Eur. J. Biochem. 197, 209–215 (1991)

001429569100228C

Structure determination of the glycans of human-serum α_1-antichymotrypsin using ^{1}H-NMR spectroscopy and deglycosylation by *N*-glycanase

Anne LAINE[1], Eric HACHULLA[1], Gérard STRECKER[2], Jean-Claude MICHALSKI[2] and Jean-Michel WIERUSZESKI[2]

[1] Unité 16 INSERM, 2 Place de Verdun Lille Cedex, France
[2] Laboratoire de Chimie Biologique et Unité Associée au Centre National de la Recherche Scientifique no. 217, Université des Sciences et Techniques de Lille Flandres-Artois, Villeneuve d'Ascq Cedex, France

(Received July 3/December 11, 1990) – EJB 90 0774

α_1-Antichymotrypsin purified from normal human serum was separated by affinity chromatography into three microheterogeneous forms on a concanavalin-A – Sepharose column: a pass-through (peak 1), a retarded (peak 2) and a bound form (peaks 3 + 4). For each form the asparagine-linked carbohydrate chains were liberated as oligosaccharides by hydrazinolysis, submitted to reduction with $NaBH_4$ after re-*N*-acetylation and further separated by affinity chromatography on a concanavalin-A – Sepharose column. The complete primary structure of the glycans was determined by high-resolution ^{1}H-NMR spectroscopy. The results indicated the presence of disialyl diantennary and of trisialyl triantennary type glycanic structures, the latter being accompanied by traces of disialylated triantennary oligosaccharide. The *N*-glycanase was used for the deglycosylation of the unfractionated α_1-antichymotrypsin; the successive removal of the N-linked complex-type oligosaccharide side chains of α_1-antichymotrypsin was studied in the presence of detergents. From these experiments it is concluded that α_1-antichymotrypsin carries four oligosaccharide side chains. Moreover our results show that the peak 1 contains four triantennary glycans, the peak 2 three triantennary and one diantennary glycans while the bound peaks 3 + 4 possess, on average, about one triantennary and three diantennary glycans per molecule. Since we showed that the peak 4 contains mostly diantennary glycans, it can be deduced that in peak 3 there are molecules carrying two triantennary and two diantennary glycans and others carrying one triantennary and three diantennary glycans.

Human α_1-antichymotrypsin is a plasma glycoprotein with a relative molecular mass (M_r) of approximately 58000 and a carbohydrate content of 24% [1]. It belongs to the superfamily of serpins (serine proteinase inhibitors) [2]. In addition to C-reactive protein and serum amyloid A protein, α_1-antichymotrypsin is one of the major acute-phase proteins [3] since its serum concentration increases more than fourfold within a few hours in response to an inflammatory stimulus [4].

The precise biological function of α_1-antichymotrypsin has not been yet determined. It is known to inhibit chymotrypsin-like proteases [5–7] by formation of equimolar complexes which, however, dissociate with time. Eriksson et al. [8] recently described four families with inherited partial α_1-antichymotrypsin deficiency in which heterozygotes have 50% of normal plasma levels. No homozygotes have yet been identified.

α_1-Antichymotrypsin has been shown to be identical to the protein termed 64DP [9] and has a high affinity for DNA-cellulose.

It has been claimed that α_1-antichymotrypsin is a component of the brain amyloid deposits in Alzheimer's disease [10] but we recently showed [11] that no distinction can be made between extracts of 14 control brains and extracts of 12 Alzheimer brains with regard to the amount of α_1-antichymotrypsin.

The complete amino acid sequence of α_1-antichymotrypsin deduced from the nucleotide sequence [12] reveals four potential glycosylation sites (in positions 8, 68, 161, 246). In a recent work Rubin et al. [13] found two other potential glycosylation sites (in positions 81 and 102) in the gene-deduced α_1-antichymotrypsin sequence they determined. But no information on the structural features of the glycans has been published so far.

α_1-Antichymotrypsin purified from the serum of a single healthy donor, in order to avoid possible genetic heterogeneity, was separated by affinity chromatography into three fractions on a column of concanavalin A (ConA)-linked to Sepharose [14]: a pass-through fraction, a retarded fraction and a bound fraction which was eluted from the column by the addition of methyl α-D-glucoside to the buffer. Our preliminary results [14] suggest that these three fractions carry glycans with decreasing degrees of branching from the ConA pass-through form to the ConA-bound form. In serum, marked variations in both the levels and the relative proportions of the three microheterogeneous forms of α_1-antichymotrypsin have been reported to occur in physiological and pathological states [15–19].

