Role of sialic acid residues in crossed immuno-affinoelectrophoresis of α_1-proteinase inhibitor

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We have investigated the importance of the degree of sialylation when an acute phase glycoprotein, α_1-proteinase inhibitor (α_1-Pi), was analysed both by affinity chromatography on concanavalin A (Con A)-Sepharose and by crossed immuno-affinoelectrophoresis (CIAE) using Con A in the first dimension. Human α_1-Pi was isolated by immunosorption chromatography and then more or less desialylated. On Con A-Sepharose chromatography no significant difference was observed in the percentage of the two fractions (retained or not retained) whatever the degree of desialylation. In contrast by CIAE this degree was largely involved in the separation of the different isoforms obtained in the first dimension.

Crossed immuno-affinoelectrophoresis; Sialic acid; α_1-Proteinase inhibitor; N-glycan; Concanavalin A

1. INTRODUCTION

In human serum several N-glycosylated proteins are acute phase reactants. In recent studies (reviewed in [1]), a number of these glycoproteins of known glycan composition, usually containing bi-, tri- and tetra-antennary complex type structures, have been investigated under various stimuli using concanavalin A (Con A) either immobilized on Sepharose 4B or present in the first dimension of a crossed immuno-affinoelectrophoresis (CIAE). This lectin recognizes high mannose type and biantennary complex type structures while tri- and tetra-antennary complex types are not recognized.

α_1-Proteinase inhibitor (α_1-Pi) contains three N-linked complex type oligosaccharide glycans [2] and belongs to the group of acute phase proteins. Tissue injury produces an increase in α_1-Pi serum level, which is often accompanied by a shift in the percentage of α_1-Pi isoforms: either toward Con A non-reactive isoforms or toward Con A reactive isoforms, depending on the method used to investigate this type of change, or the nature of the stimuli [3–7].

In this work, using α_1-Pi from the same healthy donor, we studied the modifications of the reactivity toward Con A before and after desialylation both by a chromatography process and by CIAE.

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2. MATERIALS AND METHODS

2.1. *Purification of α_1-Pi*

α_1-Pi was isolated from normal serum by immuno-purification as described earlier [7].

2.2. *Partial enzymatic desialylation*

α_1-Pi (15 mg) was incubated in a 50 mM sodium acetate buffer (pH 5.5) containing 5 mM $CaCl_2$ with 20 μU *Vibrio cholerae* neuraminidase (Calbiochem) in a volume of 6 ml at 37°C. No precipitation was observed during desialylation. At times 0, 1 h, 5 h, and 12 h, aliquots were withdrawn, then chromatographed on Con A-Sepharose or submitted to CIAE.

2.3. *Affinity chromatography on Con A-Sepharose 4B*

α_1-Pi obtained at different times of the desialylation was submitted (in triplicate) to affinity chromatography on Con A-Sepharose 4B (Pharmacia Fine Chemicals). The column (1.6 × 4 cm) was equilibrated with 50 mM Tris-HCl (pH 7.5), 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1 M NaCl buffer. Elution was carried out, first with equilibration buffer, then with 300 mM α-methylglucoside (Sigma) in the same buffer.

2.4. *Crossed immuno-affinoelectrophoresis*

Diluted aliquots of solution from native or partially desialylated α_1-Pi were analyzed by CIAE as described by Bøg-Hansen [8] with free Con A (Sigma, type IV) and antiserum anti human α_1-Pi (Hoechst-Behring).

2.5. *Analytical methods*

Quantification of α_1-Pi was performed by immunonephelometry using a Behring laser nephelometer (Hoechst-Behring) as described previously [3]. Sialic acid was analyzed by thiobarbituric acid assay [9].

3. RESULTS

Treatment of native α_1-Pi with neuraminidase for various periods of time resulted in a gradual removal of

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Table 1

Analysis of sialic acid removed during neuraminidase treatment of native α_1-Pi (time 0)

	Desialylation			
Time	0	1 h	5 h	12 h
NeuAc (μM)	–	1.7 ± 0.2	3.7 ± 0.2	5.8 ± 0.1
Percentage of desialylation	–	19.1 ± 2.8	43.8 ± 2.9	69.8 ± 1.4

Percentage of desialylation was calculated from total hydrolysis obtained by acid treatment (0.05 N H_2SO_4) at 80°C for 60 min. Results are means ± SD (n = 3)

Table 2

Percentage of non-retained α_1-Pi isoforms on Con A-Sepharose chromatography during progressive desialylation of α_1-Pi

	Desialylation			
Time	0	1 h	5 h	12 h
Non-retained α_1-Pi isoforms	12.8 ± 2.2	13.1 ± 1.9	13.0 ± 2.5	12.9 ± 2.3

Results are means ± SD (n = 3)

about 70% of the sialic acids present in native α_1-Pi (table 1). As desialylation progressed, samples were taken from the incubation mixture for chromatography on Con A-Sepharose and for CIAE.

