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INDUCTION OF α1-ANTITRYPSIN mRNA AND CLONING OF ITS cDNA

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SUMMARY

Local inflammation was inflicted in a baboon by turpentine administration in order to induce the plasma level of α1-antitrypsin, an acute phase protein synthesized in the liver. Comparison of the α1-antitrypsin mRNA activity in the induced and non-induced baboon liver indicated that the "acute phase" response to chemical-inflicted inflammation is mediated through an increase in the steady-state level of cellular mRNA. Alpha-1-antitrypsin was then enriched from the induced baboon liver to a purity of greater than 90% by specific immunoprecipitation of polysomes. Double-stranded DNA was synthesized from the enriched mRNA and inserted into the Pst I site of pBR322. Recombinant clones containing α1-antitrypsin cDNA sequences were identified by hybrid-selected translation and confirmed by DNA sequence analysis.

INTRODUCTION

Alpha-1-antitrypsin is a major plasma protease inhibitor (1-3). It is a glycoprotein synthesized in the liver and consists of a single polypeptide chain with a molecular weight of 51,000 (4,5). Individuals with α1-antitrypsin deficiency often develop chronic obstructive pulmonary emphysema and/or infantile liver cirrhosis (6-8). The genetic deficiency is characterized by the presence of a variant protein which is inherited as an autosomal recessive trait (9,10).

The plasma level of α1-antitrypsin is greatly increased under a variety of physiologic and pathologic conditions, such as local inflammation, surgery, acute and chronic infections, neoplasia, bacterial endotoxin, nephritis, pregnancy, and administration of contraceptive hormones (11). Accordingly, α1-antitrypsin is one of the typical acute phase proteins (12). In order to study

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the acute phase response and the genetic deficiency at the molecular level, an α1-antitrypsin cDNA clone has been constructed.

METHODS

Administration of Turpentine to a Baboon: A 3 year-old male baboon weighing 4.5 Kg was injected subcutaneously with 4.5 ml of 1:1 mixture of turpentine and olive oil. The animal was sacrificed after 24 hours. The liver was removed surgically, rinsed with ice-cold saline, and immediately frozen in liquid nitrogen.

Enrichment of α1-antitrypsin mRNA by Specific Polysome Immunoprecipitation: Polysomes were prepared from the baboon liver as described previously (13) and those synthesizing nascent α1-antitrypsin chains were enriched by immunoprecipitation according to the procedure of Gough and Adams (14). The RNA was analysed using a mRNA-dependent cell-free translation system derived from rabbit reticulocytes (15), and newly synthesized radioactive α1-antitrypsin was immunoprecipitated according to the procedure of Rohrschneider et al (16). The protein products were analysed by polyacrylamide gel electrophoresis (17) followed by fluorography.

Cloning of α1-antitrypsin cDNA: The synthesis and cloning of cDNA from the enriched mRNA preparation and the identification of α1-antitrypsin clones by hybrid-selected translation were essentially as reported previously (18). DNA sequencing was performed according to the procedure of Maxam and Gilbert (19), and electrophoresis on thin polyacrylamide gels was as described by Sanger and Coulson (20).

RESULTS AND DISCUSSION

Comparison of α1-antitrypsin mRNA Activities in Normal and Turpentine-Induced Baboon Liver: Total poly A-containing RNA was isolated from the liver of a normal and a turpentine-treated baboon. The RNA preparation was analyzed using a mRNA-dependent rabbit reticulocyte lysate system, employing [^{35}S] methionine. Total protein products as well as specific immunoprecipitable products were analysed by fluorography (Fig. 1). Panel A shows the profiles of total protein products synthesized in response to various quantities of total poly A-containing RNA isolated from a normal liver (lanes 2-4) and a turpentine-induced liver (lanes 12-14). It is apparent that the 69,000 dalton albumin band is much more prominent in the lanes containing samples from normal liver compared to those containing samples from induced liver. Conversely, there appears to be a relatively more intense band in the lanes containing induced liver samples with an electrophoretic mobility slightly greater than that of the endogenous 50,000 dalton reticulocyte lysate band. Since this intense