In the present paper we describe the complete primary structure of the glycans of serum α_1-antichymotrypsin as determined by high-resolution ^{1}H-NMR spectroscopy. We started from the three separated microheterogeneous forms. We also determined the proportion of di- and triantennary glycans per microheterogeneous form of α_1-antichymotrypsin. This is essential for a better understanding of the variations observed in the relative proportions of the three microheterogeneous forms [15–19].

One aim of our work was to determine the number of glycans per molecule of α_1-antichymotrypsin. We used the *N*-

Correspondence to A. Laine, Unité 16 INSERM, 2 Place de Verdun 59045 Lille Cedex, France

Abbreviation. ConA, concanavalin A.

Enzyme. *N*-Glycanase, peptide-N^4-(*N*-acetyl-β-D-glucosaminyl) asparagine amidase (EC 3.5.1.52).

glycanase [peptide N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase] from *Flavobacterium meningosepticum* for the deglycosylation of unfractionated α_1-antichymotrypsin; the successive removal of the N-linked complex-type oligosaccharide side chains was studied in the presence of detergents. This experiment was also performed with purified α_1-proteinase inhibitor used as control; this is known to possess three glycan groups/molecule [20].

MATERIALS AND METHODS

Purification of α_1-antichymotrypsin

α_1-Antichymotrypsin was purified from the serum of a healthy donor by immunoaffinity chromatography using the procedure previously described [6]. The homogeneity of the purified α_1-antichymotrypsin was assessed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) as previously described [6]: a single, rather broad, band of M_r near 58000 (59000 – 57000) was observed (see Fig. 4).

Purification of human α_1-proteinase inhibitor

Human α_1-proteinase inhibitor was also purified using immunoaffinity chromatography as in [21].

Fractionation of native α_1-antichymotrypsin by affinity chromatography on ConA – Sepharose 4B

The preparation of the three microheterogeneous forms was performed as previously described [14]. A ConA – Sepharose 4B column (25 ml gel) prepared in the laboratory was used and affinity chromatography experiments were performed according to [22]: purified α_1-antichymotrypsin (5 mg) was applied onto the column equilibrated with 0.05 M Tris/HCl pH 7.6 containing 0.15 M NaCl, 1 mM each of $MnCl_2$, $MgCl_2$, $CaCl_2$ and 0.02% NaN_3. The pass-through, or unbound, and the retarded fractions were collected when washing the column with the equilibration buffer. The ConA-bound material was desorbed by 0.02 M methyl α-D-glucoside in equilibration buffer. No additional protein was eluted with 0.2 M methyl α-D-glucoside. The fractions corresponding to each form were collected and desalted on a Bio-Gel P4 column eluted with demineralised water.

Crossed immunoaffinity electrophoresis

Crossed immunoaffinity electrophoresis was carried out as previously described [14] using free ConA placed in the first-dimension gel.

Preparation of the asparagine-linked oligosaccharides from the three microheterogeneous forms of α_1-antichymotrypsin

Oligosaccharides from the three microheterogeneous forms were released by hydrazinolysis [23], then re-*N*-acetylated [24] and reduced with $NaBH_4$. The resulting mixtures of oligosaccharides were desalted on a Bio-Gel P2 column eluted with demineralised water.

Further resolution of the oligosaccharides mixtures

For each microheterogeneous form, the separation between the diantennary and the multiantennary glycans was achieved by affinity chromatography on a small (5-ml) ConA – Sepharose (Pharmacia) column equilibrated with 5 mM sodium acetate pH 6.0 containing 0.1 M NaCl, 1 mM each of $MnCl_2$, $MgCl_2$ and $CaCl_2$. The unbound material was eluted with the equilibration buffer (5 column volumes were used). Elution of the bound material was performed by adding 0.02 M methyl α-D-glucoside to the equilibration buffer. A further elution with 0.2 M methyl α-D-glucoside in the equilibration buffer was performed to ascertain if some additional bound material could be eluted (high-mannose-type glycan, for example). Each oligosaccharide fraction was desalted on a Bio-Gel P2 column eluted with demineralised water. The only step which could be monitored by recording the absorbance at 206 or 226 nm was the desalting step.

Primary structure analysis

For ^{1}H-NMR spectroscopic analysis, the oligosaccharide fractions were repeatedly exchanged in D_20 at room temperature and at pD 7 with intermediate lyophilisation. The 400-MHz ^{1}H-NMR spectra were recorded on a Bruker AM 400-WB spectrometer operating in the pulsed-Fourier transform mode and equipped with a Bruker Aspect 3000 computer at a probe temperature of 300 K. Chemical shifts (δ) are given relative to sodium 4,4-dimethyl-4-silapentane-sulphonate, but were actually measured by reference to internal acetone in D_20: $\delta = 2.225$ ppm with an accuracy of ± 0.002 ppm.

