Conformational diseases are caused by a structural rearrangement within a protein that results in aberrant intermolecular linkage and tissue deposition. This is typified by the polymers that form with the Z deficiency variant of α₁-antitrypsin (Glu-342 → Lys). These polymers are retained within hepatocytes to form inclusions that are associated with hepatitis, cirrhosis, and hepatocellular carcinoma. We have assessed a surface hydrophobic cavity in α₁-antitrypsin as a potential target for rational drug design in order to prevent polymer formation and the associated liver disease. The introduction of either Thr-114 → Phe or Gly-117 → Phe on strand 2 of β-sheet A within this cavity significantly raised the melting temperature and retarded polymer formation. Conversely, Leu-100 → Phe on helix D accelerated polymer formation, but this effect was abrogated by the addition of Thr-114 → Phe. None of these mutations affected the inhibitory activity of α₁-antitrypsin. The importance of these observations was underscored by the finding that the Thr-114 → Phe mutation reduced polymer formation and increased the secretion of Z α₁-antitrypsin from a Xenopus oocyte expression system. Moreover cysteine mutants within the hydrophobic pocket were able to bind a range of fluorophores illustrating the accessibility of the cavity to external agents. These results demonstrate the importance of this cavity as a site for drug design to ameliorate polymerization and prevent the associated conformational disease.

Conformational diseases arise when a protein undergoes a change in size or a fluctuation in shape, which results in self-association and tissue deposition (1). This process is now recognized to underlie a whole range of diseases including the amyloidoses, prion encephalopathies, glutenine repeat diseases, and Alzheimer’s and Parkinson’s disease (2). The paradigm for the conformational diseases was provided by the serpinopathies, which result from mutations in members of the serpin superfamily. The most well characterized of these is the severe plasma deficiency that is associated with the Z allele of α₁-antitrypsin (3).

α₁-Antitrypsin is the most abundant circulating proteinase inhibitor and the archetypal member of the serpin superfamily (4, 5). Most individuals have two M α₁-antitrypsin alleles, but ~1 in 2000 are homozygous for the Z variant. The Z mutation results from a Glu → Lys substitution at amino acid 342 (6) and leads to retention of α₁-antitrypsin as inclusion bodies within the hepatocyte. These inclusions predispose to neonatal hepatitis, juvenile cirrhosis, and adult hepatocellular carcinoma (7–9). The resulting secretory defect accounts for the low circulating plasma level of α₁-antitrypsin, which is only 15% of normal in the Z homozygote. This plasma deficiency exposes the lungs to uncontrolled proteolytic attack that in turn causes early onset panacinar emphysema particularly in Z α₁-antitrypsin homozygotes who smoke (10).

The structure of α₁-antitrypsin is based on a five-stranded β-sheet A and a mobile reactive center loop (11–13). Our previous studies have shown that the Z mutation promotes opening of β-sheet A to facilitate a sequential interaction between the reactive center loop of one molecule and β-sheet A of a second, resulting in polymer formation (3, 14–16). These polymers tangle within the rough endoplasmic reticulum of hepatocytes to form the periodic acid-Schiff-positive inclusions that are associated with liver disease (3, 17). The significance of the reactive loop-β-sheet linkage was underscored by two other α₁-antitrypsin variants, Siiyama (Ser-53 → Phe) and Mmalton (Ala52 → Phe) that also resulted in hepatic inclusions and severe plasma deficiency of α₁-antitrypsin. Both of these mutants spontaneously formed polymers in vivo (18, 19). Moreover, this linkage accounts for the mild plasma deficiency observed in both S (Glu-264 → Val) and I (Arg-39 → Cys) α₁-antitrypsin (20, 21).

Further support for polymer formation as the mechanism responsible for the retention of mutant α₁-antitrypsin within hepatocytes came from studies utilizing the Xenopus oocyte expression system. Point mutations that attenuated polymerization of Z α₁-antitrypsin in vitro (14, 22) increased the secretion of Z α₁-antitrypsin in vivo (23). Our understanding of the mechanism underlying polymerization has allowed the design of strategies to prevent polymer formation (3, 16). To date, however, these have been based on peptides that bind to β-sheet A and as a consequence inactivate α₁-antitrypsin as a proteinase inhibitor. A more useful strategy would be to identify cavities in α₁-antitrypsin that can bind peptides, or their mimetics, and prevent polymerization without a loss of inhibitory activity.