When native and desialylated α_1-Pi were chromatographed on Con A-Sepharose, two fractions were obtained: the non-retained fraction containing α_1-Pi isoforms rich in triantennary complex type structures and the retained fraction containing α_1-Pi isoforms rich in biantennary complex type structures (table 2). The percentage of the non-retained isoforms remained stable whatever the degree of desialylation, and was similar to the values obtained in earlier reports [3,7].

In contrast, CIAE patterns (using free Con A in the first dimension) showed three peaks (fig.1). Before desialylation, peak 1 (component with a lower mobility) was always associated with a weakly retained component (peak 2). Its precipitation area was slightly greater than that of peak 1. The component non-retained by free Con A (peak 3) represented 15 ± 3% of the total precipitate area. During desialylation we noted a progressive decrease in the precipitation area of peak 3, from 10 ± 3% (time 1 h) to 3 ± 1% (time 12 h), while peaks 1 + 2 increased in the same proportion as that of peak 3 but with a higher quantity for peak 1 component. This modification of electrophoretic behaviour, during desialylation, was confirmed when both native and desialylated α_1-Pi were analysed by crossed immunoelectrophoresis without Con A in the first dimension (fig.1E). Desialylation of the isoforms, theoretically non-retained on Con A, produced a decrease in their mobility in the first dimension and the diffusion was also reduced in the second dimension. This explains that, during neuraminidase treatment, the precipitate area for both components (non-retained and weakly retained on Con A) decreased progressively, whereas peak 1 increased. The latter became a mixture of α_1-Pi isoforms with more or less desialylated bi- and triantennary complex type structures.

4. DISCUSSION

Acute phase glycoproteins bear bi-, tri- or tetra-antennary complex type structures. During inflammation, the concentration of several glycoproteins increases in serum in relation to both the number of antennae of the side chains [3–7] and the sialic acid content [10,11]. Over the last few years it has become feasible to determine the global change in the structure of N-glycans during different types of tissue injuries, using either chromatography on Con A-Sepharose or CIAE with free Con A in the first dimension. On this basis, for acute phase glycoproteins an increase of reactivity with Con A is generally explained as an increase in biantennary complex type structure content.

The aim of our study was to determine if a change in sialic acid content could affect the results obtained by these two methods. The data showed that the percentage of α_1-Pi isoforms non-retained on Con A-Sepharose (isoforms rich in triantennary complex type structures) was not influenced by the number of sialic acid residues. With the CIAE method the mobility of the non-retained or weakly retained isoforms is not so well established. It varied not only with the type of structures but also with the sialic acid moiety. Recently, Pos et al. [12] speculated that the difference in the degree of sialylation of α_1-acid glycoprotein could contribute to the shift in the electrophoretic mobility of different isoforms of this protein. Our results on α_1-Pi confirm this hypothesis, even if the degree of sialylation of this protein is less important than for α_1-acid glycoprotein.

Thus, on CIAE the electrophoretic behaviour of α_1-Pi is influenced in the first dimension by two parameters, the number of antennae and sialic acid residues. Moreover, the latter were also involved in the second dimension [13,14]. These two findings could explain the discrepancies observed in the same protein but for different diseases. It seems that desialylation of the protein or serum prior to electrophoresis allows more precise determination of the specific modifications of the degree of branching of the side chains. Hence results obtained on CIAE may be compared with those from chromatography, which are not influenced by the number of terminal sialic acid residues.

