.)) were homogeneous on electrophoresis in polyacrylamide gel in the presence of SDS (Laemmli 1970). The relative molecular weight of α_1-AG(-1 comp.) and α_1-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.

Desialyzation and deglycosylation with a mixture of exoglycosidases

The combined treatment of α_1-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 α_1-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 $\pm$ 0.3%, $n = 5$ to 15.5 $\pm$ 0.3%, $n = 5$. This decrease ($\sim$ 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 α_1-AG(-Sia,-Gal). Polyacrylamide gel electrophoresis in the presence of SDS (Laemmli 1970) revealed small admixtures of proteins with relative molecular weights differring significantly from that of α_1-AG. The corresponding zones apparently belong to exoglycosidases used for deglycosylation. The calculated relative molecular weight of α_1-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: α_1-AG, α_1-AG(-Sia), α_1-AG(-1 comp.), α_1-AG(-Sia,-1 comp.) and α_1-AG(-Sia,-Gal). A linear dependence of ΔA 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 ΔA in

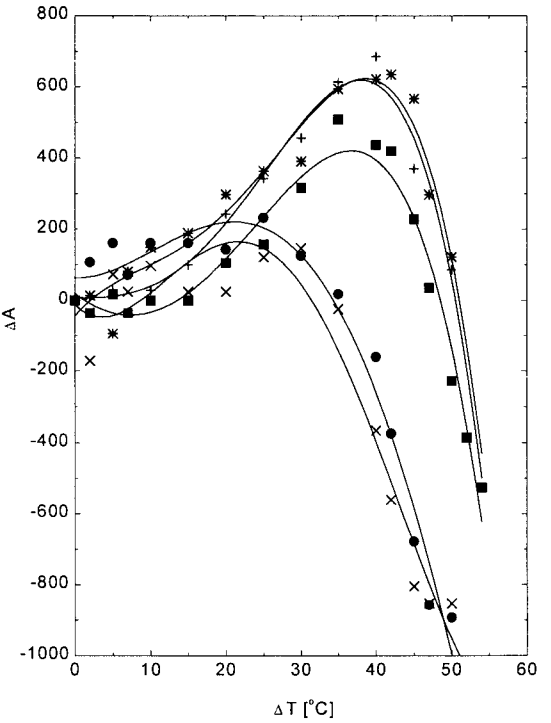

Figure 1. Temperature perturbation difference spectra (TPDS) Dependence of ΔA on ΔT at 294 nm (+), α_1-AG, (×), α_1-AG(-1 comp), (■), α_1-AG(-Sia), (●), α_1-AG(-Sia,-1 comp), (*) α_1-AG(-Sia,-Gal)

the 250–264 nm region is always higher for partially deglycosylated preparations of α_1-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 α_1-AG(-1 comp.) and α_1-AG(-Sia,-1 comp.) on one hand and α_1-AG, α_1-AG(-Sia) and α_1-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 α_1-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 α_1-AG(-Sia) the numbers of exposed tryptophyl residues decreased from 1.5–2.5 (20–40 °C) for native α_1-AG to 0.5–1.5 (20–40 °C).

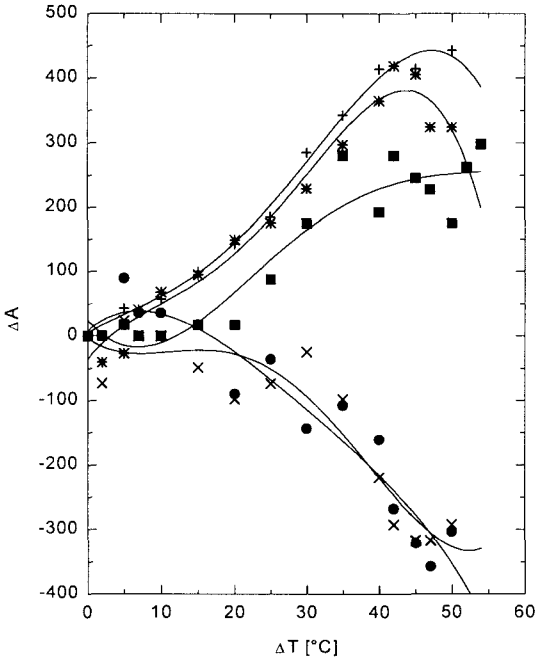

Figure 2. Temperature perturbation difference spectra (TPDS) Dependence of ΔA on ΔT at 300 nm (+), α_1-AG, (×), α_1-AG(-1 comp), (■), α_1-AG(-Sia), (●), α_1-AG(-Sia,-1 comp), (∗), α_1-AG(-Sia,-Gal)

