A Naturally Occurring Non-Polymerogenic Mutant of α1-Antitrypsin Characterized by Prolonged Retention in the Endoplasmic Reticulum

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Running Title: ER Retention of Mutant α1-AT Saarbrucken

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Summary

The classical form of α1-AT deficiency is associated with a mutant α1-ATZ molecule which polymerizes in the endoplasmic reticulum of liver cells. A subgroup of individuals homozygous for the PI Z allele develop chronic liver injury and are predisposed to hepatocellular carcinoma. In this study we evaluated the primary structure of α1-AT in a family in which three affected members had severe liver disease associated with α1-AT deficiency. We discovered that one sibling was a compound heterozygote with one PI Z allele and a second allele, the PI Z + saar allele, bearing the mutation that characterizes α1-ATZ as well as the mutation that characterizes α1-AT saarbrucken (α1-AT saar). The mutation in PI saar introduces a premature termination codon resulting in an α1-AT protein truncated for 19 amino acids at its carboxyl terminus. Studies of a second sibling with severe liver disease and other living family members did not reveal the presence of the α1-AT saar mutation and therefore do not substantiate a role for this mutation in the liver disease phenotype of this family. However, studies of α1-AT saar and α1-ATZ + saar expressed in heterologous cells show that there is prolonged intracellular retention of these mutants even though they do not have polymerogenic properties. These results therefore have important implications for further understanding the fate of mutant α1-AT molecules, the mechanism of ER retention and the pathogenesis of liver injury in α1-AT deficiency.
Introduction

A subgroup of patients (10%) with the classical form of α1-AT deficiency (homozygous for the PI Z allele) are predisposed to chronic hepatitis, cirrhosis and hepatocellular carcinoma. Because the incidence of homozygous α1-AT deficiency in Caucasian populations is relatively high (1 in 1600 to 1 in 2000 live births), this deficiency constitutes the most common genetic cause of liver disease in children and the most frequent genetic diagnosis for which children undergo liver transplantation (reviewed in 1). Deficiency of α1-AT is also associated with premature development of destructive lung disease/emphysema (1).

The PI Z allele is characterized by a single nucleotide substitution which results in the substitution of lysine for glutamate at amino acid 342 (2-4). The mutant α1-ATZ protein is retained in the endoplasmic reticulum (ER) of liver cells rather than secreted into the blood and body fluids (5,6). Circulating blood levels of α1-AT in deficient patients reach 10-15% of the levels present in the general population. Studies by Lomas and Carrell have shown that the substitution of lysine for glutamate 342 restricts the mobility of the reactive-site loop of the α1-AT molecule and brings out a tendency for α1-AT molecules to polymerize in the ER (7-9). Studies which show that the secretory defect is partially reversed by introducing another mutation which counteracts the polymerogenic properties of the α1-AT molecule (10-12) provide evidence that the polymerization is, at least in part, responsible for the retention of α1-ATZ in the ER.

Emphysema is caused by lack of α1-AT in the lung, permitting neutrophil elastase to destroy the connective tissue matrix (reviewed in 13,14). In contrast to this loss-of-function mechanism for lung injury, liver disease is thought to involve a gain-of-function mechanism wherein the retention of the mutant polymerized α1-ATZ protein in the ER invokes damage to
the affected liver cells (reviewed in 15) but the mechanism by which retention of this aggregated glycoprotein elicits liver injury is unknown. By expressing mutant α1-ATZ in skin fibroblasts from α1-AT deficient patients with liver disease (“susceptible hosts”) and comparing them to fibroblasts from α1-AT deficient individuals without liver disease (“protected hosts”) we have found that there is more efficient degradation of the mutant α1-ATZ molecule in the ER of protected hosts. These results have provided evidence for the concept that genetic/environmental mechanisms which impair the degradation pathways that constitute the quality control apparatus of the ER increase susceptibility to liver disease in α1-AT-deficient individuals (16).