Conditions for deglycosylation of α_1-antichymotrypsin and of α_1-proteinase inhibitor by N-glycanase

N-Glycanase [peptide N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase] from *Flavobacterium meningosepticum* was purchased from Genzyme. Deglycosylation was performed on denatured and native α_1-antichymotrypsin as well as on denatured and native α_1-proteinase inhibitor which is here used as a control. The enzyme reactions were carried out in 0.2 M sodium phosphate pH 8.6 at 37°C. Incubation times were varied. The reaction was stopped by the addition to an aliquot of the reaction mixture of an equal volume of sample buffer used for SDS/PAGE and heat treatment at 100°C for 3 min and the products were analysed by SDS/PAGE as previously described [6].

For denatured samples, the glycoprotein samples were boiled for 3 min in the presence of 0.8% SDS, 0.8% 2-mercaptoethanol and 8 mM *o*-phenanthroline. After cooling, 1 vol. of a solution containing 4% 2-mercaptoethanol, 5% NP 40 and 40 mM *o*-phenanthroline was added to 5 vol. of the previous reaction mixture. The final glycoprotein concentration was 0.3 mg/ml. Then 1.3 unit *N*-glycanase/60 μg glycoprotein was added and the mixtures were incubated at 37°C.

For native samples, in one set of experiments the glycoprotein samples were incubated with *N*-glycanase in the presence of 0.7% 2-mercaptoethanol, 0.8% NP 40 and 7 mM *o*-phenanthroline. Triton X-100 (0.5% final concentration) was used instead of NP 40 in another experiment; 1.3 units *N*-glycanase/60 μg glycoprotein were used and the mixtures were incubated at 37°C.

Separation of oligosaccharides was performed according to [25] by thin-layer chromatography using the solvent butanol/ethanol/acetic acid/pyridine/water (10:100:3:10:30).

Desialylation of the glycans was performed using 0.05 M CF_3COOH for 3 min at 80°C.

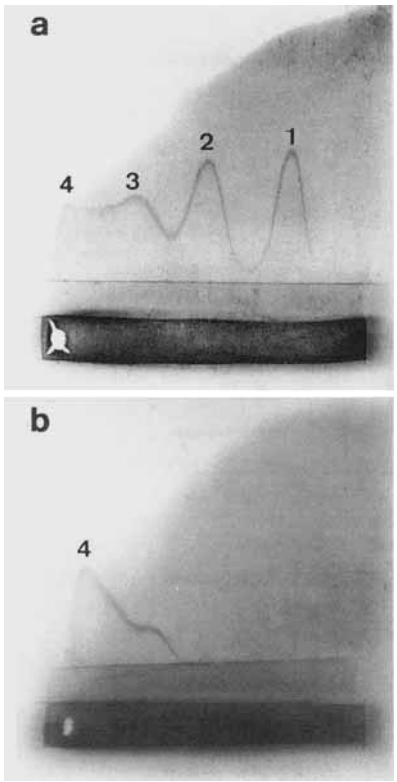


Fig 1. *Crossed immunoaffinity electrophoretic patterns of purified α_1-antichymotrypsin (a) and of the peak 4 (b) obtained by overloading the laboratory-prepared ConA – Sepharose 4B column.* Free ConA was used in the first dimension and methyl α-D-glucoside in the second dimension in addition to specific antiserum against α_1-antichymotrypsin

RESULTS AND DISCUSSION

Fractionation of native α_1-antichymotrypsin by affinity chromatography on ConA – Sepharose 4B

Purified α_1-antichymotrypsin is resolved into four peaks numbered 1 – 4 (Fig. 1a) in the presence of ConA in crossed immunoaffinity electrophoresis. As shown previously [14], three fractions of α_1-antichymotrypsin were separated upon chromatography on laboratory-made ConA – Sepharose 4B. Briefly, the pass-through fraction (fraction A) corresponds to peak 1 (about 21% of the total α_1-antichymotrypsin pattern in healthy individuals [18]) and exhibits the same migration as the peak obtained for unfractionated α_1-antichymotrypsin without ConA in the first dimension. The retarded material (fraction B) corresponds to peak 2 (about 27% of the total α_1-antichymotrypsin pattern) and the bound material (fraction C) corresponds to the peaks 3 and 4 (about 50% of the total α_1-antichymotrypsin pattern).

To separate peaks 3 and 4, the column was overloaded with 10 mg α_1-antichymotrypsin. In that case only the material exhibiting the highest affinity for the ConA – Sepharose 4B column (corresponding to peak 4) was retained and desorbed by addition of methyl α-D-glucoside (Fig. 1b). After hydrazinolysis, re-*N*-acetylation and desalting, we did not have enough material to study the carbohydrate structure using ^{1}H-NMR spectroscopic analysis; however, we consider it was not worthwhile preparing this material on a large scale since, when we overloaded the column, the separation between the fractions A and B was unsatisfactory.

