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SYNTHESIS AND SECRETION OF α_2-MACROGLOBULIN BY HUMAN LUNG FIBROBLASTS

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Summary: Synthesis and secretion of α_2-macroglobulin were studied with the human lung fibroblast cell line GM 1379. After incubation with [^{3}H]leucine the cells secreted radioactively labeled α_2-macroglobulin consisting of subunits with a molecular weight of 180,000. When the cells were treated with tunicamycin the unglycosylated α_2-macroglobulin subunit exhibited a molecular weight of 160,000. Poly(A)$^+$RNA was isolated from the cultured cells and translated in a rabbit reticulocyte lysate. From the translation products an α_2-macroglobulin species with a molecular weight of 160,000 was immunoprecipitated. The addition of pancreatic microsoms to the translation mixture resulted in the synthesis of an α_2-macroglobulin subunit which had molecular weights of 180,000. Thus, a size of approximately 160,000 for the protein moiety and 20,000 for the carbohydrate portion can be estimated for a subunit of α_2-macroglobulin from human lung fibroblasts.

Introduction: α_2-Macroglobulin (α_2M)[1] is an important plasma proteinase inhibitor which exhibits broad specificity toward endoproteinases (1). Human α_2M is a glycoprotein with a molecular weight of 720,000 and consists of four identical subunits (2-5). The major site of synthesis of this proteinase inhibitor is the liver from where it is secreted into the plasma (6-8). The proteinase inhibitor plays a predominant role in controlling proteolytic processes in plasma and extravascular tissue fluids; in particular, during inflammatory processes α_2M protects against tissue destruction (9).

[1] Abbreviation: α_2M, α_2-macroglobulin.

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Recently it has been demonstrated by cell-free translation and RNA-cDNA hybridization that human lung tissue is also capable to synthesize α_2M (10). In man an important site of action of α_2M is the lung where this proteinase inhibitor assists to prevent tissue destruction during chronic inflammation and, thus, the local production greatly benefits lung defense. However, the types of lung cells which synthesize α_2M have not been identified, although some evidence has been obtained that pulmonary macrophages (11) and lung fibroblasts (12,13) contain α_2M and, therefore, may also be the sites of synthesis. We have investigated the synthesis and secretion of α_2M by a human fibroblast cell line by labeling studies as well as cell-free translation. These results demonstrate that α_2M is in fact synthesized and secreted by these cells.

Materials and Methods: L-[^{35}S]Methionine (>600 Ci/mmol) and L-[^{3}H]leucine (140 Ci/mmol) were purchased from Amersham Buchler (Braunschweig, W.Germany), [^{14}C]formaldehyde (51 mCi/mmol) and Protosol from New England Nuclear (Boston, MA), guanidine HCl (grade I) and oligo(dT)-cellulose from Sigma (St.Louis, MO), protein A-Sepharose Cl-4B from Pharmacia (Freiburg, W.Germany), tunicamycin from Calbiochem-Behring (San Diego, CA), and media for cell culture were obtained from Gibco (Grand Island, NY). The purification of α_2M from human serum (14) and the preparation of rabbit antiserum have been described (10). Reductive methylation of α_2M with [^{14}C]formaldehyde was performed according to Jentoft and Dearborn (15).

Cell culture: Human GM 1379 lung fibroblasts were obtained from Human Cell Mutant Repository (Philadelphia, PA) and cultured in Eagle's minimum essential medium with Earle's salts, supplemented with 20 % fetal calf serum, 1 mM pyruvate, 100,000 U/l penicillin and 100 mg/l streptomycin. The cells were maintained at 37°C in a 10% CO_2 atmosphere. Confluent cultures containing approximately 2 x 10 cells per 25 cm disk were incubated with leucine- and serum-free medium for 1 h. Following this preincubation 3 ml medium containing 50 µCi [^{3}H]leucine per dish were added. The cells were labeled for 15 h and α_2M was immunoprecipitated from the medium. In some experiments the cells were incubated with tunicamycin (3 µg/ml) for 4 h and subsequently labeled with [^{3}H]leucine for 15 h in the presence of the glycosylation inhibitor.

