PURIFICATION AND CHEMICAL COMPOSITIONS OF HUMAN α_1-ANTITRYPSIN OF THE MM TYPE

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1. Introduction

It has been shown that there are genetic variants of α_1-antitrypsin, a glycoprotein found in human serum, and a causal relationship has been established between these variants and the occurrence of various pulmonary diseases [1]. For instance, subjects of homozygous ZZ have a deficiency of α_1-antitrypsin and possess only 10–15% the amount of α_1-antitrypsin of subjects homozygous for the normal type MM, and the former group is predisposed to chronic obstructive pulmonary disease such as emphysema. However. very little is known about the molecular differences of the genetic variants of α_1-antitrypsin and how the level of this serum protein is regulated. Although α_1-antitrypsin was purified from pooled human serum and some of its physical–chemical properties reported some years ago, [2, 3] the preparations used at that time were not pure and the existence of genetic variants was then not known. Recently a report [4] appeared which described the purification of α_1-antitrypsin from human serum by using the method of affinity chromatography to remove albumin. However, the authors did not present composition data of the purified product. Therefore, we decided to reinvestigate this problem, purifying α_1-antitrypsin from serum of a single individual, whose genetic type with respect to α_1-antitrypsin had been previously determined to be the normal homozygous type MM. The purified product was homogeneous as judged by disc gel electrophoresis. The molecular weight, the amino acid and carbohydrate compositions of the protein were determined.

2. Methods

The activity of trypsin was assayed by a spectrophotometric method of Schwert and Takenaka [5] using α-*N*-benzoyl-L-arginine ethyl ester-HCl as substrate. Antitrypsin activity was measured as moles of trypsin inhibited extrapolated to 100% per unit of sample tested.

α_1-Antitrypsin was isolated and purified by gel filtration and chromatography on DEAE-cellulose and hydroxyapatite. Serum (300 ml) from the same donor was passed through a column (5 × 90 cm) of Sephadex G-100 equilibrated with 0.02 M sodium phosphate buffer, pH 6.5. The fractions containing antitrypsin activity were pooled and chromatographed on a column (2.8 × 40 cm) of DEAE-cellulose (Whatman microgranular DE-52) equilibrated with the same phosphate buffer and eluted with a NaCl gradient from 0–0.2 M. The assay of α_1-antitrypsin and of albumin in this and in subsequent steps was carried out by electrophoresis in 0.05 M barbital buffer, pH 8.6, on Millipore phoroslides supplied by the Millipore Corporation. The α_1-antitrypsin fraction was eluted at 0.062 M NaCl and albumin at 0.058 M. The pooled α_1-antitrypsin fractions were dialyzed against 0.01 M Tris–HCl buffer, pH 8.9, then chromatographed on a column (2.8 × 40 cm) of DEAE-cellulose equilibrated in the same buffer and eluted with a NaCl gradient from 0–0.3 M. Under these conditions, α_1-antitrypsin was eluted at 0.086 M and albumin at 0.100 M NaCl. This step was repeated until the α_1-antitrypsin fraction obtained was essentially free of albumin. Further purification was achieved by chromatography on a column (2.4 × 40 cm) of hy-

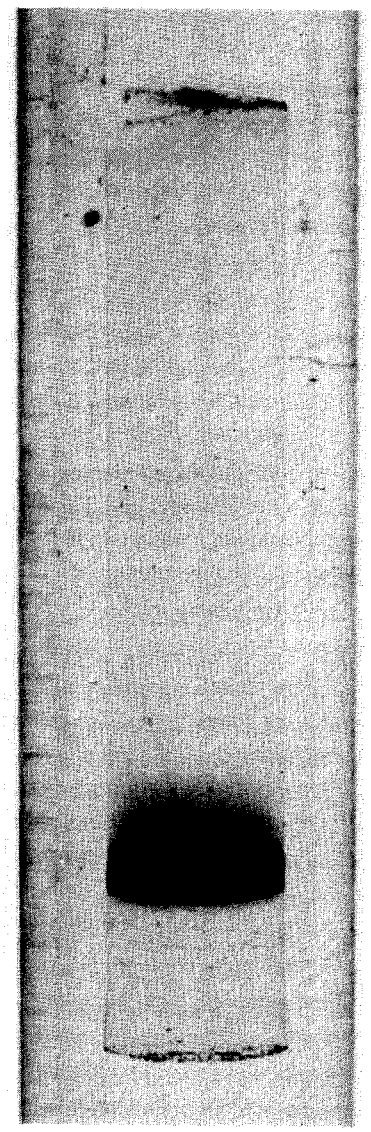

Fig. 1. Homogeneity of α_1-antitrypsin on disc polyacrylamide gel-electrophoresis. Standard disc polyacrylamide gel (7.5%) electrophoresis was carried out at pH 9.5 according to Davis [6]. Amido Black (0.1%) in 7% glacial acetic acid was used to stain the protein band. The top of the gel was the cathode denoted by (−); the bottom, the anode denoted by (+).