Fluorescence spectroscopy

Fluorescence spectra were recorded for samples of α_1-AG, α_1-AG(-1 comp.), α_1-AG(-Sia,-1 comp.) and α_1-AG(-Sia,-Gal). The fluorescence intensity at any temperature was found to be highest for α_1-AG and decreased in the order: α_1-AG(-1 comp.), α_1-AG(-Sia,-1 comp.) and α_1-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 α_1-AG(-1 comp.) and α_1-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 α_1-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,

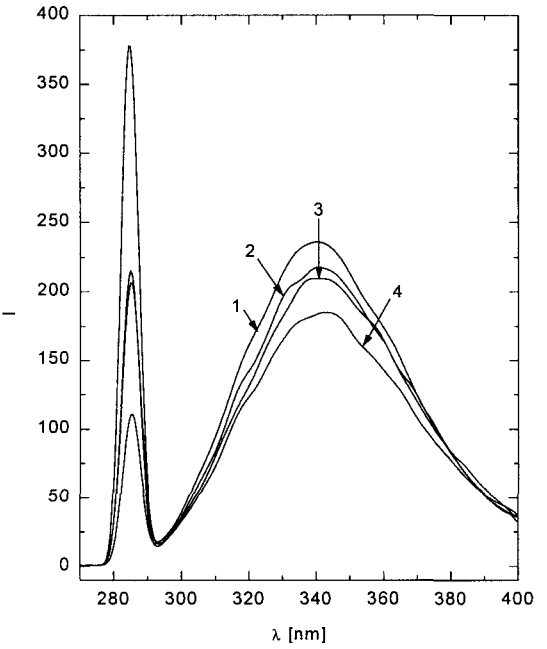

Figure 3. Fluorescence spectra at 50 °C (1), α_1-AG, (2), α_1-AG(-1 comp), (3), α_1-AG(-Sia,-1 comp), (4), α_1-AG(-Sia,-Gal)

Table 1. Parameter R of FD-spectra calculated according to Padros et al (1984)

Sample	l_1 [nm]	l_2 [nm]	h_1 [nm]	h_2 [nm]	R
α_1-AG	282	284	29	42	1 45
α_1-AG(-Sia)	281	283	57	34	0 60
α_1-AG(-Sia,-1 comp)	280	284	29	14	0 48
α_1-AG(-1 comp)	283	285	13	23	1 80
α_1-AG(-Sia,-Gal)	282	284	117	88	0 75

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 α_1-AG are given in Table 1. The values for all desialyzed prepa-

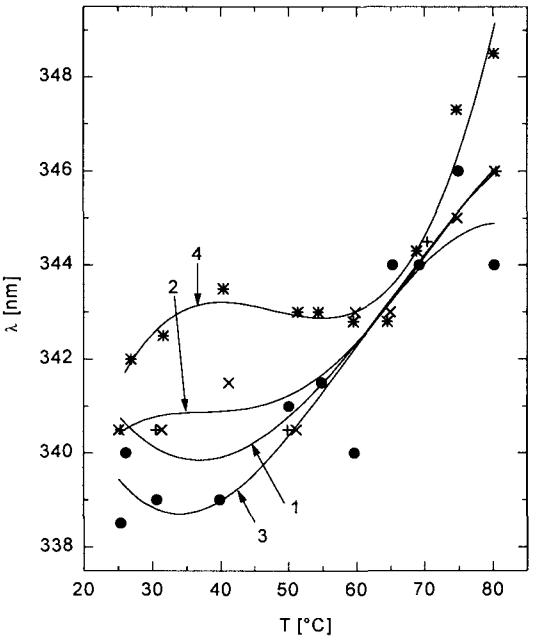

Figure 4. Fluorescence spectroscopy Temperature dependence of the emission maximum wavelenght 1 (+), α_1-AG, 2 (×), α_1-AG(-1 comp), 3 (•), α_1-AG(-Sia,-1 comp), 4 (*), α_1-AG(-Sia,-Gal)