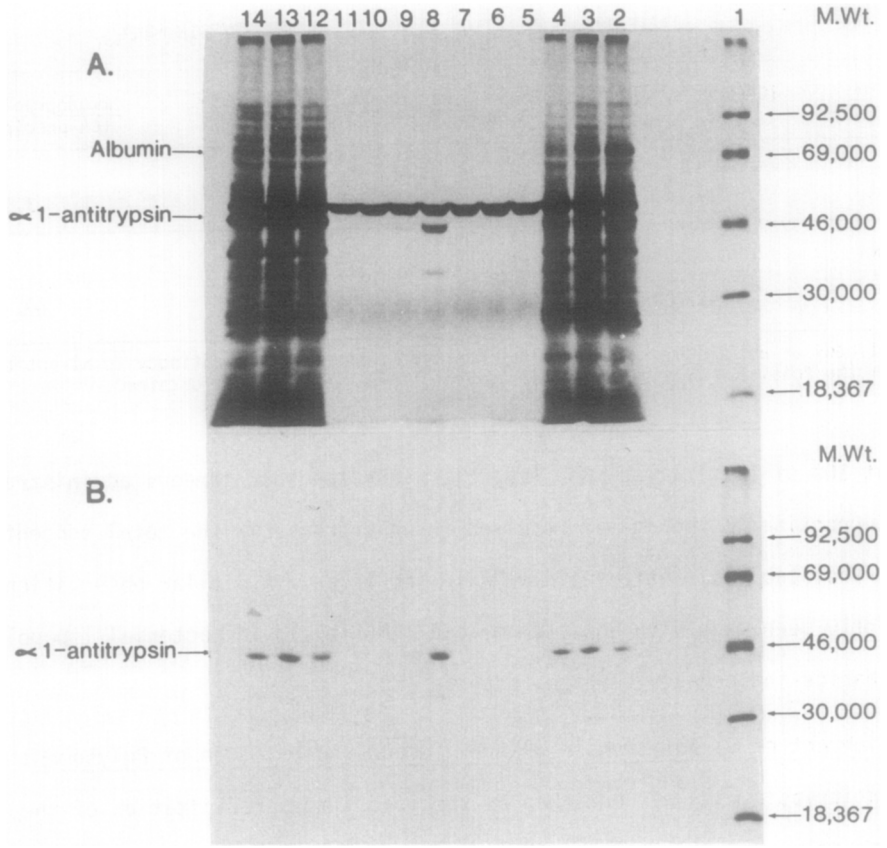

Figure 1: Analysis of αl-antitrypsin mRNA contents by cell free translation followed by SDS-polyacrylamide gel electrophoresis and fluorography. Panel A, total translation products; Panel B, translation products immunoprecipitated with a specific goat antibody to human αl-antitrypsin. Lane 1, [^{14}C]labeled protein markers; lanes 2, 3 and 4 are products synthesized in response to 1.0, 0.5 and 0.25 μg of total poly A-containing RNA from a normal baboon liver; lanes 5-11 are various elution fractions from an oligo dT-cellulose column after enrichment of αl-antitrypsin mRNA from total liver polysomes by specific immunoprecipitation; lanes 12, 13 and 14 are products synthesized in response to 0.25, 0.5 and 1.0 μg of total poly A-containing RNA from a turpentine-induced baboon liver.

band is immunoprecipitable with a specific antibody to αl-antitrypsin (Fig. 1B), there appears to be an increase of translatable αl-antitrypsin mRNA activity in the induced baboon liver. The difference in the content of αl-antitrypsin mRNA between the two baboon livers cannot be entirely incidental due to variation between different animals, since the αl-antitrypsin band generated from the induced baboon liver RNA is more prominent than the albumin band, which normally is the most abundant mRNA in the liver and constitutes