In this study we examined a family with two sibs having severe liver disease associated with homozygous α1-AT deficiency. The mother had died of presumed idiopathic liver disease many years earlier (α1-AT phenotype unknown). We found that one of the sibs was a compound heterozygote, with one PI Z allele and one allele with both the substitution that characterizes the PI Z allele and the substitution that characterizes the PI saarbrucken (saar) allele. Although the studies of this family do not substantiate the idea that the PI saar allele is associated with liver injury, studies of the intracellular fate of this mutant have important implications for understanding the mechanism of liver disease in α1-AT deficiency.
Experimental Procedures

Patients. The index patient, patient #1, developed GI bleeding at 48 years of age. She was found to have esophageal varices and a diagnosis of α1-AT deficiency with a PI Z genotype and blood levels of α1-AT that were 8% normal. She initially underwent variceal sclerotherapy but later required a distal splenorenal shunt at age 50 for recurrent variceal bleeding. Symptoms of chronic liver failure necessitated a liver transplant at age 56 years. There was no history of significant alcohol exposure. The native liver was characterized by cirrhosis and PAS positive, diastase-resistant globules in liver parenchymal cells.

Her brother, patient #2, was discovered to have splenomegaly at age 38 years. For unknown reasons he underwent a splenectomy shortly thereafter. At age 41 years he developed intestinal bleeding and was found to have esophageal and gastric varices. He was found to be homozygous for PI Z allele with blood α1-AT levels 11% normal. He underwent a gastric devascularization procedures at that time. A liver biopsy showed chronic hepatitis with PAS-positive, diastase-resistant globules in liver cells. He had recurrent gastrointestinal bleeding, developed hepatic encephalopathy and died at age 46 after declining liver transplantation. He was not known to have much in the way of alcohol exposure. The mother of patients #1 and #2 died from severe liver failure of unknown etiology. There was no history of alcohol use for the mother. Another brother (patient #3) and two other sisters (patients #4 and #5) were available for further studies.

Sequence analysis. For the index patient, patient #1, total cellular RNA was isolated from her native liver by guanidine isothiocyanate extraction and ethanol precipitation (17). Poly A + RNA was purified with Oligotex Column Kit (QIAGEN) and then subjected to RT-PCR using primer A1-1 (sense) -- 5’CTGTCTCCTCAGCTTCAGGCA-3’ and primer A1-1A
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(antisense) -- 5’ATCACATGCAGGAGGACCA-3’. The 1.4 kb PCR fragment was gel purified and subjected to direct sequence analysis. This PCR fragment was also subcloned into the pGME-T vector so that the sequence of each of the two α1-AT alleles could be determined. A total of eight subclones were subjected to sequence analysis, confirming the presence of compound heterozygosity with four subclones having identical sequence for each of the two alleles.

Genomic DNA was isolated from skin fibroblasts of patient #2 and from blood of patients #3, 4, and 5 with the DNeasy Tissue Kit (QIAGEN). This DNA was subjected to PCR for exon V using primer A1-G5 (sense) -- 5’-CTGGGATCAGCCTTACAACGTGT-3’ and A1-GR (antisense) -- 5’-TTCCCATGAAGAGGGGAGACTTG-3’. A 210-base pair PCR product was gel purified for direct sequencing. To confirm the mutations, sequencing reactions in both directions were analyzed.

**Cell lines, metabolic labeling, immunoprecipitation, SDS-PAGE.** Overlap PCR methods (18) were used to generate α1-AT saar with wild type α1-AT cDNA (16) as template and to generate α1-ATZ + saar with mutant α1-ATZ cDNA (16) as template. The inner primer pair was sense: 5’-TCAGTCCCTTTTCTCGATGGT-3’ and antisense: 5’-TCAGTCCCTTTTCTCGATGGT-3’. The outer primer pair was sense: 5’-CTGACTAGTGACAGTGAATCGAC-3’ and antisense: 5’-GACTAGTTGAGGAGCGAGG-3’. Direct sequence analysis of each cDNA showed the presence of the intended mutation without any unintended mutation. These cDNA constructs were subcloned into the pRc/RSV expression vector (Invitrogen, Carlsbad, California) to generate heterologous cell lines with stable expression of human wild type α1-AT, mutant α1-ATZ, mutant α1-AT saar and mutant α1-ATZ + saar. Chinese hamster ovary (CHO K1) cells
were separately transfected by the calcium phosphate DNA-precipitation method with each of these vectors and candidate clones selected in G418. For pulse labeling, each of these cell lines was incubated at 37°C for 3 hr in Trans³⁵S label (250 µcl/ml DMEM lacking methionine). For pulse-chase radiolabeling, each of the cell lines was incubated at 37°C for 1 hr in Trans³⁵S label (250 µcl/ml DMEM lacking methionine) for the pulse period, rinsed extensively and then incubated at 37°C in complete DMEM for the chase period. At specified time intervals extracellular (EC) medium was harvested and cells (IC) lysed in PBS/1% Triton X-100/0.5% deoxycholic acid/2mM phenylmethylsulfonic acid. EC and IC samples were clarified, immunoprecipitated and immunoprecipitates analyzed by SDS-PAGE/fluorography exactly as described previously (16,19). Aliquots of each sample were subjected to trichloroacetic acid precipitation to ensure that there were equivalent levels of incorporation of radiolabel in each cell line at time 0 IC. Separate gels were also subjected to PhosphorImager (Molecular Dynamics) analysis.