By thin-layer chromatography we demonstrated that the oligosaccharide chains contained in peak 4 were mostly diantennary glycans.

Isolation of the oligosaccharides from the three microheterogeneous forms

Fraction A consists of a large amount of unretained material on the ConA – Sepharose column. Nevertheless to ensure that we did not neglect one glycanic structure, fractions eluted using 0.02 M and 0.2 M methyl α-D-glucoside in the buffer were analysed by ^{1}H-NMR spectroscopy.

Fractions B and C contained both unretained and retained materials. According to the absorbance, there was much more unretained than retained material in B and much more retained than unretained material in C.

400-MHz ^{1}H-NMR spectroscopy

The ^{1}H-NMR analyses (Fig. 2) of the different carbohydrate chains released by hydrazinolysis and fractionated on ConA – Sepharose indicated the presence in the retained material eluted using 0.02 M methyl α-D-glucoside of disialyl diantennary and, in the unretained material, of trisialyl triantennary type glycanic structures, the latter being accompanied by traces of disialylated triantennary oligosaccharide. By ^{1}H-NMR spectroscopy, we demonstrated that for each microheterogeneous form there was no glycan material in the fractions eluted from the ConA – Sepharose column (Pharmacia) using 0.2 M or 0.02 M methyl α-D-glucoside when fraction A was subjected to affinity chromatography on the ConA – Sepharose column.

The diantennary structure is α-2,6-sialylated, as shown by the characteristic resonances of the NeuAc H-3a and H-3e atoms (see Table 1). The resonance positions of the other structural reporter group signals are identical to those described for the reference compound [26].

The trisialylated glycan structures possess α-2,6 and α-2,3-linked sialic acid, in the ratio 2:0.8, as shown by the typical H-3a and H-3e resonances (Table 1). Gal-6 and Gal-6′ are both α-2,6-sialylated ($\delta_{H1} = 4.444$ ppm). This is also confirmed by the absence of NAc signals at 2.042 – 2.046 ppm, indicative of C-3 sialylated Gal-6 and Gal-6′, and the presence of NAc resonances at 2.069 and 2.064 ppm, indicative of C-6 sialylated Gal-6 and Gal-6′. The occurrence of the β-1,4-linked GlcNAc-7 was established on the basis of the chemical shift of the H-2 signal of Man-4, found at $\delta = 4.220$ ppm [26, 27]. By difference, the third NeuAc residue must be α-2,3-linked to Gal-8. This is confirmed by the set of the H-1 resonances of Gal-8 and GlcNAc-7, and by the NAc signal of GlcNAc-7 (Table 1). Traces of material with asialo Gal-8 residue are deduced from the Man H-1 signal at $\delta = 5.104$ ppm and from the Gal-8 H-1 signal at $\delta = 4.469$ ppm. The structure of the di- and triantennary oligosaccharide units are classical ones which occur frequently in glycoproteins [28].

Deglycosylation with N-*glycanase*

Deglycosylation of α_1-proteinase inhibitor. In our study the reaction of *N*-glycanase on purified α_1-proteinase inhibitor was used as a control. Working on rat α_1-proteinase inhibitor Steube et al. [29] showed that endoglucosaminidase F removed the carbohydrate side chains of the glycoprotein resulting in differently glycosylated forms which were separated electrophoretically. α_1-Proteinase inhibitor carrying three, two, one

