In vitro synthesis of M and Z forms of human α_1-antitrypsin

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mRNA was prepared from autopsy liver samples from a homozygote for α_1-antitrypsin deficiency (*PiZZ*) and from a normal (*PiMM*) subject. Both preparations gave equivalent synthesis of α_1-antitrypsin in a wheat germ cell-free system. This suggests that the deficiency of plasma α_1-antitrypsin associated with the Z variant is due to a failure of processing and secretion of the protein rather than of its synthesis. It is likely that it is the resultant intracellular accumulation of the Z protein rather than a deficiency of protease inhibitor that is the primary cause of the liver pathology associated with this variant.

α_1AT synthesis *α1AT deficiency* *Human liver mRNA*

1. INTRODUCTION

Some 1:2000 Northern Europeans are homozygotes [1] for the Z variant of the plasma protease inhibitor, α_1-antitrypsin (α_1AT). These homozygotes are denoted as genotype PiZZ as compared to the homozygote for the normal M gene, *PiMM*. The Z abnormality results in both an intrahepatic accumulation of α_1AT and a gross decrease in its concentration in the plasma. Clinically, the major consequence for the *PiZZ* homozygote is a predisposition to premature emphysema but there is also an appreciable occurrence of liver disease, particularly in the neonate. It has been suggested that this liver disease results from the lowered levels of inhibitor [1] but we believe [2] that it is more likely to be due to the accumulation of Z α_1AT within the hepatocyte. This implies that the abnormal Z protein is synthesized at the same rate as the normal M protein but that a partial blockage occurs in the subsequent processing and secretion of the Z α_1AT. Support for this is provided by studies reported here that show equivalent in vitro synthesis of α_1AT using mRNA extracted from liver of a normal (*PiMM*) and an abnormal (*PiZZ*) individual.

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2. MATERIALS AND METHODS

Liver samples were obtained at autopsy within 1 h of death and immediately frozen in liquid nitrogen and stored at −80°C. Donors:

(i) Female died aged 45 of CO_2 narcosis due to advanced emphysema. α_1AT phenotype PiZ by 2-dimensional agarose electrophoresis, repeated plasma concentration of 18% of normal pool indicating genotype *PiZZ*. There was no history of previous jaundice or liver disease. There was no evidence of a current acute phase reaction, nor had she been on oestrogen medication. The liver had normal gross appearance, microscopically there was some portal tract fibrosis with numerous large intracellular inclusions of α_1AT confirmed by immunofluorescence.

(ii) Male aged 14 died from multiple injuries 4 days after a road traffic accident; previously in good health; α_1AT phenotype PiM; plasma concentration 240% of normal pool indicated genotype *PiMM* and was compatible with a brisk acute phase reaction following trauma. The liver had normal appearance and microscopy.

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Oligo(dT)-cellulose was from Sigma (St Louis MO), L-[4,5-^{3}H]leucine (136 Ci/mmol) from Amersham International (Bucks), L-[^{35}S]methionine (1021.4 Ci/mmol) from New England Nuclear (Waltham MA), rabbit antisera to human α_1AT was from DAKO (Copenhagen), goat anti-rabbit serum was from Wellcome (Beckenham).

Liver mRNA was isolated using a technique modified from that in [3,4]. The poly(A)$^+$ RNA was isolated using oligo(dT)-cellulose affinity chromatography. Typically, ~1400 A_{260} total nucleic acid was obtained from 10 g tissue and from this 3–11 A_{260} units of poly(A)$^+$ RNA (mRNA) was isolated.

M and Z mRNA samples were translated in a wheat germ cell-free system, prepared as in [5] and the assay conditions were similar to those in [6]. Synthesis was performed in a final volume of 50 μl or 100 μl at 21 °C for 90 min. Either L-[^{35}S]methionine or L-[4,5-^{3}H]leucine was employed as a radiolabel, at 1–10 μCi/assay. Total mRNA activity was measured by trichloroacetic acid precipitation of polypeptides, then liquid scintillation counting. The presence of α_1AT was determined by immunoprecipitation using a double antibody technique modified from that in [7]. If L-[4,5-^{3}H]leucine was the radiolabel, the pellets were solubilized with NCS before adding toluene-based scintillation fluid.