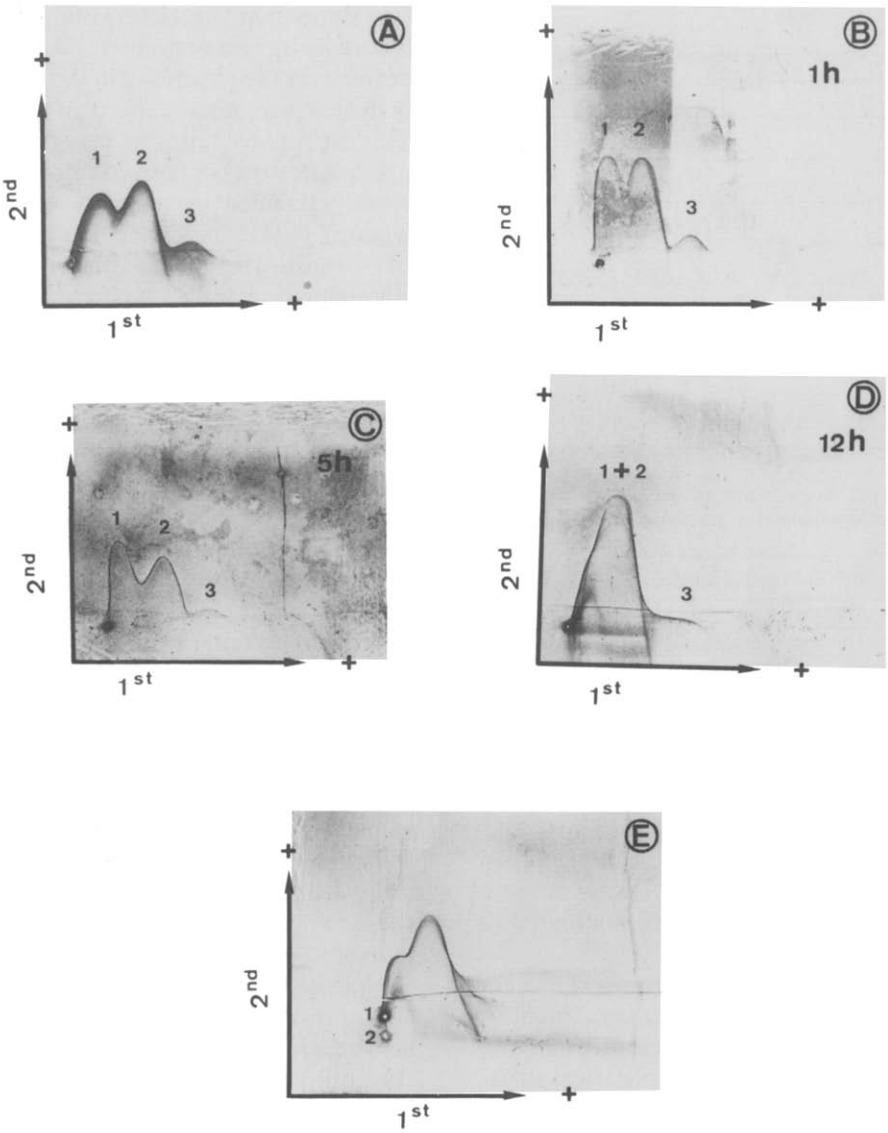

Fig.1. Crossed immuno-affinoelectrophoresis patterns with free Con A in the first dimension (2 mg/ml) then α-methylglucoside (58 mg/ml) and antiserum anti α_1-Pi (5 μl/ml) in the second dimension. Electrophoresis in the first dimension was carried out with a voltage of 10 V/cm for 2 h and in the second dimension with 2 V/cm for 18 h. α_1-Pi precipitate pattern before desialylation (A) and after 1 h (B), 5 h (C) and 12 h (D) in contact with 20 μU of neuraminidase. (E) Crossed immunoelectrophoresis without Con A in the first dimension. Lane 1: α_1-Pi (solution at 4 mg/ml) incubated with neuraminidase for 12 h. Lane 2: α_1-Pi (solution at 0.5 mg/ml) without desialylation.

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REFERENCES

[1] Hatton, M.W.C., März, L. and Regoeczi, E. (1983) Trends Biochem. Sci. 8, 287–291.
[2] Jeppsson, J.O., Lilja, H. and Johansson, M. (1985) J. Chromatogr. 327, 173–177.
[3] Mallet, B., Franc, J.L., Miquel, M. and Arnaud, C. (1987) Clin. Chim. Acta 167, 247–257.
[4] Hansen, J.E.S., Jensen, S.P., Norgaard-Pedersen, B. and Bøg-Hansen, T.C. (1986) Electrophoresis 7, 180–183.
[5] Vaughan, L., Lorier, M.A. and Carrell, R.W. (1982) Biochim. Biophys. Acta 701, 339–345.
[6] Laine, A., Hachulla, E. and Hayem, A. (1989) Electrophoresis 10, 227–233.
[7] Lejeune, P.J., Mallet, B., Farnarier, C. and Kaplanski, S. (1989) Biochim. Biophys. Acta 990, 122–127.
[8] Bøg-Hansen, T.C. (1973) Anal. Biochem. 56, 480–488.
[9] Warren, L. (1959) J. Biol. Chem. 234, 1971–1975.
[10] Schamberger, R.J. (1984) J. Clin. Chem. Clin. Biochem. 22, 647–651.
[11] Turner, G.A., Skillen, A.W., Buamah, P., Guthrie, D., Welsh, J., Harrison, J. and Kowalski, A. (1985) J. Clin. Pathol. 38, 588–592.
[12] Pos, O., Drechou, A., Durand, G., Bierhuizen, M.F.A., Van der Stelt, M.E. and Van Dijk, W. (1989) Clin. Chim. Acta 184, 121–132.
[13] Bordas, M.C., Biou, D.R., Feger, J.M., Durand, G.M., Joziasse, D.H. and Van den Eijnden, D.H. (1981) Clin. Chim. Acta 116, 17–24.
[14] De Jonc, G. and Van Eijk, H.G. (1988) Electrophoresis 9, 589–598.