α_1-Antitrypsin and serum albumin mRNA accumulation in normal, acute phase and ZZ human liver

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Received 30 July 1985

α_1-Antitrypsin and albumin mRNA levels of 4 human livers were assessed using a newly sequenced cDNA clone of the carboxyterminal third of α_1-antitrypsin and a previously cloned albumin cDNA sequence. The relative concentration of α_1-antitrypsin mRNA was the same in poly(A)-containing RNA isolated from acute phase (MM) and α_1-antitrypsin deficient (ZZ) individuals. In the acute phase liver relative to the normal (MM) liver, total RNA extracts showed a marked decrease in albumin mRNA concentration but no increase in α_1-antitrypsin mRNA. The ZZ liver showed decreased total and poly(A)-containing RNA content but the same proportion of α_1-antitrypsin to albumin mRNA as in the normal (MM) liver. This supports other evidence that ZZ α_1-antitrypsin deficiency is due to a defect in polypeptide processing (secretion) rather than a deficiency in mRNA accumulation.

Z human α_1-antitrypsin *Acute phase protein* *Liver mRNA* *cDNA sequence α_1-antitrypsin*

1. INTRODUCTION

α_1-Antitrypsin is a plasma glycoprotein of M_r 51000 and has a prime function as an inhibitor of leukocyte elastase (see [1]). In humans, the plasma concentration of α_1-antitrypsin undergoes about a 3-fold increase in the acute phase state that accompanies severe trauma or active inflammation. Decreased concentrations of plasma α_1-antitrypsin levels (15% of normal) occur in homozygotes (PiZZ) for the severe, Z, deficiency allele, which differs from the normal M in the point mutation 342 Glu→Lys. In PiZZ individuals, hepatocytes contain aggregates of incompletely processed Z α_1-antitrypsin which accumulate in the secretory pathway at the level of the endoplasmic reticulum (see [1,2]). Affected individuals are prone to liver disease, in particular chronic active hepatitis and cryptogenic cirrhosis [3,4]. Studies using mRNA directed cell-free synthesis show that Z α_1-antitrypsin mRNA is translated in vitro as efficiently as normal (M) α_1-antitrypsin mRNA, and that the Z and M polypeptides undergo equivalent initial post- or co-translational addition of oligosaccharide side-chains [5,6]. Studies using Xenopus oocytes as a surrogate system to examine aspects of secretion show that, as in the human liver, secretion of the Z polypeptide is blocked in the secretory pathway prior to its entry into the Golgi apparatus [7,8]. Such observations imply that the reduced levels of plasma Z α_1-antitrypsin reflect a defect in the secretion of the synthesized protein, but they do not exclude the possibility that there may be a decrease in Z mRNA levels. It also remains to be established whether increased synthesis of α_1-antitrypsin in the acute phase reflects increased mRNA levels or increased utilization of existing mRNA (see [9]).

To examine the relative levels of α_1-antitrypsin mRNA in M and Z adult human livers and in liver

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

tissue from patients in acute phase, we have cloned and characterised a cDNA fragment representing part of the coding region and the complete 3′-untranslated region of human α_1-antitrypsin mRNA. Using this, and a human albumin cDNA probe, we have compared α_1-antitrypsin and albumin mRNA levels in RNA isolated from normal (PiMM), acute phase (PiMM) and PiZZ human liver tissue. The results highlight the problems associated with the comparative quantitation of mRNA levels in normal and diseased tissues, but indicate that the reduced levels of plasma α_1-antitrypsin associated with the Z allele are not due to a decrease in α_1-antitrypsin mRNA accumulation relative to normal tissue.

2. MATERIALS AND METHODS

2.1. *Materials*

All chemicals and enzymes were obtained from sources described elsewhere [10,11], [γ-^{32}P]ATP (3000 Ci/mmol) and [α-^{32}P]dCTP (400 Ci/mmol) were obtained from Amersham. Liver samples were obtained at autopsy within 1 h of death, snap-frozen in liquid N_2 then stored until required at −80°C. The α_1-antitrypsin specific oligonucleotide was a gift of Dr S. Humphries, and the human albumin cDNA clone, a gift of Dr Richard Lawn, Genentech, USA.

