N-terminal amino acid sequences of precursor and mature forms of α-1-antitrypsin

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α-1-Antitrypsin is found in hepatocytes as a high-mannose glycoprotein (M_r 49000), extracellularly as a complex-type glycoprotein (M_r 54000). Deglycosylation of both forms with peptide: N-glycosidase led to proteins of identical app. M_r (41000). The sequence of 26 N-terminal amino acids of rat α_1-antitrypsin was determined. A high content of polar amino acids was found. The partially characterized presequence of in vitro synthesized α_1-antitrypsin showed a cluster of hydrophobic amino acids. A pre-peptide of 24 amino acids is proposed. There is no evidence for the existence of a propeptide.

α_1-Antitrypsin *Edman degradation* *In vitro translation* *Prepeptide* *Hepatocyte primary culture* *Deglycosylation*

1. INTRODUCTION

α-1-Antitrypsin is the major plasma proteinase inhibitor in man and in a variety of animal species. It is a complex-type glycoprotein [1,2], which is synthesized in the liver [3] and secreted into the blood stream. Usually secretory glycoproteins are processed in both their carbohydrate moieties and their protein backbones. In [4] we described the existence of two differently glycosylated forms of α_1-antitrypsin in rat hepatocytes. A high mannose glycoprotein of an app. M_r 49000 was found to be the precursor of a complex-type glycoprotein of an app. M_r 54000. To understand the processing of the protein part of α_1-antitrypsin during its biosynthesis, in vitro translation studies have been carried out recently [5,6]. These studies suggested the existence of a higher M_r precursor with an amino-terminal extension of about 20 amino acids. In order to characterize this prepeptide, we have compared the N-terminal amino acid sequence of α_1-antitrypsin purified from rat serum with the partially characterized N-terminal amino acid sequences of α_1-antitrypsin synthesized in rat hepatocyte primary cultures and in an in vitro translation system.

2. MATERIALS AND METHODS

L-[2,3-^{3}H]Alanine (30–50 Ci/mmol), [4,5-^{3}H]-leucine (40–60 Ci/mmol), L-[^{35}S]methionine (>1000 Ci/mmol) were purchased from the Radiochemical Centre (Amersham), protein A–Sepharose CL-4B, activated thiol–Sepharose 4B, and con A–Sepharose were obtained from Pharmacia (Freiburg), tunicamycin was from Calbiochem-Behring (Giessen). For the purification of α_1-antitrypsin rat serum was subjected to a 50% and a subsequent 80% ammonium sulfate precipitation, followed by affinity chromatography, first on activated thiol–Sepharose 4B as in [7], second by affinity chromatography on con A–Sepharose as in [8], and third by preparative SDS–polyacrylamide gel electrophoresis [9]. The polyacrylamide slice containing α_1-antitrypsin was cut from the gel, homogenized, mixed with Freund's complete

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adjuvant and used for the immunization of rabbits. Rat hepatocyte primary cultures were prepared as in [4,5]. Rat liver poly(A)$^+$RNA was isolated from polysomes by phenol extraction as in [10], followed by affinity chromatography on oligo(dT)-cellulose [11,12]. Cell-free protein synthesis was carried out in a wheat germ system [13]. Immunoprecipitation of α_1-antitrypsin was done essentially as in [14]. SDS–polyacrylamide slab gel electrophoresis was carried out as in [9], fluorography as in [15]. Alpha-1-antitrypsin synthesized in vitro or in vivo in the presence of [^{3}H]leucine or [^{3}H]alanine was precipitated with 2 mg of whale myoglobin and subjected to automatic Edman degradation with a Beckman Spinco Sequencer 890S following the method in [16]. The radioactivity of the thiazolinone derivatives of each degradation step was measured. In order to define the degradation step, an aliquot of each fraction was converted to the corresponding phenylthiohydantoin derivative and separated on a reversed phase column by isocratic high-pressure liquid chromatography [17].

