N-terminal amino acid sequences of precursor and mature forms of \(\alpha-1\)-antitrypsin

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\(\alpha-1\)-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 \(\alpha-1\)-antitrypsin was determined. A high content of polar amino acids was found. The partially characterized presequence of in vitro synthesized \(\alpha-1\)-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.

1. INTRODUCTION

\(\alpha-1\)-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 \(\alpha-1\)-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 \(\alpha-1\)-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 \(\alpha-1\)-antitrypsin purified from rat serum with the partially characterized N-terminal amino acid sequences of \(\alpha-1\)-antitrypsin synthesized in rat hepatocyte primary cultures and in an in vitro translation system.

2. MATERIALS AND METHODS

\(L-\left[2,3^-{\text{H}}\right]\)Alanine (30–50 Ci/mmol), \(\left[4,5^-{\text{H}}\right]\)leucine (40–60 Ci/mmol), \(L-\left[^{35}\text{S}\right]\)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 \(\alpha-1\)-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, and con A–Sepharose were obtained from Pharmacia (Freiburg), tunicamycin was from Calbiochem-Behring (Giessen). For the purification of \(\alpha-1\)-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, and second by affinity chromatography on con A–Sepharose as in \([8]\), and third by preparative SDS–polyacrylamide gel electrophoresis \([9]\). The polyacrylamide slice containing \(\alpha-1\)-antitrypsin was cut from the gel, homogenized, mixed with Freund’s complete
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 α1-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 [3H]leucine or [3H]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 α1-antitrypsin, synthesized either in hepatocyte primary cultures or in a cell-free wheat germ system. Two different M<sub>r</sub> forms of α1-antitrypsin were found in hepatocytes and their medium. When glycosylation was inhibited by tunicamycin, only one M<sub>r</sub> form of α1-antitrypsin (M<sub>r</sub> 41000) was obtained in hepatocytes and their medium. To find out whether the high mannose type glycoprotein (M<sub>r</sub> 49000) and the complex type (M<sub>r</sub> 54000) glycoprotein contain polypeptide chains of identical M<sub>r</sub>, both forms of α1-antitrypsin were deglycosylated by peptide: N-glycosidase from almond emulsin. Enzymatic deglycosylation led to polypeptides of identical electrophoretic mobility undistinguishable from that of unglycosylated α1-antitrypsin, synthesized in tunicamycin-treated hepatocytes (M<sub>r</sub> 41000). On the other hand, in vitro synthesized α1-antitrypsin exhibited an app. M<sub>r</sub> of 43000.

To characterize the N-terminal extension of the in vitro synthesized α1-antitrypsin, we have first determined the N-terminal amino acid sequence of mature α1-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 α1-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 α1-antitrypsin.

N-Terminal amino acid sequences of rat α1-anti-
Fig. 2. N-terminal amino acid sequence of rat and human $\alpha_1$-antitrypsin. Rat $\alpha_1$-antitrypsin was prepared and subjected to automatic Edman degradation as in section 2. The amino acid sequence of human $\alpha_1$-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 $\alpha_1$-antitrypsin.

When $\alpha_1$-antitrypsin, synthesized in vitro in the presence of either $[^3]$Hleucine or $[^3]$Halanine 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 $\alpha_1$-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 $\alpha_1$-antitrypsin translated in vitro. Alpha-1-antitrypsin was translated in a cell-free system derived from wheat germ in the presence of $[^3]$Hleucine (A) or $[^3]$Halanine (B), and subjected to 30 Edman degradation cycles.
methionine residue, have been found in wheat germ and rabbit reticulocyte lysates [25].

If it is assumed that alanine at position 26 of the \( \alpha_1 \)-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 \( \alpha_1 \)-antitrypsin (fig.1). This assumption is further supported by the high sequence homology with respect to alanine and leucine between the \( \alpha_1 \)-antitrypsin prepeptides of man and rat (fig.4).

To study the possibility that the intracellular \( \alpha_1 \)-antitrypsin may exist as a proprotein as in the case of albumin, we have labeled \( \alpha_1 \)-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 \( \alpha_1 \)-antitrypsin purified from rat serum, we conclude that \( \alpha_1 \)-antitrypsin does not exist in a proprotein form in the cells.

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