2.2. *Construction of human acute phase liver cDNA library*

Total RNA was isolated from frozen human liver, as described elsewhere [5], and poly(A)-containing RNA isolated using affinity chromatography on oligo(dT)-cellulose. The construction of cDNA libraries has been described in detail previously. Briefly, DNA complementary to human liver poly(A)-containing RNA was synthesized using *E. coli* DNA polymerase I (Klenow fragment), the double stranded cDNA (ds cDNA) treated with S_1 nuclease to remove the single-stranded hairpin loop, and ds cDNA greater than 400 bp then inserted into the *PstI* site of pAT153 using G-C tailing [10–14]. The resultant chimaeric plasmids were used to transform *E. coli* RRI. Screening in situ of 1000 colonies immobilised on nitrocellulose was carried out using an oligonucleotide (3′ GGACCTACTGTAATTTC 5′) complementary to the 3′ untranslated region of the α_1-antitrypsin mRNA (see [16]). The oligonucleotide was ^{32}P-labelled using T_4 polynucleotide kinase at 37°C for 30 min in a final volume of 27.5 μl in 50 mM imidazole-HCl, pH 6.6, containing 10 mM magnesium chloride, 5 mM DTT, 0.1 mM spermidine, 250 μM ADP and 3 μM [γ-^{32}P]ATP (3000 Ci/mmol) to a final specific activity of 2×10^7 cpm/μg. Nitrocellulose filters were prehybridized for 18 h at 50°C in 3 × SSC and 0.1% (w/v) SDS, then for 1 h at 37°C in 6 × SSC, 0.2% (w/v) SDS, 0.05% (w/v) sodium pyrophosphate, 5 × Denhardt's solution and yeast total RNA at 100 μg/ml. Hybridization was then carried out for 18 h at 37°C in 10 ml of 6 × SSC, 1 × Denhardt's, 0.05% (w/v) sodium pyrophosphate, 100 μg/ml yeast total RNA and ^{32}P-labelled oligonucleotide (10^7 cpm). Filters were washed at 37°C for 30 min with 4 changes of 6 × SSC and 0.05% (w/v) sodium pyrophosphate, using 200 ml/wash, then air dried, and autoradiographed for 18 h at −70°C using Kodak X-Omat RP film and Kodak Lanex Super Screens. Plasmid DNA was prepared from colonies giving a strong signal, and one, phAPL 511, containing the largest inserted cDNA sequence (460 bp), was chosen for nucleotide sequence analysis by the chemical cleavage method of Maxam and Gilbert [15]. Sequence analysis was performed on both strands across all restriction sites used for radiolabelling.

2.3. *RNA blotting*

RNA concentrations were determined using the orcinol reaction [17]. The size and relative distribution of α_1-antitrypsin and albumin mRNA in total RNA and poly(A)-containing RNA isolated from human liver samples was determined by RNA blotting after separation on formaldehyde gels as described elsewhere [10], using either nick-translated α_1-antitrypsin cDNA, or a human albumin cDNA clone (pF47, see [20]). Autoradiographs were scanned using a Quickscan Junior TLC scanning densitometer after different exposure times to ensure that in subsequent quantitative calculations signal intensity was proportional to relative mRNA concentration.

3. RESULTS

To investigate the relative levels of intact α_1-antitrypsin mRNA in the livers of individuals of

M and Z phenotypes, and in the acute phase state, we have constructed a small human cDNA library (6000 recombinants) using total poly(A)-containing RNA from an α_1-antitrypsin phenotype PiMM human liver obtained at autopsy from a patient with an acute phase reaction following trauma. Candidate α_1-antitrypsin cDNA clones were isolated using a synthetic oligonucleotide probe (see section 2.2), one of which, phAPL511, was shown by nucleotide sequence analysis (fig.1) to comprise the carboxyl terminal coding region (amino acids 268–394) and the 3′-untranslated region of the α_1-antitrypsin mRNA. The nucleotide and derived amino acid sequence was in agreement with other published work [18,19].