3. RESULTS AND DISCUSSION

Fig.1 shows a fluorogram of a SDS–polyacrylamide slab gel with radioactively labeled α_1-antitrypsin, synthesized either in hepatocyte primary cultures or in a cell-free wheat germ system. Two different M_r forms of α_1-antitrypsin were found in hepatocytes and their medium. When glycosylation was inhibited by tunicamycin, only one M_r form of α_1-antitrypsin (M_r 41000) was obtained in hepatocytes and their medium. To find out whether the high mannose type glycoprotein (M_r 49000) and the complex type (M_r 54000) glycoprotein contain polypeptide chains of identical M_r both forms of α_1-antitrypsin were deglycosylated by peptide: N-glycosidase from almond emulsin. Enzymatic deglycosylation led to polypeptides of identical electrophoretic mobility undistinguishable from that of unglycosylated α_1-antitrypsin, synthesized in tunicamycin-treated hepatocytes (M_r 41000). On the other hand, in vitro synthesized α_1-antitrypsin exhibited an app. M_r of 43000.

To characterize the N-terminal extension of the in vitro synthesized α_1-antitrypsin, we have first determined the N-terminal amino acid sequence of mature α_1-antitrypsin purified from rat serum. Fig.2 shows the first 26 amino acids. The high content of polar amino acid residues is remarkable. The direct comparison with the sequence of human α_1-antitrypsin shows homology only in 5 positions (1, 2, 4, 12 and 14). However, homology in 17 amino acid residues is found when two insertions – one of 5 and one of 1 amino acid – are assumed in human α_1-antitrypsin.

N-Terminal amino acid sequences of rat α_1-anti-

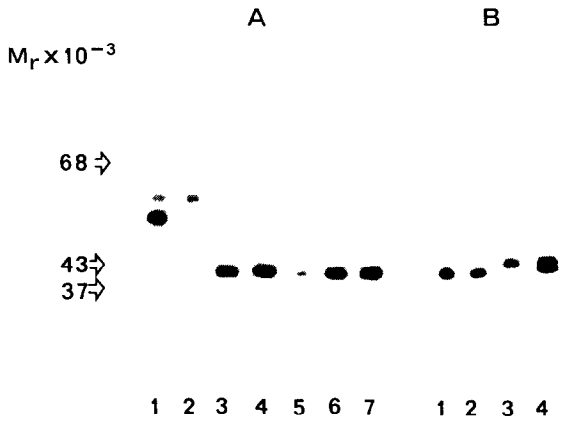

Fig.1. Synthesis of α-1-antitrypsin in rat hepatocyte monolayers and in a cell-free system. (A) Rat hepatocyte monolayers were prepared as in [4,5]. About 3.9×10^6 cells of about 25 mg wet wt/dish were incubated in modified Waymouth medium [18] containing 25 μCi of [^{35}S]methionine for 3 h at 37°C. Alpha-1-antitrypsin was immunoprecipitated from a $12000 \times g$ supernatant of the cells (lane 1) after homogenization in 25 mM Tris–HCl buffer (pH 7.5), 20 mM NaCl, 1% deoxycholate (Na^+) and 1% Triton X-100 and from the medium (lane 2). Alpha-1-antitrypsin immunoprecipitated from cells (lane 4) and medium (lane 5) after incubation with 10 units of peptide: N-glycosidase from almonds [19,20] in a total volume of 0.1 ml of 10 mM acetate (Na^+) (pH 5.1) and 0.1% sodium dodecyl sulfate at 37°C for 24 h. Alpha-1-antitrypsin immunoprecipitated from hepatocytes treated with tunicamycin (3 μg/ml medium) for 1 h and labeled with [^{35}S]methionine (25 μCi per dish) in the presence of tunicamycin (lane 3); (lane 6) same as lane 3, except that α_1-antitrypsin was treated with 10 units of peptide: N-glycosidase; (lane 7) mixture of lanes 3 and 5; (B) Alpha-1-antitrypsin from tunicamycin-treated (3 μg/ml) hepatocytes (lane 1) and their medium (lane 2); α_1-antitrypsin synthesized by in vitro translation of poly(A)$^+$RNA in a wheat germ system (lane 3); mixture of lanes 2 and 3 (lane 4). The M_r markers used were bovine serum albumin (68000), ovalbumin (43000), and alcohol dehydrogenase from yeast (37000).

