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COMPLETE AMINO ACID SEQUENCE OF HUMAN α_1-MICROGLOBULIN

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SUMMARY

Complete amino acid sequence of human α_1-microglobulin has been established. It is composed of 167 amino acid residues and contains three carbohydrate attachment sites. No amino acid sequence heterogeneity was found.

Introduction

α_1-Microglobulin is a glycoprotein, present in serum and other body fluids (1-3). It was originally isolated from the urine of the patient of tubular proteinuria (4). The molecular weight of α_1-microglobulin has been reported 25,000-30,000 and contains about 20% carbohydrate (1-5).

It shows a single band on SDS polyacrylamide gel electrophoresis, but shows charge heterogeneity by agarose gel electrophoresis or isoelectric focusing (1,2). This charge heterogeneity is not diminished even after completely desialylated; therefore, it was assumed to be derived from amino acid sequence heterogeneity (1,2).

The present communication reports on the complete amino acid sequence of α_1-microglobulin and we have not found any amino acid sequence heterogeneity.

Materials and Methods

The isolation procedure of α_1-microglobulin from the pooled urine of the patients with marked tubular proteinuria was described in the previous paper (5).

α_1-Microglobulin was reduced with dithiothreitol and then carboxymethylated with iodoacetic acid in the presence of 6 M guanidine-HCl.

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Digestions of S-RCM-protein by trypsin (Wothington, TPCK), chymotrypsin (Wothington) and *Staphylococcal aureus* V-8 protease (Miles) were carried out in 0.1 M NH_4HCO_3 at 37°C for 5-48 h. Peptic digestion was carried out in diluted formic acid, pH 2.0, at 37°C for 5 h. Citraconylation was performed according to the method of Atassi and Habeeb (6), and then citraconylated protein was digested by trypsin at 37°C for 5 h. CNBr digestion was performed in 70% formic acid at room temperature for 20 h.

A first separation of trypstic, chymotryptic and peptic peptides was obtained by gel filtration on a column (2.2 x 140 cm) of Sephadex G-25 superfine eqilibrated with 0.05 M NH_4HCO_3. *S. aureus* protease peptides and tryptic peptides of citraconylated protein were separated by a column (2.2 x 90 cm) of Sephadex G-50 superfine and CNBr peptides were separated by a column (3 x 140 cm) of Sephadex G-50 superfine eqilibrated with 0.05 M NH_4HCO_3.

The peptides were purified by high voltage paper electrophoresis at pH 5.5 or 2.0 followed by descending chromatography in the second dimension in n-butanol/pyridine/acetic acid/water (15/10/3/12 by volume).

Amino acid analysis was carried out with Hitachi 835-50 Amino Acid Analyzer. Glucosamine was eluted between methionine and isoleucine, and galactosamine was between leucine and tyrosine.

Amino-terminal sequence of S-RCM-protein was determined by Edman method (7). Sequences of small peptides were determined by subtractive Edman degradation (8) or dansyl-Edman method (9).

Carboxy-terminal residues were determined by carboxypeptidase A, B (Worthington) or Y (Sigma) digestion.

Results

Most of the amino acid sequences were determined using tryptic peptides. The overlaps of tryptic peptides were obtained by chymotryptic, *S. aureus* protease, peptic and CNBr peptides and also subpeptides of CNBr peptides. The results are shown in Fig. 1.

Three carbohydrate attachment sites were found; one was threonine at position 5, in which was found galctosamine by amino acid analysis, and the others were asparagine at positions 17 and 86, in which was found glucosamine.

No radioactive carboxymethylcysteine was obtained by iodo-^{14}C-acetic acid labelling without prior reduction with dithiothreitol in the presence of 6 M guanidine-HCl. Therefore, cysteines at positions 62 and 157 are connected by disulfide bond.