Electrophoresis utilised 10–20% SDS–polyacrylamide vertical slab gels [8] for fluorography [6] gels were impregnated with 20%, 2,5-diphenyloxazole (PPO).

3. RESULTS

Incubation of the mRNA prepared from both the normal (*PiMM*) and abnormal (*PiZZ*) livers in a wheat germ cell-free system resulted in the synthesis of a number of proteins as expected from the translation of heterogeneous liver mRNA (fig.1). The presence in each instance of α_1AT was shown by a single band on gel electrophoresis after antibody precipitation. Confirmation that the synthesis was mRNA-dependent was given by the dose–response curves shown in fig.2a. Both the Z and M liver extracts gave essentially the same synthesis of α_1AT in relation to the total amount of RNA per assay (fig.2b).

In repeated experiments, α_1AT consistently formed 1.5–3.0% of the cell-free translation prod-

Table 1

Percentage of total translation products precipitated with α_1-antitrypsin antibodies

Liver mRNA	Experiment 1	Experiment 2	Experiment 3	Experiment 4
PiMM	2.5%	3.1%	1.7%	2.0%
PiZZ	2.2%	2.9%	1.4%	1.7%

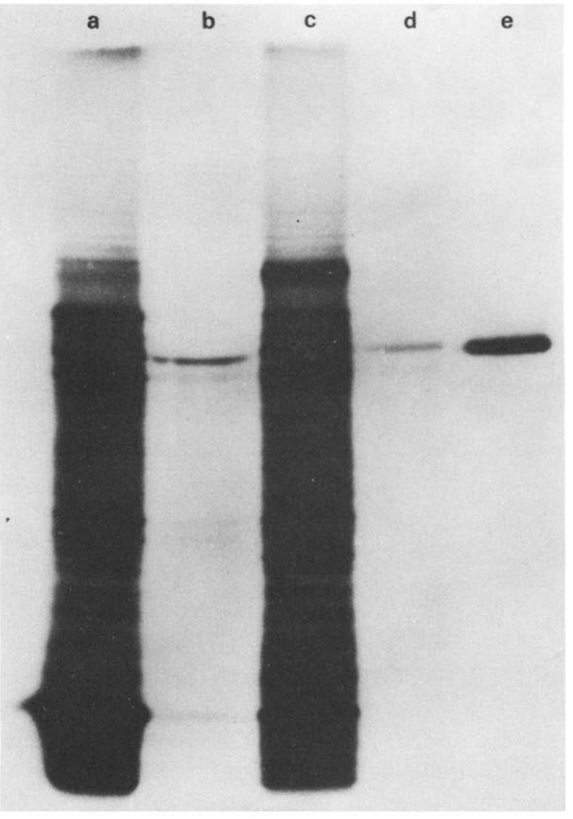

Fig.1. Autoradiograph of polyacrylamide gel electrophoresis of wheat germ incubates. Incubates with mRNA from the MM liver (a) and the ZZ liver (c) gave similar translation products, the differences in intensity being those expected of acute and non-acute phase proteins. (b) (MM) and (d) (ZZ) show single products obtained by α_1-antitrypsin–antibody precipitation of samples of each of the two incubates. (e) is a marker of plasma α_1-antitrypsin (during storage the glycosylated protein is subjected to degradation which lowers its M_r to 47 000 approximating that of the unprocessed polypeptide). A control lane (not shown) without added mRNA gave no visible activity.