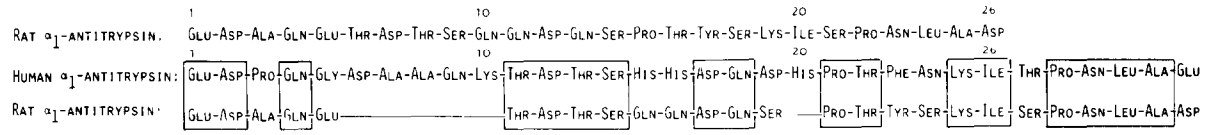

Fig.2. N-terminal amino acid sequence of rat and human α-1-antitrypsin. Rat α_1-antitrypsin was prepared and subjected to automatic Edman degradation as in section 2. The amino acid sequence of human α_1-antitrypsin is taken from [21].

trypsin have been described in [6,22]. The 4 amino acids determined in [22] agree with our results. Arginine has been found in positions 6 and 8 [6]. In our studies threonine was identified at these positions. In contrast to our sequence data, as well as those in [6,22], Roll and Glew alanine was found [23] as the amino terminus of rat α_1-antitrypsin.

When α_1-antitrypsin, synthesized in vitro in the presence of either [^{3}H]leucine or [^{3}H]alanine was subjected to radioactive Edman degradation, leucine was found in positions 8–11, 14, 17 and 22, and alanine in positions 1, 12, 18, 23 and 26 (fig.3). In eukaryotic cells methionine is the first amino acid of all newly-synthesized proteins. However, from the fact that in our preparation alanine was found in the first Edman degradation cycle, it must be concluded that methionine had been removed from the amino terminus of the α_1-antitrypsin precursor. Similar observations were made in [24] with preovomucoid synthesized in a reticulocyte lysate. Aminopeptidase activities likely to be responsible for the removal of the initiator