We have used this cloned α_1-antitrypsin cDNA sequence, and as a control, a cloned human albumin cDNA sequence [20], to investigate mRNA levels in total RNA and poly(A)-containing RNA prepared from 4 human liver samples. Three of these were from individuals of MM phenotype. One (A.W.) was in severe acute phase, and another in mild acute phase (G.W.) as judged by elevated plasma C-reactive protein levels (table 1), a well characterised acute phase reactant (see [21]). The third (W.P.) was normal by clinical criteria (table 1).

In the acute phase individual, plasma α_1-antitrypsin and orosomucoid levels were also elevated, plasma albumin remained in the normal range, whilst prealbumin and transferrin levels were decreased when compared with the normal and mild acute phase plasma levels. Clinical parameters on the ZZ patient (F.K.) were less complete. The patient had decreased plasma α_1-antitrypsin, but was not in acute phase as judged by normal levels of other serum proteins (table 1). Immunocytochemical examination of the liver revealed numerous and large intracellular inclusions of α_1-antitrypsin involving 25% of hepatic cells. Yields of total and poly(A)-containing RNA varied considerably between liver samples. A high yield was obtained from acute phase liver (A.W.), but much reduced levels (2–3-fold) from the ZZ tissue (F.K.) (table 1).

Initial investigations compared by RNA blotting (fig.2A), the relative concentration of α_1-antitrypsin mRNA in poly(A)-containing RNA isolated from the acute phase tissue (A.W.) and the ZZ tissue (F.K.). In each preparation a single RNA species was observed of about 1400 nucleotides in length. Surprisingly, in spite of the 13-fold difference in plasma α_1-antitrypsin levels between the two patients, and the 3-fold decrease in yield of the poly(A)-containing RNA isolated from the ZZ tissue compared with the acute phase MM (table 1), relative α_1-antitrypsin mRNA concentrations differed only marginally. Quantitation by densitometry showed a 30% decrease in α_1-antitrypsin mRNA levels in the acute phase when compared

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        270                                     280                                     290
    Thr His Asp Ile Ile Thr Lys Phe Leu Glu Asn Glu Asp Arg Arg Ser Ala Ser Leu His Leu Pro Lys Leu Ser Ile Thr Gly Thr
5'- ACC CAC GAT ATC ATC ACC AAG TTC CTG GAA AAT GAA GAC AGA AGG TCT GCC AGC TTA CAT TTA CCC AAA CTG TCC ATT ACT GGA ACC
    1                                                   50

            300                                     310                                     320
    Tyr Asp Leu Lys Ser Val Leu Gly Gln Leu Gly Ile Thr Lys Val Phe Ser Asn Gly Ala Asp Leu Ser Gly Val Thr Glu Glu Ala
    TAT GAT CTG AAG AGC GTC CTG GGT CAA CTG GGC ATC ACT AAG GTC TTC AGC AAT GGG GCT GAC CTC TCC GGG GTC ACA GAG GAG GCA
                100                                                     150

                330                                     340                                     350
    Pro Leu Lys Leu Ser Lys Ala Val His Lys Ala Val Leu Thr Ile Asp Glu Lys Gly Thr Glu Ala Ala Gly Ala Met Phe Leu Glu
    CCC CTG AAG CTC TCC AAG GCC GTG CAT AAG GCT GTG CTG ACC ATC GAC GAG AAA GGG ACT GAA GCT GCT GGG GCC ATG TTT TTA GAG
                                                                                            250

                        360                                     370                                     380
    Ala Ile Pro Met Ser Ile Pro Pro Glu Val Lys Phe Asn Lys Pro Phe Val Phe Leu Met Ile Glu Gln Asn Thr Lys Ser Pro Leu
    GCC ATA CCC ATG TCT ATC CCC CCC GAG GTC AAG TTC AAC AAA CCC TTT GTC TTC TTA ATG ATT GAA CAA AAT ACC AAG TCT CCC CTC
                                            300

                    390
    Phe Met Gly Lys Val Val Asn Pro Thr Gln Lys Stop                                                    Probe
    TTC ATG GGA AAA GTG GTG AAT CCC ACC CAA AAA TAA CTGCCTCTCGCTCCTCAACCCCTCCCCTCCATCCCTGGCCCCCTCCCTGGATGACATTAAAGAAGGG
    350                                             400                                                 450

    TTGAGCTGG   Poly A -3'
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Fig.1. Nucleotide sequence of human α_1-antitrypsin cDNA cloned into phAPL511.