TABLE I: TRANSLATION ASSAY FOR α1-ANTITRYPSIN mRNA

RNA	TCA-Precipitable cpm	Immunoprecipitation cpm*	Immunoprecipitable cp / TCA-precipitable cpm
total liver polysome RNA	182,000	7,050	3.8%
enriched mRNA for α1-anti-trypsin	43,200	15,500	36%

*When no antibody or antithrombin III antibody was used instead of the antibody to α1-antitrypsin, 0.2-0.5% of the total TCA precipitable radioactivity ([^{3}H] leucine) was obtained.

about 10% of total liver mRNA (13,21). Thus the subcutaneous administration of turpentine to the animal resulted in an increase in the total concentration of translatable α1-antitrypsin mRNA in the liver. A similar observation has recently been made with hepatic amyloid mRNA levels in bacterial lipopolysaccharide-induced acute-phase response (22).

Enrichment of α1-antitrypsin mRNA by Immunoprecipitation of Polysomes: Alpha-1-antitrypsin mRNA was enriched by specific immunoprecipitation of the total liver polysomes of a turpentine-induced baboon. The extent of enrichment of the mRNA was estimated by cell-free translation. Incorporation of labeled amino acid into tricholoracetic acid-insoluble material represented total mRNA activity and α1-antitrypsin mRNA activity was determined by measuring the radioactivity that had been incorporated into antibody-precipitable material. As shown in Table I, 3.8% of total product synthesized with liver polysomal RNA was precipitated by the antibody to α1-antitrypsin. After enrichment by the polysome immunoprecipitation procedures, 36% of the product was precipitated by the antibody, indicating that a 10-fold enrichment of the specific mRNA had been achieved.

Total translation products as well as the specific immunoprecipitable material were also analysed by SDS-polyacrylamide gel electrophoresis followed by fluorography. In addition to the endogenous reticulocyte lysate band at 50,000 daltons, the presence of a single major band at 46,000 daltons and an

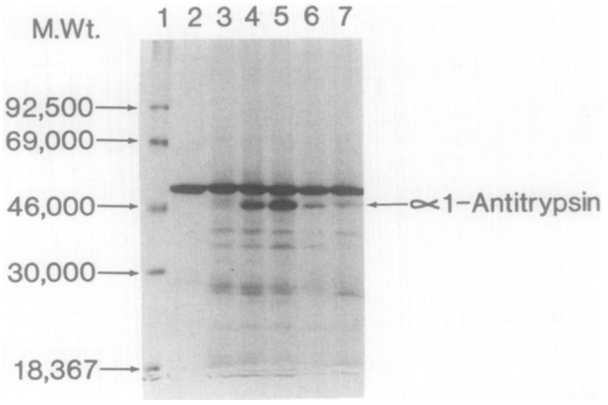

Figure 2: Hybrid-selected translation analysis of various cDNA clones by SDS-polyacrylamide gel electrophoresis and fluorography. Lane 1, [^{14}C]-labeled protein markers; lane 2, mock translation; lane 3, total translation products directed by RNA selected by hybridization with pBR322 DNA; lane 4, with pBα1a1 DNA; lane 5, with pBα1a3 DNA; lanes 6 and 7, with DNAs from non-specific clones.

additional minor band at about 35,000 daltons are evident (Fig. 1A, lane 8). Both of these bands are α1-antitrypsin since each is immunoprecipitable by the specific antibody (Fig. 1B, lane 8). The 35,000 dalton band is probably an incomplete translation product. Non-glycosylated human α1-antitrypsin has a molecular weight of 46,000 daltons. Judging from the profile of total protein products synthesized in the cell-free translation system, the purity of α1-antitrypsin mRNA in the polysome-enriched RNA preparation appeared to be greater than 90% by this assay.