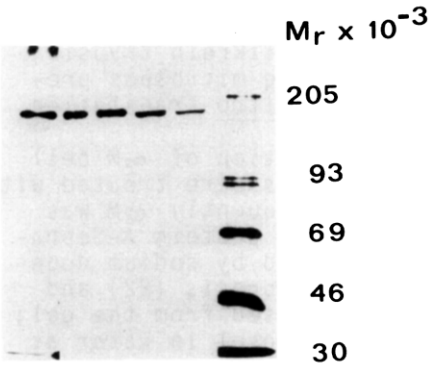
Isolation of poly(A)$^+$RNA and in vitro translation: Poly(A)$^+$RNA from cultured lung fibroblasts was prepared by isolation of total RNA according to Alvino et al. (16) and chromatography on oligo(dT)-cellulose (16). 5 µg of poly(A)$^+$RNA were incubated for 120 min in 100 µl nuclease-treated rabbit reticulocyte lysate (18) containing 75 µCi [^{35}S] methionine, 100 units RNase inhibi-

tor from human placenta (19), 0.01 µg of pepstatin, chymostatin, leupeptin and antipain each, and 1 unit of kallikrein trypsin inhibitor (Trasylol). In some experiments 4 Eq microsoms prepared from dog pancreas were added to the in vitro translation system (20).

Immunoprecipitation: For the immunoprecipitation of α_2M cell culture media and in vitro translation mixtures were treated with preimmune serum and protein A-Sepharose; subsequently α_2M was immunoprecipitated with specific antiserum and protein A-Sepharose (21). The immunoprecipitates were analyzed by sodium dodecyl sulfate 7.5 % polyacrylamide gel electrophoresis (22) and fluorography (23). Radioactive bands were excised from the gel; the gel slices were solubilized with 90 % Protosol in water at 40°C overnight and counted for radioactivity.

Results: The synthesis and secretion of α_2M by cultured human lung fibroblasts was investigated by labeling the cells with [^{3}H] leucine and subsequent immunoprecipitation of α_2M from the culture medium with a specific antiserum. The immunoprecipitates were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Figure 1). Human lung fibroblasts secreted radioactively labeled α_2M which consisted of subunits with an apparent molecular weight of 180,000 (lane 2). An identical electrophoretic mobility was observed for [^{14}C]methylated α_2M from human plasma (lane 1). The specificity of the immunoprecipitation was further demonstrated by competition of unlabeled α_2M with the radioactive protein synthesized by cultured lung fibroblasts (lanes 3-5). In order to estimate the sizes of the protein and carbohydrate moieties of α_2M synthesized by human lung fibroblasts, synthesis of α_2M was studied in the presence of the glycosylation inhibitor tunicamycin (Figure 2). In addition to the mature protein (lane 6) cells treated with tunicamycin also secreted a protein with an apparent molecular weight of 160,000 (lane 7).

The synthesis of α_2M was also investigated by cell-free translation of poly(A)$^+$RNA from human lung fibroblasts in a rabbit reticulocyte lysate (Figure 2). Polypeptides up to high molecular weights were synthesized (lane 1). α_2M was isolated from the total translation products by immunoprecipitation and exhibited



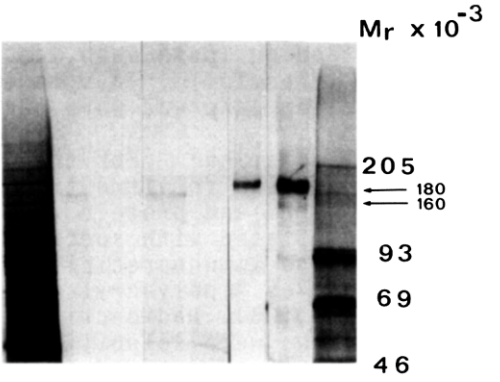


Figure 1: Synthesis and secretion of α_2-macroglobulin by human lung fibroblasts.
Approximately 2 x 10 cells were labeled with [^{3}H]leucine for 15 h. Secreted α_2M was immunoprecipitated from the culture medium and analyzed by sodium dodecyl sulfate 7.5 % polyacrylamide gel electrophoresis: [^{14}C]Methylated α_2M from human serum (lane 1); immunoprecipitation of α_2M from culture medium of human lung fibroblasts (lane 2) and immunoprecipitation in the presence of 10 µg, 40 µg and 60 µg unlabeled α_2M, respectively (lanes 3-5). Molecular weight markers (lane 6): myosin (205,000), phosphorylase b (two bands, 92 - 94,000), bovine serum albumin (69,000), ovalbumin (46,000), and carbonic anhydrase (30,000).