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 Dunach 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 α_1-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 α_1-AG changed only slightly, and after cooling

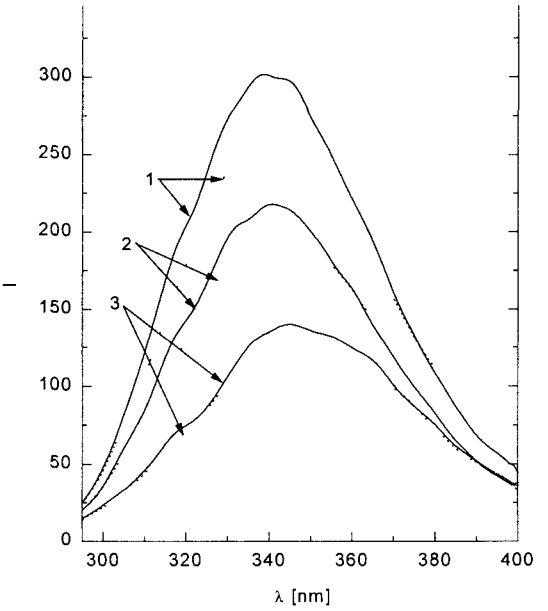

Figure 5. Fluorescence emission spectra of α_1-AG(-Sia,-1 comp) at different temperatures Increasing temperature (1) —— 25 °C, (2) —— 50 °C, (3) —— 75 °C Decreasing temperature (1) 25 °C, (2) 50 °C, (3) 75 °C

Table 2. The content of ordered secondary structures in native and partially deglycosylated α_1-AG preparations

Sample	α-helix	β-structure	β-turn
α_1-AG	7%	58%	12%
α_1-AG(-1 comp)	5%	23%	22%
α_1-AG(-Sia)	4%	20%	21%
α_1-AG(-Sia,-1 comp)	4%	4%	27%
α_1-AG(-Sia,-Gal)	5%	12%	23%

down the CD-spectra became indistinquishable 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, α_1-AG is a typical serum glycoprotein with a relatively very high content of these components. Therefore

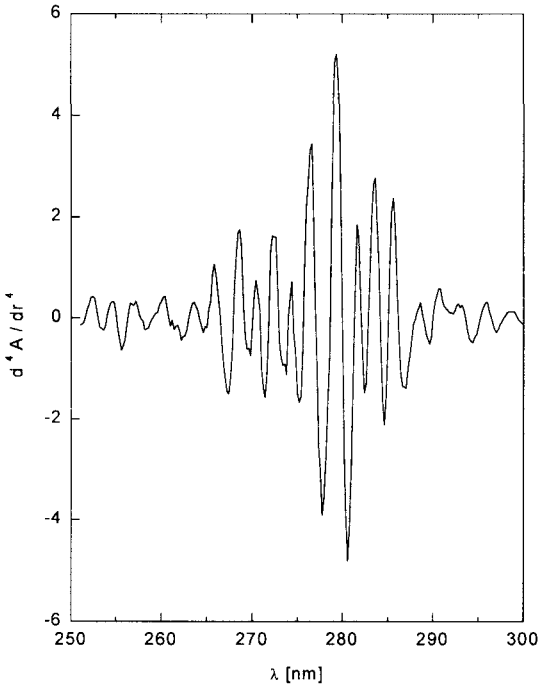

Figure 6. The fourth-derivative of α_1-AG absorption spectrum (FD-spectrum).

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 α_1-AG was not checked for sialic acid content(Kawahara et al. 1973). Partially deglycosylated α_1-AG was also studied with respect to its optical rotation. However, no significant influence of saccharide components on this property of α_1-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 α_1-AG. Since that time it has been recommended to denature α_1-AG before PNGase F treatment to achieve optimum deglycosylation (Tarentino et al. 1985).