Table 1

Donors		F.K.	W.P.	G.W.	A.W.
Sex/age		F 46	M 20	M 18	M 14
Phenotype		ZZ	MM	MM	MM
	Normal range				
α_1-AT (% normal pool)	75–125	18	54	113	240
Orosomucoid (% normal pool)	75–125	100	43	94	220
Haptoglobin (% normal pool)	60–140	100	16	65	115
Albumin (g/l)	35–53	35	25	30	30
C-reactive pool (mg/l)	10	N.D.	8	75	350
Prealbumin (g/l)	0.2–0.3	0.17	0.13	0.14	0.10
Transferrin (g/l)	2.0–3.0	2.9	1.7	1.6	1.1
RNA (mg/g liver)	–	0.30	0.95	0.97	1.44
Poly(A)$^+$ RNA (μg/g liver)	–	20			60
Relative α_1-AT mRNA content per unit RNA	100%	10	100	90	62
Relative albumin mRNA content per unit RNA	100%	10	100	68	16

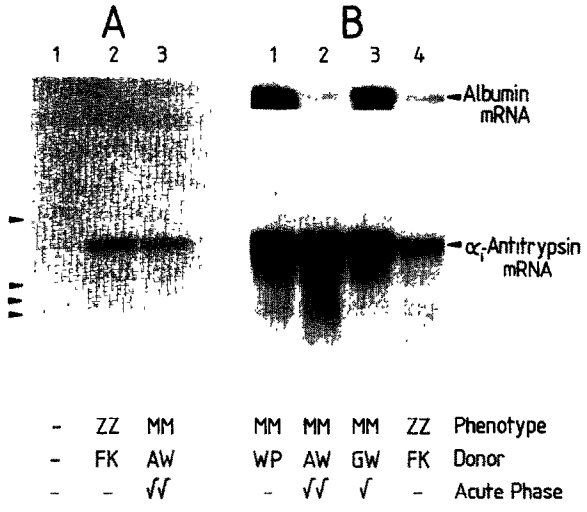

Fig.2. Accumulation of α_1-antitrypsin and albumin mRNA in normal, acute phase and ZZ human liver. (A) Poly(A)-containing RNA isolated from human placenta (1 μg, tract 1), ZZ human liver (1 μg, tract 2) and acute phase human liver MM (1 μg, tract 3) were separated by electrophoresis under denaturating conditions on a 1.0% (w/v) agarose gel, then blotted onto a Pall Biodyne nylon membrane and the α_1-antitrypsin mRNA identified by hybridization to a ^{32}P-labelled phAPL511 cDNA probe (spec. act. 2.5 × 10^8 cpm/μg, exposure time 15 min). Arrows indicate the relative mobility of DNA fragments of: 1631, 517, 396 and 298 nucleotides electrophoresed in parallel. (B) Total RNA from 4 human liver samples were separated by electrophoresis (5 μg each) and the relative amounts of α_1-antitrypsin mRNA present in each determined by blotting as described above, using a ^{32}P-labelled phAPL511 α_1-antitrypsin cDNA probe (spec. act. 3 × 10^8 cpm/μg, exposure time 60 min). The membrane was then washed in 90% formamide at 37°C for 2 h to remove the hybridized cDNA, and then reprobed using a ^{32}P-labelled pF47 albumin cDNA probe (spec. act. 8 × 10^7 cpm/μg, exposure time 60 min) see inset. MM phenotype (tract 1), MM acute phase (tract 2), MM mild acute phase (tract 3) and ZZ phenotype (tract 4).

with ZZ tissue. No α_1-antitrypsin mRNA was present in placental poly(A)-containing RNA run in parallel. As an extension of these observations we have investigated the relative levels of α_1-antitrypsin and albumin mRNA in total RNA preparations isolated from the 4 tissues described. This demonstrated (fig.2B), in contrast to the results obtained using poly(A)-containing RNA, variation in the relative concentration of α_1-antitrypsin mRNA levels between different preparations. In particular the relative concentration of α_1-antitrypsin RNA in the ZZ phenotype (F.K.) was 5-fold lower than the normal (W.P.). Both acute phase and mild acute phase preparations had reduced α_1-antitrypsin mRNA levels when compared with the normal (fig.2B and table 1). Reanalysis of the same filter using the albumin cDNA probe showed equally contrasting results. Relative albumin mRNA levels in the acute phase tissue were 6-fold lower than in the normal tissue, whilst in the mild acute phase, a slight decrease was also apparent (fig.2B and table 1). However, in the ZZ tissue (F.K.), in keeping with α_1-antitrypsin mRNA result, the relative albumin mRNA levels were 10-fold lower, when compared with the normal tissue (W.P.).