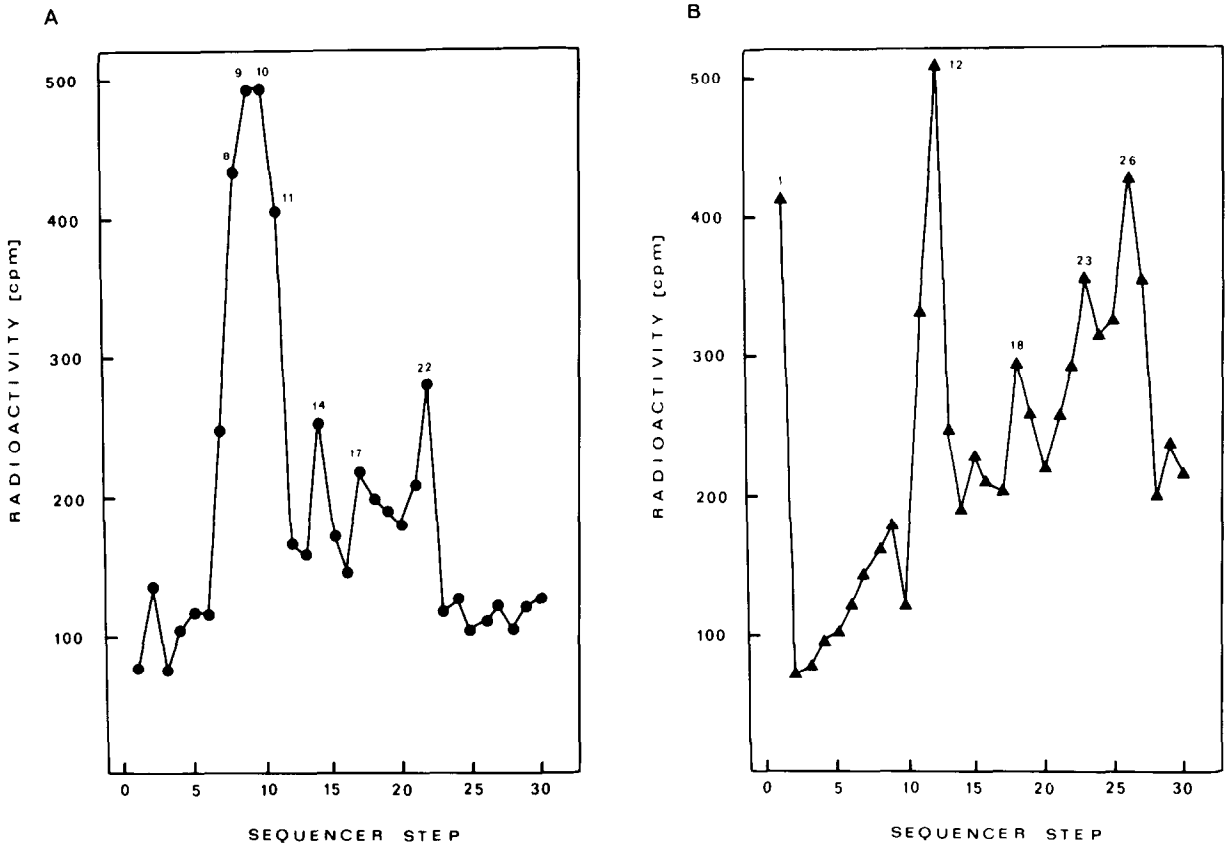

Fig.3. N-terminal sequence analysis of α-1-antitrypsin translated in vitro. Alpha-1-antitrypsin was translated in a cell-free system derived from wheat germ in the presence of [^{3}H]leucine (A) or [^{3}H]alanine (B), and subjected to 30 Edman degradation cycles.

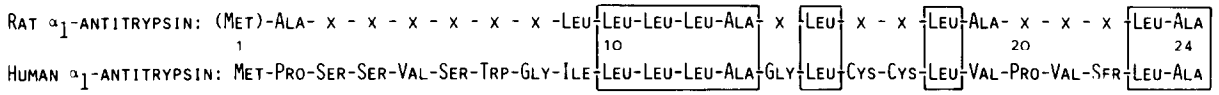

Fig.4. Comparison of the prepeptides of rat and human α-1-antitrypsin. The partial amino acid sequence of rat α_1-antitrypsin is taken from fig.3. The amino acid sequence of the prepeptide of human α_1-antitrypsin has been deduced from the nucleotide sequence of a cloned human α_1-antitrypsin gene [21].

methionine residue, have been found in wheat germ and rabbit reticulocyte lysates [25].

If it is assumed that alanine at position 26 of the α_1-antitrypsin, synthesized in vitro (fig.3B) corresponds to the alanine at position 3 in the mature protein (fig.2), a prepeptide of 24 amino acids – including the N-terminal methionine – exists. A prepeptide of this length can be expected from the differences in app. M_r of in vitro synthesized and unglycosylated mature α_1-antitrypsin (fig.1). This assumption is further supported by the high sequence homology with respect to alanine and leucine between the α_1-antitrypsin prepeptides of man and rat (fig.4).

To study the possibility that the intracellular α_1-antitrypsin may exist as a proprotein as in the case of albumin, we have labeled α_1-antitrypsin in hepatocyte primary cultures with [^{3}H]leucine and subjected it to automatic Edman degradation. Radioactively-labeled leucine was found in position 24. Since leucine is also present at position 24 of the mature α_1-antitrypsin purified from rat serum, we conclude that α_1-antitrypsin does not exist in a proprotein form in the cells.

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