In our experiments we easily prepared completely desialyzed α_1-AG. However, even prolonged treatment of native or desialyzed α_1-AG with PNGase F resulted in the removal of only one of the five saccharide components of α_1-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,

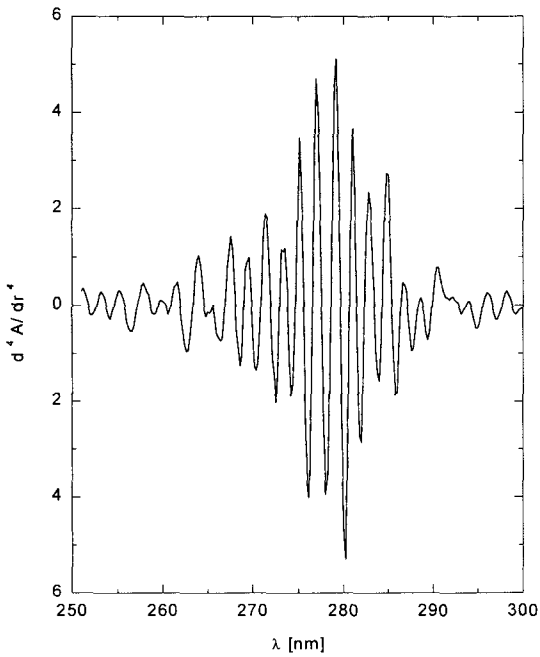

Figure 7. The fourth-derivative of α_1-AG(Sia) absorption spectrum (FD-spectrum)

deglycosylation of desialyzed α_1-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 α_1-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 α_1-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

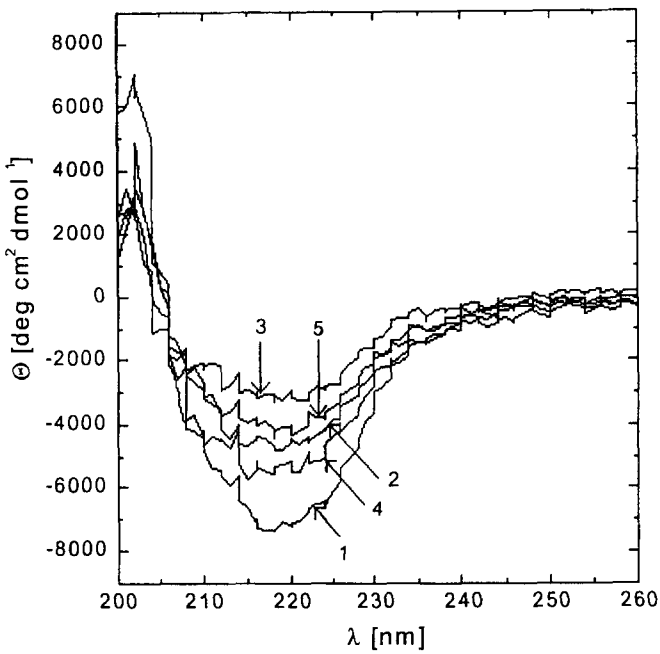

Figure 8. Circular dichroic (CD) spectra at 25 °C (1), α_1-AG, (2), α_1-AG(-1 comp), (3), α_1 AG(-Sia,-1 comp), (4), α_1-AG(-Sia,-Gal), (5), α_1-AG(-Sia)

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 α_1 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 α_1-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 α_1-AG structure than total desialyzation of all saccharide components

The effect of partial deglycosylation of α_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 α_1-AG has not yet been established. However, it is a well-known fact that desialyzation of this glycoprotein results in the deblocking of antigenic determinants (Schwick et al. 1977). Sialic acid-deficient α_1-AG has been found in the sera or urine of patients with Hodgkin's disease, psoriatic arthritis, diabetes, neoplastic disease, and chronic myeloic leukemia (Schmid et al. 1964). α_1-AG with altered saccharide components was also found in human bile (Abei et al. 1994).