For Western blot analysis cell were lysed in PBS/1% Triton X-100/0.5% deoxycholic acid/2mM phenylmethylsulfonic acid and cell lysates subjected to SDS-PAGE directly or to immunoprecipitation followed by SDS-PAGE. Blots were incubated with biotinylated goat-antihuman α1-AT, then with peroxidase labeled strepavidin and developed with the Supersignal substrate (Pierce Chemicals, Rockford, IL).

**Preparation of soluble and insoluble fractions from cell lysates.** Cell lysates were passed through a 25-gauge needle ten times on ice (20). Insoluble material was recovered by centrifugation at 16,000 xg for 15 minutes. Pellets were solubilized in 50 µl 50 mM Tris-HCl, pH 6.8, 5% SDS, 10% glycerol with 1 minute of sonication followed by 10 minutes of boiling.
**Immunofluorescence.** Cells were plated on glass slides, fixed, permeabilized and stained with anti-α1-AT IgG as primary and rhodamine-conjugated anti-Ig as secondary and anti-GRP78/BiP Ig as primary with Cy2-conjugated anti-Ig as secondary, exactly as previously described (16). Cells stained with primary or secondary antibody alone were included as controls for background.
Results

Identification of the PI Z + saar allele. Analysis of the coding sequence of the α1-AT gene in the index patient, patient #1, revealed on one allele the substitution of E342 GAG to K342AAG, the substitution which characterizes the PI Z allele (Figure 1). On the other allele there was the substitution of E342GAG to K342AAG and a nucleotide insertion at the E363 codon. The latter insertion results in a frameshift with premature termination at codon 376 and truncation of 19 amino acids from the carboxyl terminus (Figure 2). Because this allele had the A213 polymorphism it is classified as the α1-AT null saarbrucken variant. Thus the index patient had one PI Z allele and one PI Z + saar allele. Analysis of the complete coding sequence of the α1-AT gene for the brother, patient #2, who died from liver failure, revealed the E342GAG to K342AAG substitutions on both alleles, indicating that he is homozygous for PI Z. Analysis of the coding region of exon V of the α1-AT gene for patients #3, 4, and 5 revealed E342GAG on one allele and K342AAG on the other allele, indicating that they are PIM Z heterozygotes (Figure 3). There was no evidence for the nucleotide insertion that characterizes the PI saar allele in patients #2, 3, 4, or 5.

Fate of α1-AT saar and α1-ATZ + saar in transfected CHO cells. To determine the fate of these mutants, we generated stable transfected cell lines which express wild type α1-AT, α1-ATZ, α1-AT saar and α1-ATZ + saar. First, we subjected these cell lines to continuous pulse labeling to determine the relative electrophoretic mobility in the cells (IC) and the cell culture fluid (EC) (Figure 4). The results show in IC for wild type α1-AT (M) the presence of ~52 and 55 kD polypeptide. These correspond to the partially and fully glycosylated intracellular intermediates, respectively, that are usually observed for wild type α1-AT. The relative electrophoretic migration of the fully glycosylated 55 kD polypeptide corresponds with
that of mature α1-AT found in the EC fluid (center panel). For α1-AT saar there is a single
50 kD polypeptide, reflecting faster electrophoretic migration due to carboxyl terminal
truncation. For α1-ATZ a single 52 kD polypeptide reflecting the partially glycosylated
intermediate, accumulates in the IC. For α1-ATZ + saar a single truncated 50 kD polypeptide is
present. The greater intensity of α1-AT saar, α1-ATZ, and α1-ATZ + saar than wild type α1-
AT M suggests the possibility that there is intracellular accumulation of all these mutants. The
results of the EC samples show a marked decrease in the amount of the mutants, suggesting
defective secretion. Longer fluorographic exposure of the EC (right panel) shows a trace amount
of a slightly faster migrating polypeptide for saar, a little bit more of the 55 kD polypeptide for Z
but no detectable polypeptide for Z + saar.