Figure 2: Synthesis and glycosylation of α_2-macroglobulin by a cell-free translation system and by human lung fibroblasts.
Cell-free synthesis of α_2M in a reticulocyte lysate was directed by poly(A)$^+$RNA from human lung fibroblasts and glycosylation was stimulated by addition of pancreatic microsomes. About 8 x 10 cpm of [^{35}S]radioactivity were incorporated into trichloroacetic acid-precipitable protein and used for immunoprecipitation of α_2M. Human lung fibroblasts were labeled with [^{3}H]leucine and the effect of tunicamycin on glycosylation was studied. Secreted α_2M was isolated from the cell culture medium by immunoprecipitation. The immunoprecipitates were analyzed by sodium dodecyl sulfate 7.5 % polyacrylamide gel electrophoresis: Total translation products of poly(A)$^+$RNA from human lung fibroblasts (lane 1); immunoprecipitation of α_2M (lane 2), and immunoprecipitation in the presence of 100 µg unlabeled α_2M (lane 3); immunoprecipitation of α_2M synthesized in the presence of pancreatic microsomes (lane 4), and immunoprecipitation in the presence of 100 µg unlabeled α_2M (lane 5); immunoprecipitation of α_2M from the culture medium of human lung fibroblasts (lane 6), and immunoprecipitation of α_2M from the culture medium of cells treated with tunicamycin (lane 7). Molecular weight markers (lane 8).

an apparent molecular weight of 160,000 (lane 2). When unlabeled α_2M was added prior to the immunoprecipitation competition with the radioactive α_2M was observed (lane 3). Addition of pancreatic microsomal membranes to the cell-free translation system yielded an α_2M species with an apparent molecular weight of 180,000 which is the result of core glycosylation (lanes 4,5).

Table I

Rates of synthesis and secretion of α_2-macroglobulin by human lung fibroblasts

Experiment	Total radioactivity (cpm)	Immunoprecipitable radioactivity (cpm)	(%)
Cell-free translation	7 500 000[a]	1 150	0.015
Cell culture	460 000[b]	15 600	3.3

[a] Total trichloroacetic acid-precipitable [^{35}S]radioactivity per 150 µl translation mixture containing 7.5 µg of poly(A)$^+$RNA.

[b] Total trichloroacetic acid-precipitable [^{3}H]radioactivity per 3 ml cell culture medium.

The rates of synthesis and secretion of α_2M by human lung fibroblasts were estimated from the cell-free translation and cell culture experiments (Table I). The rate of synthesis was estimated from the translability of mRNA in a cell free system; approximately 0.015% of the synthesized protein corresponded to α_2M. Of the proteins secreted by cultured lung fibroblasts about 3.3% was identified as α_2M.

Discussion: The synthesis of α_2M by cultured human lung fibroblasts has been demonstrated by incorporation of radioactively labeled amino acids into newly synthesized protein and by cell-free translation of mRNA isolated from the cultured cells. These results suggest that within human lung tissue fibroblasts are a site of synthesis for this important proteinase inhibitor. α_2M synthesized and secreted by human lung fibroblasts exhibits a moleular weight of 180,000 per subunit. In the presence of the glycosylation inhibitor tunicamycin and unglycosylated species with a molecular weight of 160,000 is released from the cells. A molecular weight of 160,000 has also been observed for the cell-free synthesized protein, and when the cell-free translation is carried out in the

presence of pancreatic microsomes a core-glycosylated form with a molecular weight of 180,000 is found. Thus, subunits of α_2M from human lung fibroblasts consist of a protein moiety of about 160,000 and a carbohydrate moiety of approximately 20,000. A strikingly similar composition has been found for α_2M synthesized by human liver (2-5).

The observation that human lung fibroblasts synthesize α_2M appears to be of importance for the control of proteolytic processes in lung tissue. Fibroblasts also possess specific receptors which recognize α_2M-proteinase-complexes and mediate the uptake of these complexes by endocytosis (3). The synthesis and secretion of α_2M and the receptor-mediated endocytosis of α_2M-proteinase-complexes provide a mechanism of modulating proteolytic processes in the alveolar microenvironment and thus play an important role in lung defense.

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