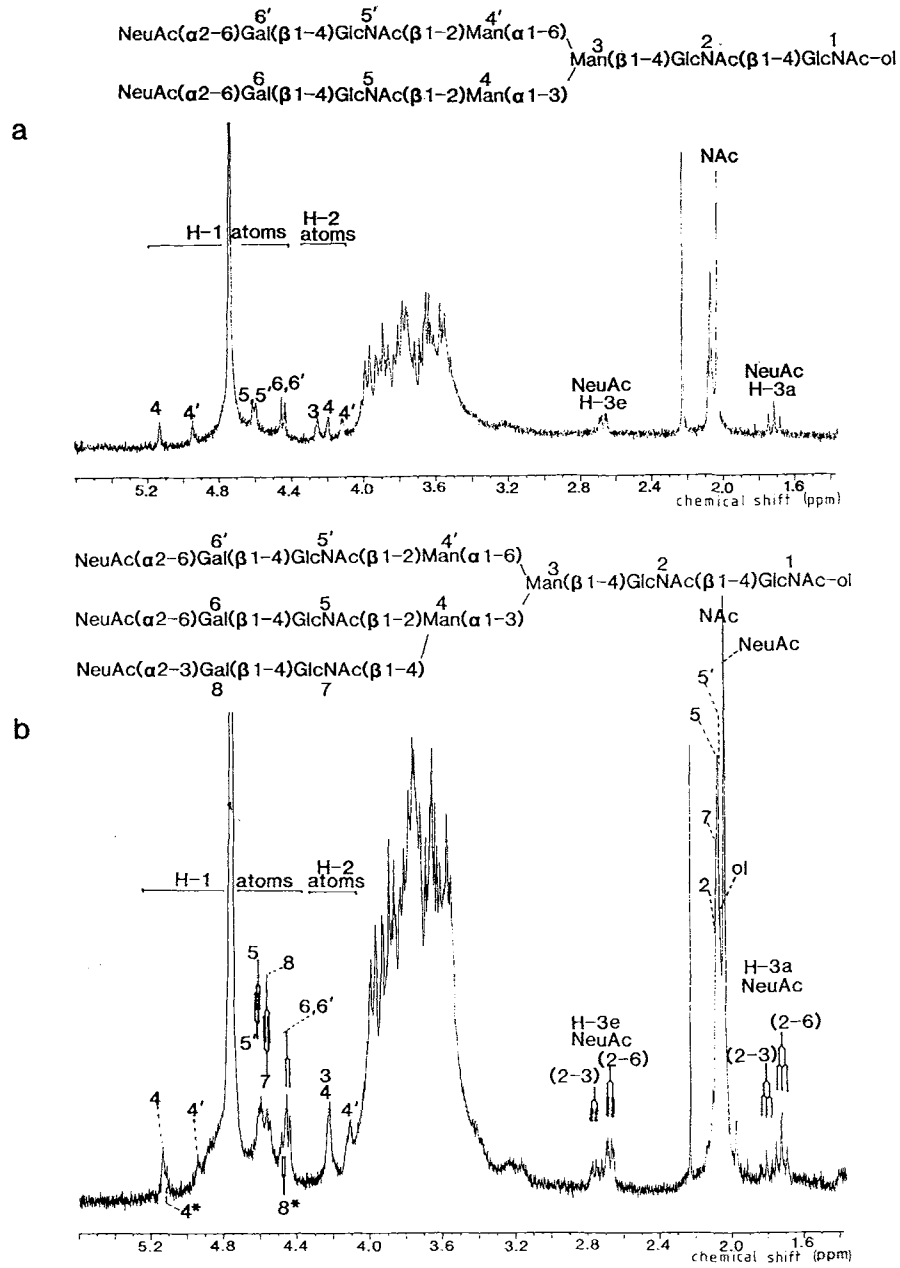
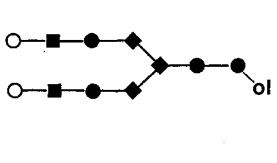
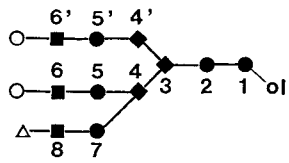
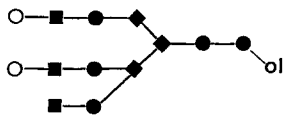


Fig. 2. *400-MHz ^{1}H-NMR spectra and primary structures of the diantennary (a) and of the triantennary (b) glycans from normal human serum α_1-antichymotrypsin.* In (b) asterisks are related to a triantennary glycan with a non-sialylated Gal-8 residue

and zero oligosaccharide side chain(s) were visualized by these authors. Fig. 3 demonstrates that denatured (Fig. 3a) and native (Fig. 3b) glycoprotein have different extents of deglycosylation. Three bands having an apparent M_r smaller than that of native α_1-proteinase inhibitor are observed in Fig. 3a, no change occurs when the incubation time is increased. An apparent M_r of about 44000 was estimated for the smallest band. Thus α_1-proteinase inhibitor carrying two, one and zero oligosaccharide chain(s) are visualized. Fig. 3a shows that α_1-proteinase inhibitor forms containing one and zero carbohydrate side chain represent the major deglycosylation products of denatured α_1-proteinase inhibitor. Fig. 3b shows that when deglycosylation has been performed on native α_1-proteinase inhibitor (with NP 40 as detergent) undeglycosylated glycoprotein remains even after a 24-h incubation and that an α_1-proteinase inhibitor form with one carbohydrate side chain represents the major deglycosylation product. Therefore denatured α_1-proteinase inhibitor is more easily deglycosylated than native. Moreover we demonstrated that the conditions of the deglycosylation were suitable to determine the number of glycan groups/molecule.