4. DISCUSSION

The data we have obtained have a number of interesting facets. On the basis of Northern blot analysis in the acute phase liver, albumin mRNA levels decrease about 5-fold, but there is little evidence to suggest that the increase in plasma α_1-antitrypsin is a result of increased mRNA levels. Decreased albumin synthesis and mRNA levels, and increased acute phase protein synthesis have previously been reported in acute phase murine liver [22]. Decreased albumin synthesis, paralleled by increased synthesis of acute phase proteins, has been described in acute phase rat liver [9]. Thus in humans, in common with animal model systems in the acute phase state, decreased plasma albumin levels are a consequence of decreased liver albumin mRNA levels. However, the results obtained here suggest that the increased plasma levels of α_1-antitrypsin in the acute phase state, induced by trauma, is not a result of increased α_1-antitrypsin mRNA accumulation and presumably, therefore, reflects an increased rate of protein synthesis. These results should be interpreted with caution as the acute phase response is short lived with the plasma changes occurring on a slower and later timescale, i.e. the α_1-antitrypsin mRNA may have already fallen to normal after a transient rise in concentration. This interpretation would fit with studies on chemically induced acute phase and normal baboon liver, which report that increased α_1-antitrypsin synthesis is mediated through an increase in steady state levels of cellular mRNA [25].

Interpretation of the data obtained from the comparison of tissue from PiMM and PiZZ individuals is more complex. PiZZ liver contained reduced amounts of total RNA when compared with PiMM liver, the yield of total RNA was 3–4-fold lower than the normal MM phenotype tissue examined. Since a good correlation exists between the ZZ phenotype and liver disease, in particular cirrhosis [3,4,20,24], it seems likely that lower RNA yields associated with PiZZ liver tissue reflect the extent of cell damage and loss due to cirrhosis. This would explain the apparent disparity in relative α_1-antitrypsin concentrations when comparing poly(A)-containing RNA and total RNA isolated from PiZZ (F.K.) and PiMM (A.W.) tissue. The equivalence of α_1-antitrypsin mRNA concentration in the poly(A)-containing RNA populations, will reflect synthesis only by healthy hepatocytes in PiZZ and PiMM tissue, whilst the relative concentration of α_1-antitrypsin mRNA in total RNA reflects a contribution from healthy and cirrhosed tissue. The paralleled decrease in albumin mRNA in the PiZZ tissue validates this argument since both proteins are produced together in the same hepatocytes (Bathurst, I.C. and Lorier, M., unpublished). Thus total α_1-antitrypsin mRNA levels in PiZZ tissue are reduced when compared to normal PiMM liver tissue as are albumin mRNA levels, but the relative concentration of antitrypsin mRNA and albumin mRNA in viable hepatocytes in the PiZZ liver tissue, probably differs little from hepatocytes in the PiMM tissue.

We conclude that decreased circulating plasma levels of α_1-antitrypsin in the PiZZ individual are not due to a defect in mRNA accumulation in healthy hepatocytes, but must reflect in part a defect in secretion of α_1-antitrypsin, since plasma albumin levels are maintained in spite of the paralleled decrease in the relative concentration of α_1-antitrypsin and albumin mRNA when compared with normal tissue. Recent analysis of total RNA isolated from liver tissue of a 7 year old female PiZZ patient (see [7]) confirmed this view (Riley, J.H. and Craig, R.K., unpublished).

ACKNOWLEDGEMENTS

This work was supported in part by the National Children's Health Research Foundation and the Medical Research Council of New Zealand. We thank Dr Richard Lawn, Genentech for the gift of the albumin cDNA clone.

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