Discussion

The amino-terminal sequence of α_1-microglobulin has been reported from several laboratories (10-14); however, all reports missed the presence

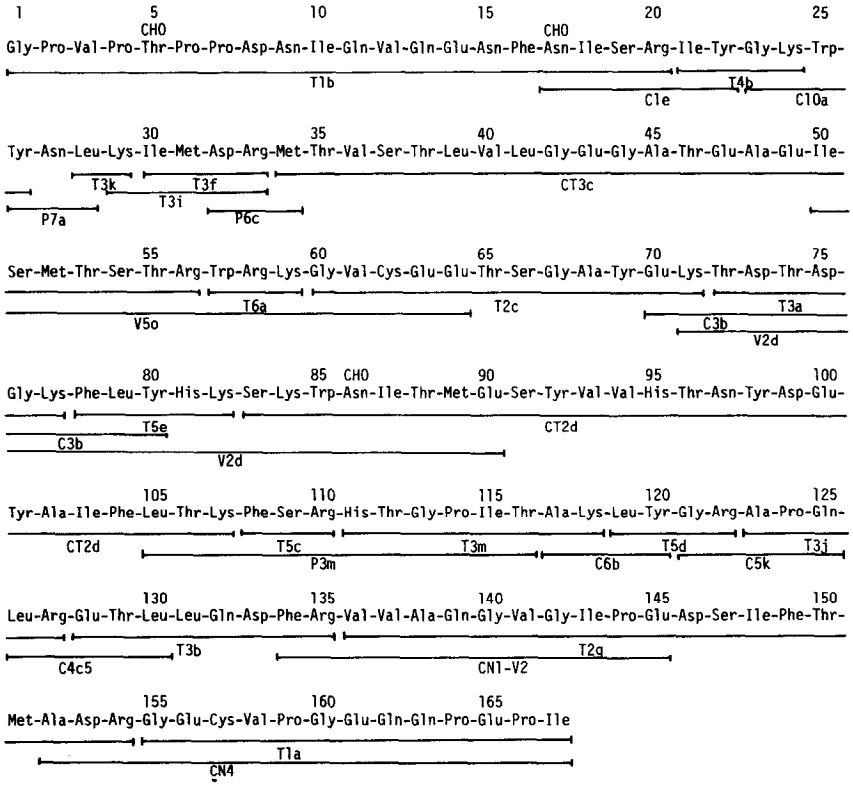

Fig. 1 Summary of the amino acid sequence determination of human α_1-microglobulin. T=trypsin, C=chymotrypsin, P=pepsin, V=*Staphylococcal aureus* V-8 protease, CT=trypsin after citraconylation, CN=CNBr peptides. CHO=carbohydrate attachment site.

of two carbohydrate attachment sites in the amino-terminal region, and confused identification of amino acids around the carbohydrate attachment sites (10,12,14).

α_1-Microglobulin is also called protein HC (heterogeneous in charge) and shows charge heterogeneity by agarose gel electrophoresis or isoelectric focusing (1,2). This charge heterogeneity was not diminished even after completely desialylated; therefore, charge heterogeneity might be derived from amino acid sequence heterogeneity (1,2). First we have expected the amino acid sequence heterogeneity and we have determined most of the sequences of peptides by subtractive Edman degradation to

distinguish from the background spots; however, no sequence heterogeneity was found.

α_1-Microglobulin contains brown color material and this color was not removed by treatment with 6 M guanidine-HCl, or CNBr digestion in 70% formic acid. Therefore, a chromophore material covalently binds to protein or carbohydrate moiety. We have not clearly identified yet, but the chromophore material seems to move with the carbohydrate bound asparagine at position 86.

The α_1-microglobulin which we used in sequence determination was prepared from the pooled urine. Therefore, an apparent charge heterogeneity of this protein so far observed is not due to the inter-individual difference; moreover, it does not seem to reside in either the polypeptide chain nor the carbohydrates, suggesting to be related with chromophore material(s).

The relationship between HLA antigens and α_1-microglobulin has been discussed (11). However, any significant sequence homology was not found between these two proteins by computer analysis.

Biological role of α_1-microglobulin is still obscure.

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