Deglycosylation of α_1-antichymotrypsin. Fig. 4a shows the results obtained when the deglycosylation was performed on denatured α_1-antichymotrypsin. Undeglycosylated glycoprotein is visualized even after a 48-h incubation. Three major bands are observed (M_r approximately 56000, 52500, 48500) together with a faint one of M_r approximately 44500. Fig. 4b shows that deglycosylation of native α_1-antichymotrypsin (with NP 40 as detergent) occurred more slowly but became more efficient when the incubation time was increased. Only a faint band of undeglycosylated α_1-antichymotrypsin remained after the 24-h incubation. The four bands which are visualized,

Table 1. *^1H chemical shifts of the structural-reporter group protons for the oligosaccharide-alditols isolated from serum α_1-antichymotrypsin* (●) GlcNAc; (◆) Man; (■) Gal; (○) NeuAc(α2−6); (△) NeuAc(α2−3). n.d. = not determined

Reporter group	Residue	Chemical shift in		
				
		ppm		
H-1	2	4.640	n.d.	n.d.
	3	4.781	n.d.	n.d.
	4	5.133	5.132	5.104
	4′	4.946	4.938	4.938
	5	4.604	4.595	4.595
	5′	4.604	4.604	4.604
	7	–	4.554	n.d.
	6	4.443	4.444	4.444
	6′	4.443	4.444	4.444
	8	–	4.548	4.469
H-2	3	4.255	4.220	4.220
	4	4.195	4.220	4.220
	4′	4.116	4.105	4.105
H-3a	NeuAc(α2−3)	–	1.803	–
	NeuAc(α2−6)	1.716	1.718	1.718
H-3e	NeuAc(α2−3)	–	2.758	–
	NeuAc(α2−6)	2.672	2.672	2.672
NAc	1-ol	2.057	2.056	2.056
	2	2.082	2.082	2.082
	5	2.070	2.069	2.069
	5′	2.064	2.064	2.064
	7	–	2.074	n.d.
	NeuAc	2.030	2.030	2.030

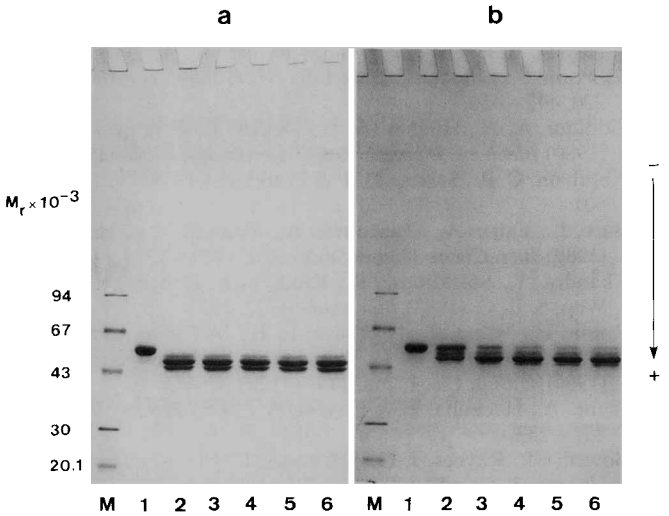


Fig. 3. *Time course of deglycosylation of α_1-proteinase inhibitor when denatured (a) or native (b) as indicated in Materials and Methods.* Lane M, M_r markers from Pharmacia treated with 2-mercaptoethanol (phosphorylase *b* 94000, albumin 67000, ovalbumin 43000, carbonic anhydrase 30000, trypsin inhibitor 20100 and α-lactalbumin 14400). Lane 1, α_1-proteinase inhibitor incubated at 37°C for 24 h without *N*-glycanase; lanes 2−6, α_1-proteinase inhibitor incubated at 37°C with *N*-glycanase for 1 h (2), 3 h (3), 6 h (4), 9 h (5), 24 h (6). After heat treatment at 100°C for 3 min, the samples were subjected to SDS/PAGE (5−25% gradient) and subsequently stained with Coomassie blue R 250