droxyapatite (Bio-Gel HT, Bio Rad Lab.) in 0.005 M potassium phosphate buffer, pH 6.5. A linear gradient from 0.005 M–0.3 M of the same buffer was passed through the column. Aliquots of the collected fractions were analyzed by electrophoresis at pH 9.5 on 7.5% polyacrylamide gel by the standard method of Davis [6]. The gels were stained for protein with 0.1% Amido black and for α_1-antitrypsin with a special stain [7]. Fractions containing α_1-antitrypsin were eluted at 0.085 M of potassium phosphate. The pooled fractions were concentrated on a small column (0.9 X 40 cm) of DEAE-cellulose and eluted by a NaCl gradient from 0–0.3 M as described above. This last step removed traces of albumin and also concentrated the purified protein. The final recovery of α_1-antitrypsin was calculated to be about 10%.

3. Results

On standard disc gel electrophoresis (fig. 1) the purified α_1-antitrypsin appeared as a single band which also showed antitrypsin activity as determined by the enzyme stain [7]. This purified product contained no demonstrable amount of albumin when examined by immunodiffusion with various concentrations of antialbumin antibodies. The protein was considered pure on the basis of these criteria. The molecular weight of the protein determined by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate and dithiothreitol [8] is 54 000. The following proteins were used as molecular weight markers in this experiment: α-globulin, bovine albumin, ovalbumin, chymotrypsinogen and cytochrome *c*. A single band was observed under these dissociating conditions which indicated that α_1-antitrypsin consists of a single polypeptide chain. The purified α_1-antitrypsin possessed full biological activity and it inhibited trypsin on a mole to mole basis. In this experiment, the amount of tyrpsin was standardized by using Shaw's inhibitor [9] and the inhibition experiment was carried out as described above. As determined by the method of acid starch gel electrophoresis described by Talamo et al. [10] both the starting material and the principle product were of type MM, indicating that purification steps apparently did not alter nor remove any components of the protein molecule. α_1-Antitrypsin contains 12% carbohydrates as determined by the method of Park and Johnson [11]. The complete carbohydrate and amino acid compositions of this glycoprotein are presented in table 1.

Table 1
Amino acid[a] and carbohydrate[b] compositions of human α_1-antitrypsin, Type MM.

Amino acids	Residues/Molecule	Amino acids	Residues/Molecule	Carbohydrates	Residues/Molecule
Asp	42.5	Met	8.6	Hexoses	16.4
Thr	33.2	Ile	18.2	Fucose	1.1
Ser	27.2	Leu	43.9	*N*-Acetyl-glucosamine	10.6
Glu	54.0	Tyr	6.0	Sialic Acid	7.0
Pro	18.7	Phe	28.3		
Gly	22.1	Lys	36.5		
Ala	26.6	His	12.4		
Val	23.8	Arg	8.1		
Cys	1.2	Trp	2.2		

[a] Protein samples were hydrolyzed in 6 N HCl *in vacuo* at 110°C for 20 and 60 hr. After the acid had been evaporated, the hydrolyzates were analyzed on a Technicon amino acid autoanalyzer [13]. The reported value for each amino acid except threonine and serine was the average of duplicate analysis obtained from hydrolyzates of the two time intervals. The values for threonine and serine were those extrapolated to zero time of hydrolysis. Value for tryptophan was obtained from a separate sample of protein which had been hydrolyzed in 4.2 N NaOH at 110°C for 16 hr, and analyzed on the Technicon amino acid autoanalyzer [14].

[b] Analysis of carbohydrates was carried out according to the procedures described by Winzler [15]. The constituents of the hexoses were identified by thin-layer chromatography to be galactose and mannose (Butanol: pyridine: 0.1 N HCl; 5:3:2, v/v/v). *N*-Acetyl-glucosamine was determined as hexosamine using glucosamine as standard. Subsequently, it was observed that only glucosamine was present in the protein hydrolyzate and that both galactosamine and mannosamine were absent. These results were obtained from analysis on the Technicon amino acid autoanalyzer [16] and by thin-layer chromatography.

For all calculations, the molecular weight of the protein was assumed to be 54 000 and the protein concentration was determined by the method of Lowry et al. [12], with ovomucoid as standard.

4. Discussion

By using the preparative method described in this paper, it is possible to obtain α_1-antitrypsin of high purity from a single genetic type (MM). Preliminary characterizations of this protein have been carried out and herein reported. These results have made it possible to initiate many new studies on this protein, such as amino acid and carbohydrate sequence, and investigations of the interaction mechanism between α_1-antitrypsin and proteases, and more importantly, this research provides a basis for the determination of the molecular differences of the various genetic types of α_1-antitrypsin. Finally, it is hoped that from these data a better understanding of the relationship between emphysema and other chronic obstructive pulmonary diseases and the genetic typing of α_1-antitrypsin may result.

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