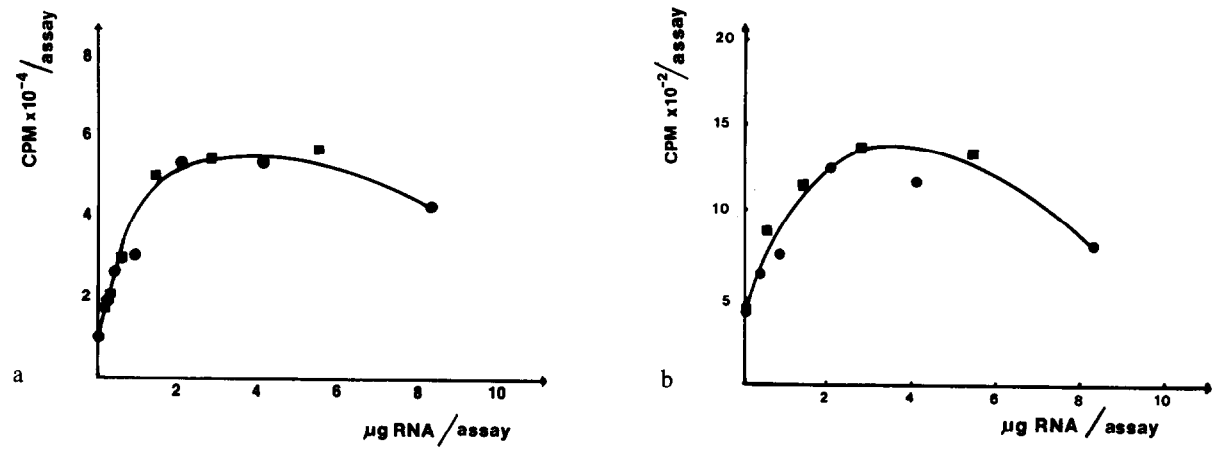

Fig.2. Wheat germ cell-free synthesis using extracted liver mRNA: MM liver extract (•); ZZ liver extract (▪). Increasing amounts of liver mRNA were added to fixed volumes of the assay system. (a) The total incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable polypeptides. There is a characteristic saturation concentration and an identical stimulation of synthesis by both MM and ZZ liver mRNA. (b) The incorporation of [^{3}H]leucine into antibody-precipitable α_1-antitrypsin. There is again no difference between the MM and ZZ mRNA preparations implying that the level of α_1AT mRNA is the same in both livers.

ucts. The figures for the two mRNA extracts were similar, but that from the M was always slightly higher (table 1).

4. DISCUSSION

The structure and abnormalities of α_1AT have recently been reviewed [9]. The common Z variant results in a plasma concentration in the *PiZZ* homozygote of some 15% of that of the *PiMM* normal. Studies of the circulating half-life of the Z protein have excluded an increased rate of removal from the plasma as a significant contributor to this deficit [10]. The fault must therefore be due to either decreased synthesis or decreased secretion. Here, we have used the wheat germ system to examine the first step in synthesis of the protein; the mRNA-directed formation of the unprocessed polypeptide. The results obtained show equivalent synthesis of the Z and M polypeptides. The slightly increased production of the M protein (table 1) is in keeping with the acute phase state of the *PiMM* donor who died shortly after major trauma and was observed to have a raised plasma α_1AT concentration.

The conclusions from these results, that the deficiency of the Z protein is due not to its decreased synthesis but to a defect in subsequent processing and secretion of the polypeptide, is in keeping with other findings. These show that although some of the Z antitrypsin is secreted [11] in its fully glycosylated form [12] into plasma, some of it also accumulates in the hepatocyte at the junction of the rough and smooth endoplasmic reticulum [13]. This material has an immature carbohydrate composition [14,15], is relatively insoluble and will not be detectable by standard electrophoresis [11]. Presumably, the initial glycosylation of the Z polypeptide has occurred but not the final pruning and modification of carbohydrate sidechains that normally occurs just prior to secretion.

It seems probable that the liver complications of α_1AT deficiency are a consequence of the intracellular accumulation of the abnormal protein, since they are usually seen only with those variants that give hepatic inclusions [2]; i.e., PiZ rather than PiS or PiNull. Since the Z variant is present in the plasma at 15% of the concentration of the M, the results from this paper imply that 85% of the product of the Z allele is not secreted and will require disposal by the cell. This must impose a stress on the ZZ hepatocyte.

An important practical point arises from the reported therapeutic use of danazol [16] to give

raised plasma concentrations of α_1AT. This will cause increased synthesis of α_1AT, but as well as giving increased secretion can also be expected to increase the load of accumulated protein with a consequent increased risk of liver pathology in the *PiZZ* individual.

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