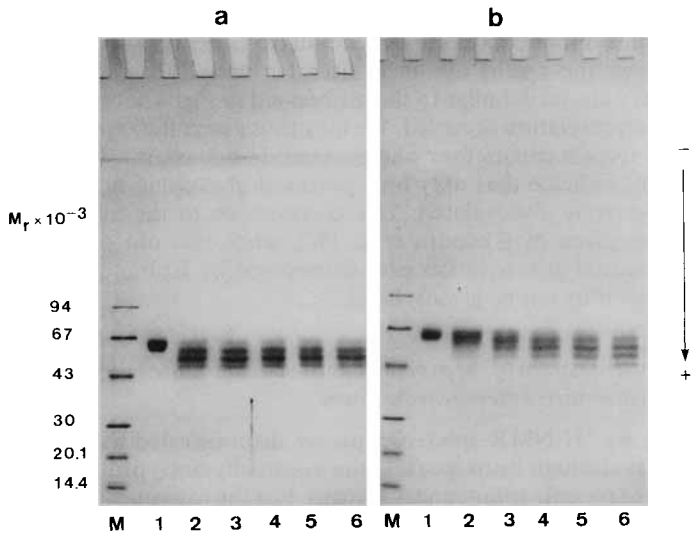


Fig. 4. *Time course of deglycosylation of α_1-antichymotrypsin when denatured (a) or native (b) as indicated in Materials and Methods.* Lane M, M_r markers as in Fig. 3. Lane 1, α_1-antichymotrypsin incubated at 37°C for 24 h without *N*-glycanase; lanes 2−6, α1-antichymotrypsin incubated at 37°C with *N*-glycanase for 1 h (2) 3 h (3), 6 h (4), 9 h (5), 24 h (6). The samples were treated, analysed and stained as in Fig. 3

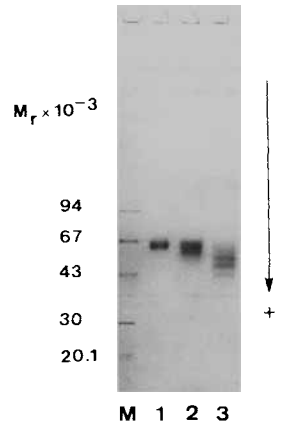


Fig. 5. *Study of the deglycosylation of* α_1*-antichymotrypsin by* N*-glycanase in presence of Triton X-100 as detergent as indicated in Materials and Methods.* Lane M, M_r markers as in Fig. 3. Lanes 1–3, α_1-antichymotrypsin incubated at 37°C (1) for 24 h without *N*-glycanase, (2) for 1 h with *N*-glycanase, (3) for 24 h with *N*-glycanase. The samples were treated, analysed and stained as in Fig. 3

of M_r approximately equal to those estimated in Fig. 4a, can be assigned to differently glycosylated forms of α_1-antichymotrypsin. By analogy with the results obtained with α_1-proteinase inhibitor, we assume that the smallest band obtained (M_r near 44500) may be assigned to the deglycosylated α_1-antichymotrypsin since this M_r is in accordance with the apparent M_r determined by Rubin et al. [13] for the recombinant α_1-antichymotrypsin they purified from one *Escherichia coli* expression system. The three other bands of apparent M_r 56000, 52500 and 48500 may be assigned to α_1-antichymotrypsin carrying three, two and one oligosaccharide side chain(s) respectively. Five possible α_1-antichymotrypsin forms are detected, indicating that native α_1-antichymotrypsin must in fact carry four oligosaccharide side chains. Steube et al. [29] demonstrated that deglycosylation of rat α_1-proteinase inhibitor was increased in the presence of 0.5% Triton X-100 instead of NP 40. We performed the deglycosylation of native α_1-antichymotrypsin in the presence of 0.5% Triton X-100. Fig. 5 shows the results obtained after 1-h and 24-h incubations. They are very similar to those observed in Fig. 4b. No further deglycosylation occurred. We may thus assert that α_1-antichymotrypsin carries four oligosaccharide side chains. These results indicate that only four potential glycosylation sites are effectively glycosylated. This corresponds to the number of sites given by Chandra et al. [12], while two out of the six potential glycosylation sites determined by Rubin et al. [13] must thus not be glycosylated.

Determination of the proportions of di- and triantennary glycans in each microheterogeneous form

By ^{1}H-NMR spectroscopy, we demonstrated that in the pass-through form (peak 1) the α_1-antichymotrypsin molecules carry only triantennary glycans. For the two other microheterogeneous forms, the glycans separated for the study by ^{1}H-NMR spectroscopy were pooled, desialylated and analysed by thin-layer chromatography. By densitometry it was possible to determine that the retarded fraction (peak 2) consists of molecules carrying three triantennary and one diantennary glycans and that the retained fraction (peaks 3 + 4) consists of molecules carrying, on average, one triantennary and three diantennary glycans. Since we showed that peak 4 consists mostly of diantennary glycans, we can conclude that peak 3 (which is almost half of the whole area of the peaks 3 + 4) is made up of a mixture of α_1-antichymotrypsin molecules carrying two triantennary and two diantennary glycans for some of them and one triantennary and three diantennary glycans for the others.

Each potential glycosylation site is able to carry one diantennary or one triantennary glycan since we can find molecules with four diantennary glycans and others with four triantennary glycans. We have no information to forecast the situation in other physiological or in pathological states such as those previously studied [16–19]. The number of α_1-antichymotrypsin molecules with a different arrangement of the glycans is multiple. Although we have not precisely determined the type of glycosylation for each potential glycosylation site in the microheterogeneous forms which contain both di- and triantennary glycans, our results favour random glycosylation rather than the existence of glycan uniformity on a single peculiar peptide chain as previously suggested by Bayard and Kerckaert [30].