Next we examined the kinetics of secretion of α1-AT in these cell lines using pulse-chase
radiolabeling (Figure 5). For wild type α1-AT, ~52 and 55 kD polypeptides are present at time 0
IC. These polypeptides disappear between 1 and 4 hours of the chase period coincident with the
appearance of the mature 55 kD polypeptide EC. For α1-ATZ the ~52 kD polypeptide is present
at time 0 IC. It disappears from IC much more slowly than wild type α1-AT. A lesser amount
of the mature 55 kD polypeptide appears EC and it appears later in the chase period. These
results show that the genetically engineered cell lines faithfully recapitulate the known abnormal
fate of α1-ATZ in the classical form of α1-AT deficiency with defective secretion and
intracellular retention. For α1-AT saar and α1-ATZ + saar the single ~50 kD polypeptide
undergoes even less disappearance from IC during the chase period and no α1-AT is detected
EC. These results indicate that truncation of the carboxyl terminus of α1-AT results in
intracellular retention. When the Z allele is truncated there appears to be even less secretion of
the protein.
In order to more definitively compare the fate of α1-ATZ and α1-AT saar, we carried out pulse-chase radiolabeling for even longer time intervals (Figure 6). For α1-ATZ the results show slow but progressive disappearance from IC between 2 and 8 hrs of the chase period with a small amount of mature α1-AT appearing EC between 5 and 8 hrs. For α1-AT saar there is very relatively little disappearance from IC throughout the chase period but some disappearance does occur between 5 and 8 hrs indicating that intracellular retention of this mutant is as great as, or even greater than, that of α1-ATZ. Again, no detectable α1-AT saar is observed in the EC fluid even after 8 hrs of the chase period. Results for α1-ATZ + saar were similar to these for α1-AT saar (data not shown).

**Intracellular localization and solubility of α1-AT saar and α1-ATZ + saar.** In order to determine whether α1-AT saar and α1-ATZ + saar accumulate in the ER, the stable transfected CHO cell lines were subjected to immunofluorescence with antibody to α1-AT and antibody to GRP78/BiP as a marker for the ER (Figure 7A). The results show that antibody to α1-AT only faintly stains the perinuclear region in CHO cells transfected with wild type α1-AT. However, in CHO cells transfected with α1-ATZ, α1-AT saar and α1-ATZ + saar, antibody to α1-AT brightly stains the cytoplasm in a reticular pattern accentuated in the perinuclear region. Staining with antibody to GRP78/BiP stains an identical region in CHO cells transfected with wild type α1-AT, α1-ATZ, α1-AT saar and α1-ATZ + saar. In Figure 7B, staining for α1-AT co-localized with that for BiP by double-label immunofluorescence in CHO cells transfected with α1-ATZ + saar. Staining for α1-AT also co-localized with that for BiP in CHO cells transfected with α1-ATZ and α1-AT saar (data not shown). These results provide evidence that α1-AT saar and α1-ATZ + saar are retained in the ER.
Next we examined the detergent-soluble and insoluble fractions from transfected CHO cells to determine whether $\alpha$1-AT saar formed insoluble aggregates. Each of the cell lines was subjected to a pulse-chase experiment and then the cell lysates were analyzed for $\alpha$1-AT after separation into soluble and insoluble fractions (Figure 8A). The results show that insoluble material is only present in CHO cells expressing mutant $\alpha$1-ATZ. There is no detectable $\alpha$1-AT in the pellet from CHO cells expressing wild type $\alpha$1-AT, $\alpha$1-AT saar or $\alpha$1-ATZ + saar. The separation of soluble and insoluble fractions from cells expressing mutant $\alpha$1-ATZ provides particularly informative results: in the soluble fraction $\alpha$1-ATZ is retained between time 0 and time 1 hr of the chase period and then progressively disappears from 1 to 2 hr and then from 2 to 4 hr of the chase period; in contrast, there is a marked increase in $\alpha$1-ATZ accumulating in the insoluble fraction from time 0 to 1 hr and then even more from 1 to 2 hr of the chase period with some disappearance beginning between 2 and 4 hrs. The lack of $\alpha$1-ATZ + saar in the insoluble fraction implies that truncation at the carboxyl terminus converts the $\alpha$1-ATZ variant into a nonpolymerogenic mutant.