Cloning of α1-antitrypsin cDNA and Its Identification: The α1-antitrypsin mRNA was employed for synthesis of its cDNA which was inserted into the Pst I site pf pBR322. Recombinants were identified by screening the transformants using [^{32}P]cDNA synthesized from the purified α1-antitrypsin mRNA preparation. Recombinants containing α1-antitrypsin DNA sequences were identified initially by hybrid-selected translation. Plasmid DNA isolated from several randomly-selected clones were immobilized and allowed to hybridize to completion with total baboon liver poly A-containing RNA. Hybridized RNA was eluted from the affinity resin by thermal denaturation and analysed in a cell-free translation system (Fig. 2). Using pBR322 as a control, the only promi-

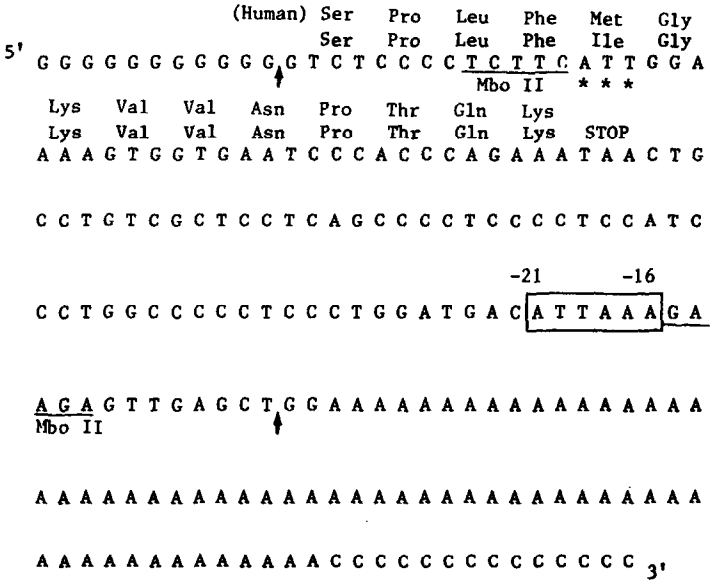

Figure 3: Nucleotide sequence of inserted DNA in pBaα1a1 and comparison of the deduced amino acid sequence to that of human α1-antitrypsin. The Mbo II recognition sequences are underlined and their respective cleavage sites are shown by arrows. The position where the ile-met substitution occurred between the two proteins is indicated by the asterisks, and the canonical ATTAAA hexanucleotide is boxed in.

nent band observed in the fluorogram was the 50,000 dalton reticulocyte lysate band (lane 3). Using RNA bound to pBaα1a1 DNA and pBaα1a3 DNA (lanes 4 and 5) however, there is obviously an additional α1-antitrypsin band, indicating that these two clones contain α1-antitrypsin DNA sequences.

In order to demonstrate unambiguously the fidelity of these clones, the nucleotide sequence of the inserted DNA in pBaα1a1 was determined. A comparison of the amino acid sequence deduced from the nucleotide sequence of the clone with the known amino acid sequence of human α1-antitrypsin (23) is shown in Fig. 3. Although the inserted DNA is rather short in this particular clone, it codes for 14 amino acids preceding the termination codon UAA, and the sequence matches perfectly with the amino acid sequence at the carboxyl terminus of human α1-antitrypsin. The only exception occurred at position number 10 from the carboxyl terminus of the protein, where an isoleucine residue (ATT)

in the baboon protein has been substituted by a methionine residue (ATG) in the human protein (Fig. 3). Comparison of the codons of the two amino acids has revealed an apparent T to G transversion at the third nucleotide position.

Although the α1-antitrypsin cDNA clone contains only a short DNA insert, it can be used as a specific hybridization probe for construction of long cDNA clone as well as for screening of a human genomic DNA library in order to identify clones containing the chromosomal α1-antitrypsin gene. The availability of these nucleic acid probes will provide the necessary tools for characterization of α1-antitrypsin deficiency at the gene level, and the development of analytical methods for prenatal diagnosis of the genetic disorder which is associated with pulmonary emphysema in man.

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