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References

Abei M , Nuutinen H , Kawczak P , Schwarzendrube J , Pillay S P , Holzbach R T (1994) Identification of human biliary α_1-acid glycoprotein as a cholesterol crystallization promoter Gastroenterology **106**, 231—238

Ashwell G , Harford J (1982) Carbohydrate-specific receptors of the liver Annu Rev Biochem **51**, 531—534

Davis B J (1964) Disc electrophoresis II Method and application to human serum proteins Ann NY Acad Sci **121**, 404—427

Dubois J , Gilles K A , Hamilton J K , Rebers P A , Smith F (1956) Colorimetric method for determination of sugars and related substances Anal Chem **28**, 350—356

Dunach M , Sabes M , Padros E (1983) Fourth-derivate spectrophotometry analysis of tryptophan environment in proteins Eur J Biochem **134**, 123—128

Fournet B , Montreuil J , Strecker G , Dorland L , Haverkamp J , Vliegenthart J F G , Binette J P , Schmid K (1978) Determination of the primary structures of 16 asialo-carbohydrate units derived from human plasma α_1-acid glycoprotein by 360-MHz ^{1}H NMR spectroscopy and permethylation analysis Biochemistry USA **17**, 5206—5214

Hu A S L , Wolfe R G , Reithel F J (1959) The preparation and purification of β-galactosidase from *Escherichia coli*, ML 308 Arch Biochem Biophys **81**, 500—507

Karpenko V , Kalous V (1977) The behaviour of native orosomucoid in acid and alkaline medium Collect Czech Chem Commun **42**, 45—55

Karpenko V , Pavlíček Z , Kalous V (1968) Behaviour of human orosomucoid in acid media Biochim Biophys Acta **154**, 245—247

Karpenko V , Šinkorová L , Janáčková L (1993) Behaviour of human blood serum orosomucoid (α_1-acid glycoprotein) in the presence of small aliphatic alcohols Collect Czech Chem Commun **58**, 2701—2714

Kawahara K , Ikenaka T , Nimberg R B , Schmid K (1973) The effect of the sialyl residues on the thermodynamic properties of α_1-acid glycoprotein Biochim Biophys Acta **295**, 505—513

Kodíček M , Infazón A , Karpenko V (1995) Heat denaturation of human orosomucoid in water / methanol mixtures Biochim Biophys Acta **1246**, 10—16

Laemmli U K (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4 Nature (London) **227**, 680—685

Levitt M , Chothia C (1976) Structural patterns in globular proteins Nature (London) **261**, 552—558

Nicola N A , Leach S J (1976) Interpretation and application of thermal difference spectra of proteins Int J Peptide Prot Res **8**, 393—415

N-Linked Oligosaccharide Profiling Kit, Instruction Manual, Catalog No 170—6501, Bio-Rad, U S A

Ornstein L (1964) Disc electrophoresis I Background and theory Ann NY Acad Sci **121**, 321—349

Padros E , Dunach M , Morros A , Sabes M Manosa J (1984) Fourth derivate spectrophotometry of protein Trends Biochem Sci **9**, 508—510

Schmid K , Kamiyama S (1963) Studies on the structure of α_1-acid glycoprotein Biochemistry USA **2**, 271—275

Schmid K , Burke J F , Debray-Sachs M , Tokita K (1964) Sialic acid – deficient α_1-acid glycoprotein produced in certain pathological states Nature (London) **204**, 75—76

Schmid K , Chen L H , Occhino J L , Foster J A , Sperandio K (1976) Topography of human plasma α_1-acid glycoprotein Biochemistry USA **15**, 2245—2254

Schulze H E , Heremans J F (1966) Molecular Biology of Human Plasma Proteins, p 203, Elsevier, Amsterdam

Schwick H G , Heide K , Haupt H (1977) α_1-Acid glycoprotein In The Glycoconjugates (Eds M I Horowitz and W Pigmann) **1**, pp 268—269, Academic Press, New York, San Francisco, London

Spiro R G (1962) Studies on the monosaccharide sequence of the serum glycoprotein fetuin J Biol Chem **237**, 646—652

Spiro R G (1966 a) Analysis of sugars found in glycoproteins Methods Enzymol **8**, 3—26

Spiro R G (1966 b) Characterization of carbohydrate units of glycoproteins Methods Enzymol **8**, 26—52

Svobodová X , Karpenko V , Kalous V (1977) On the kinetics of the alkaline denaturation of orosomucoid Collect Czech Chem Commun **42**, 1742—1753

Tarentino A L , Góme C M , Plummer, Jr T H (1985) Deglycosylation of asparagine-linked glycans by peptide N-Glycosidase F Biochemistry USA **24**, 4665—4671

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