Soluble and insoluble fractions were also subjected to Western blot analysis to determine steady state levels of soluble and insoluble $\alpha$1-AT (Figure 8B). The results show that all of the wild type $\alpha$1-AT, mutant $\alpha$1-AT saar and mutant $\alpha$1-ATZ + saar partitions into the soluble fraction. Mutant $\alpha$1-AT saar and $\alpha$1-ATZ + saar migrate faster than wild type $\alpha$1-AT and mutant $\alpha$1-ATZ as would be expected from carboxyl terminal truncation. Increased levels of soluble mutant $\alpha$1-AT saar, $\alpha$1-ATZ and $\alpha$1-ATZ + saar compared with soluble wild type $\alpha$1-AT is expected because of the intracellular accumulation of all of these mutants. Only mutant
α1-ATZ appears in the insoluble fraction (right panel). Densitometric analysis of the results of 5 different samples indicates that 17.0 ± 1.9% of α1-ATZ is found in the insoluble fraction at steady state.
Discussion

Extensive clinical experience with \( \alpha_1 \)-AT deficiency has shown that there is marked variability in the development and severity of liver disease among homozygotes for the \( \text{PI Z} \) allele, patients with the classical form of this disease. Prospective nationwide screening studies carried out in Sweden have confirmed that only \( \sim 10\% \) of this population develops clinically significant liver disease (21,22). Using a genetic complementation approach we have found that there is less efficient ER degradation of \( \alpha_1 \)-ATZ in cells from some \( \alpha_1 \)-AT-deficient individuals that are susceptible to liver disease (16), and therein, that these individuals have a lesser inherent capacity to respond to, and/or protect themselves from, the hepatotoxic circumstances posed by ER retention of mutant \( \alpha_1 \)-ATZ. In this study we examined a potentially informative family in which a pair of sibs had severe liver disease associated with the classical form of \( \alpha_1 \)-AT deficiency. There was also a history of severe idiopathic liver disease in the mother which could not be attributed to known hepatotoxic exposures including alcohol. Unfortunately, we have been unable to establish a skin fibroblast cell line from the index patient to determine whether she has delayed ER degradation of \( \alpha_1 \)-ATZ, but we do know that skin fibroblasts from her brother have delayed ER degradation of \( \alpha_1 \)-ATZ (16). We decided to analyze the sequence of the \( \alpha_1 \)-AT gene in these sibs. To our surprise the index patient from the family has an unusual compound heterozygous mutation of the \( \alpha_1 \)-AT with one \( \text{PI Z} \) allele and a second allele with a combination of the \( \text{Z} \) and saar mutations, the \( \text{PI Z + saar} \) allele. This result raised the possibility that the presence of a \( \text{PI saar} \) allele, or a \( \text{PI Z + saar} \) allele, represented a genetically linked mechanism for susceptibility to liver disease. Although we cannot completely exclude this possibility it is unlikely because one of these alleles was not found in the brother, patient #2, in
whose fibroblasts there is delayed ER degradation of \( \alpha 1 \)-ATZ. Thus, one of these alleles is not even linked to the liver disease phenotype in this family.

The presence of the saar mutation on one of the \( \alpha 1 \)-AT alleles of patient #1 also prompted us to examine the fate of \( \alpha 1 \)-AT saar in a heterologous mammalian cell expression system. The results show that there is prolonged ER retention of \( \alpha 1 \)-AT saar, perhaps even more prolonged than that of \( \alpha 1 \)-ATZ, even though it does not form insoluble polymers. Previous studies have shown that there is ER retention of the PI Clayton allele, which has the same truncation on the background of another polymorphic variant of \( \alpha 1 \)-AT (23), \( \alpha 1 \)-AT Hong Kong in which the carboxyl terminus is truncated by sixty-one amino acids (24), and a recombinant \( \alpha 1 \)-AT molecule in which the carboxyl terminus is truncated by four amino acids (25). It was particularly interesting to observe that when the \( \alpha 1 \)-ATZ molecule is truncated, as it would be in the PI Z + saar allele found in our index patient, it no longer forms insoluble aggregates but is retained in the ER for a prolonged, perhaps even more prolonged, time interval.