This work was supported by *la Fondation pour la Recherche Médicale.*

REFERENCES

1. Laine, A. & Hayem, A. (1981) *Biochim. Biophys. Acta 668*, 429–438.
2. Travis, J. & Salvesen, G. S. (1983) *Annu. Rev. Biochem. 52*, 655–709.
3. Kushner, I. (1982) *Ann. N.Y. Acad. Sci. 389*, 39–48.
4. Aronsen, K. F., Ekelund, G., Kindmark, C. O. & Laurell, C. B. (1972) *Scand. J. Clin. Lab. Invest. 29 (Suppl. 124)*, 127–136.
5. Travis, J., Bowen, J. & Baugh, R. (1978) *Biochemistry 17*, 5651–5656.
6. Laine, A., Hayem, A. & Davril, M. (1984) in *Marker proteins in inflammation* (Arnaud, P., Bienvenu, J. & Laurent, P., eds) vol. 2, pp. 171–179, W. de Gruyter, Berlin.
7. Laine, A., Davril, M., Rabaud, M., Vercaigne-Marko, D. & Hayem, A. (1985) *Eur. J. Biochem. 151*, 327–331.
8. Eriksson, S., Lindmark, B. & Lilja, H. (1986) *Acta Med. Scand. 220*, 447–453.
9. Siddiqui, A. A., Hughes, A. E., Davies, R. J. H. & Hill, J. A. (1980) *Biochem. Biophys. Res. Commun. 95*, 1737–1742.
10. Abraham, C. R., Selkoe, D. J. & Potter, H. (1988) *Cell 52*, 487–501.
11. Buée, L., Laine, A., Delacourte, A., Flament, S. & Han, K. K. (1989) *Biol. Chem. Hoppe-Seyler 370*, 1229–1234.
12. Chandra, T., Stackhouse, R., Kidd, V. J., Robson, K. J. H. & Woo, S. L. C. (1983) *Biochemistry 22*, 5055–5061.
13. Rubin, H., Wang, Z., Nickbarg, E. B., McLarney, S., Naidoo, N., Schoenberger, O. L., Johnson, J. L. & Cooperman, B. S. (1990) *J. Biol. Chem. 265*, 1199–1207.
14. Laine, A., Hachulla, E. & Hayem, A. (1989) *Electrophoresis 10*, 227–233.
15. Bowen, M., Raynes, J. G. & Cooper, I. (1982) in *Lectins* (Bøg-Hansen, T. C., ed.) vol. 2, pp. 403–411, W. de Gruyter, Berlin.
16. Raynes, J. (1982) *Biomedicine 36*, 77–86.
17. Damgaard, A. M., Heegaard, P. M., Hansen, J. E. & Bøg-Hansen, T. C. (1986) *Protides Biol. Fluids 34*, 449–452.
18. Hachulla, E., Laine, A. & Hayem, A. (1988) *Clin. Chem. 34*, 911–915.
19. Hachulla, E., Laine, A. & Hayem, A. (1990) *Clin. Sci. 78*, 557–564.
20. Carrell, R. W., Jeppsson, J. O., Vaughan, L., Brennan, S. O., Owen, M. C. & Boswell, D. R. (1981) *FEBS Lett. 135*, 301–303.
21. Davril, M., Laine, A. & Hayem, A. (1987) *Biochem. J. 245*, 699–704.

22. Bayard, B. & Kerckaert, J. P. (1977) *Biochem. Biophys. Res. Commun. 77*, 489–495.
23. Bayard, B. & Fournet, B. (1975) *Carbohyd. Res. 46*, 75–86.
24. Reading, G. L., Penhoet, E. & Ballou, C. (1978) *J. Biol. Chem. 253*, 5600–5612.
25. Bayard, B., Kerckaert, J. P., Roux, D. & Strecker, G. (1979) *Protides Biol. Fluids 27*, 153–156.
26. Vliegenthart, J. F. G., Dorland, L. & Van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 209–374.
27. Spik, G., Debruyne, V., Montreuil, J., Van Halbeek, H. & Vliegenthart, J. F. G. (1985) *FEBS Lett. 183*, 65–69.
28. Montreuil, J. (1982) in *Comprehensive biochemistry*, vol. 19B/II, pp. 1–188, Elsevier, Amsterdam.
29. Steube, K., Gross, V. & Heinrich, P. C. (1985) *Biochemistry 24*, 5587–5592.
30. Bayard, B. & Kerckaert, J. P. (1980) *Biochem. Biophys. Res. Commun. 95*, 774–784.