These results provide further evidence that there are mechanisms by which mutant \( \alpha 1 \)-AT molecules are retained in the ER other than the polymerization mechanism which has been previously correlated with ER retention of \( \alpha 1 \)-ATZ (7-9). By comparing the effects of the polymerogenic mutant \( \alpha 1 \)-ATZ to those of nonpolymerogenic mutants, such as \( \alpha 1 \)-AT saar and \( \alpha 1 \)-ATZ + saar, which are retained in the ER to a similar extent, we may be able in future studies to ascertain whether it is the intrinsic polymerogenic properties that are hepatotoxic in some patients with \( \alpha 1 \)-AT deficiency.
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Figure Legends

**Figure 1. Sequence analysis of patient #1.** RT-PCR fragment from native liver of patient #1 was gel purified and directly sequenced (panel a). The PCR product was then subcloned and the sequence of subclones revealed two different alleles (panel b). The actual sequence data is shown in panel c. Arrows point to mutations.

**Figure 2.** Map of the exon structure of the α1-AT gene showing the locations of the mutations that characterize the PI Z and PI saar alleles in exon V.

**Figure 3. Pedigree of family in which PI Z + saar allele was discovered.** The dark areas represent the PI Z alleles, hatched areas represent PI Z + saar alleles and clear areas represent PIM alleles. Two possible genotypes for the parents are depicted.

**Figure 4. Biosynthesis of wild type (M) and mutant α1-AT molecules.** Stable transfected CHO cells were subjected to pulse radiolabeling for 3 hrs. The extracellular medium (EC) was harvested and cell lysates (IC) prepared and then subjected to immunoprecipitation and SDS-PAGE/fluorography. The relative electrophoretic migration of molecular mass markers is indicated at the right margin. The left and center panels were subjected to fluorography for 20 hrs. The right panel shows the EC samples for the same gel exposed to fluorography for 3 days.

**Figure 5. Secretion of wild type and mutant α1-AT molecules.** Stable transfected CHO cells were subjected to a pulse-chase radiolabeling protocol (pulse 1 hr) and analyzed exactly as described in the legend to Figure 4 and in Experimental Procedures.

**Figure 6. Comparison of the fates of mutant α1-ATZ and saar over prolonged time intervals.** Stable transfected CHO cells were subjected to a pulse chase radiolabeling protocol (pulse 1 hr) with an extended chase period.
Figure 7. Immunolocalization of wild-type and mutant α1-AT molecules. A) Stable transfected CHO cells were stained with anti-α1-AT (left) and anti-BiP (right) antibodies. B) CHO cells transfected with α1-ATZ + saar were stained with anti-α1-AT (left) and anti-BiP (center) and the images merged (right).

Figure 8. Solubility of wild type and mutant α1-AT molecules. A) Stable transfected CHO cells were subjected to a pulse-chase radiolabeling protocol (pulse 1 hr). The cells were lysed and the cell lysates separated into soluble and insoluble fractions exactly as described in Experimental Procedures. These sample were analyzed exactly as described in the legend to Figure 4 and in Experimental Procedures. B) Stable transfected CHO cells were grown to confluence and then lysed for Western blot analysis exactly as described in Experimental Procedures.
Figure 1

(a) Pt #1 (fragment-direct)

(b) Pt #1(Z) (subclone)

(c) Pt #1(Z+Saar) (subclone)
Figure 2

Figure 3
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Figure 4

Figure 5
Figure 6
Figure 7A

| Primary | Anti $\alpha_1$AT | Anti - BiP |
|---------|-------------------|------------|
| secondary | Rhodamine anti Ig | Cy2 anti Ig |

- **Wild Type $\alpha_1$AT**
- **$\alpha_1$ATZ**
- **$\alpha_1$AT Saar**
- **$\alpha_1$ATZ + Saar**
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Figure 7B

Primary: anti - α1,AT
Secondary: Rhodamine anti IG

Primary: anti - BiP
Secondary: Cy2 - anti IG

Merge

α1ATZ + Saar

Figure 8A

| Chase (hrs) | Soluble | Insoluble | Mr (x10^-3) |
|------------|---------|-----------|-------------|
|            | 0 1 2 4 | 0 1 2 4   | -68 -46     |
| Wild type α1,AT |  | | |
| α1ATZ      |  | | -68 -46 |
| α1,AT Saar |  | | -68 -46 |
| α1ATZ+Saar |  | | -68 -46 |
Acknowledgements

The authors are indebted to Marilyn Maksin for preparing the manuscript. The studies were supported by grants from the NIH and the Alpha-1 Foundation.
A naturally occurring non-polymerogenic mutant of alpha1-antitrypsin characterized by prolonged retention in the endoplasmic reticulum
Li Lin, Bela Schmidt, Jeff Teckman and David H. Perlmutter

J. Biol. Chem. published online June 26, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105226200

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