Our high resolution crystal structure of α₁-antitrypsin revealed a large hydrophobic cavity bounded by strand 2 of β-sheet A and helices D and E (Fig. 1). The cavity is present in monomeric α₁-antitrypsin but is obliterated during polymerization (24). This cavity could provide an ideal target for drug...
design to prevent polymer formation and the associated liver disease (11, 13, 24). We have used site-directed mutagenesis to explore the role of this surface cavity in the conformational transitions of α1-antitrypsin in vitro and in vivo.

EXPERIMENTAL PROCEDURES

Mutagenesis, Expression, and Purification of Recombinant α1-Antitrypsin—Pittsburgh α1-antitrypsin (Met-358 → Arg) was used as the wild type protein. Replacement of the P1 methionine to arginine renders the protein a potent inhibitor of thrombin rather than neutrophil elastase. It otherwise has the same biophysical properties and rate of polymerization as Met-358 α1-antitrypsin (15, 25). The α1-antitrypsin mutants (Leu-100 → Phe, Thr-114 → Phe, Gly-117 → Phe, Leu-100 → Cys/Cys-232 → Ser, and Thr-114 → Cys/Cys-232 → Ser) were prepared by site-directed mutagenesis, and the sequences were confirmed by automated DNA sequence analysis. The α1-antitrypsin variants were cloned into the pET16b plasmid and transformed into BL21(DE3) Escherichia coli, and expression was induced with 0.4 mM isopropyl-p-β-thiogalactoside. Recombinant α1-antitrypsin was harvested by centrifugation after 4 hours of induction (data not shown).

Purification of Cavity Mutants—Wild type, Leu-100 → Cys/Cys-232 → Ser, Thr-114 → Cys/Cys-232 → Ser, and Gly-117 → Phe α1-antitrypsin were purified from the supernatant following lysis of the E. coli (11). However, this approach relies on glutathione affinity chromatography, and the α1-antitrypsin cavity mutants were purified from the pQE31 vector that contained an amino-terminal MRSHHHHHHHH tag. Recombinant proteins were then purified from the soluble fraction of E. coli lysate by HitTrap Ni-chelating and Q-Sepharose column chromatography as detailed previously (26). The proteins were dialyzed into 50 mM Tris, 50 mM KCl, pH 7.4, and purity was confirmed by 12% (w/v) SDS-PAGE.

Characterization of Recombinant Wild Type and Mutant α1-Antitrypsin—The recombinant proteins were characterized by non-denaturing and 0–8 M transverse urea gradient PAGE. Inhibitory activity was calculated by incubating bovine α-chymotrypsin (5 pmol) of known active site (27) with increasing concentrations of α1-antitrypsin (estimated active site concentration of 0.1 μM) in a total volume of 100 μl with 0.03 mM sodium phosphate, 0.16 mM NaCl, 0.1% (w/v) PEG 4000, pH 7.4, reaction buffer. The reaction proceeded for 10 min at room temperature, and the residual proteolytic activity was determined by the addition of the substrate succinyl-l-alanyl-l-alanyl-propyl-l-pheynalanlyl-p-nitroanilide to a final concentration of 0.16 mM. The change in the A280 over 3 min was observed. Active site values were obtained by plotting residual proteolytic activity against the volume of α1-antitrypsin and extrapolating to the x intercept (28). Binary complexes were formed by incubating 50–100 fold molar excess of the antithrombin 12-mer peptide (P1–27; Ac-Ser-Glu-Ala-Ala-Ala-Ser-Thr-Ala-Val-Val-Ile-Ala-OH) or α1-antitrypsin 6-mer peptide (P1–27; Ac-Phe-Leu-Glu-Ala-Ile-Gly-OH) with each α1-antitrypsin variant at 0.5 mg/ml in 50 mM KCl, pH 7.4, at 37 °C for up to 48 h. Samples at different time points were assayed on a 7.5% (w/v) non-denaturing gel containing 8 M urea (16). All proteins were visualized by Coomasie Blue or silver staining. The melting temperature and far ultraviolet (250–195 nm) CD spectrum were obtained for each α1-antitrypsin mutant as described previously (14).

RESULTS

Three residues of α1-antitrypsin were selected for site-directed mutagenesis in order to explore the role of the cavity in polymer formation. Leu-100 on hD and Thr-114 on s2A have side chains that point into the hydrophobic pocket, whereas Gly-117 is located at the base of the cavity on s2A (Fig. 1B). Introducing large phenylalanine residues, or cysteine residues that could be labeled with bulky fluorophores, at these sites was predicted to fill the cavity and mimic the effect of binding a small molecule inhibitor. The one naturally occurring cysteine at position 232 in α1-antitrypsin was replaced by a serine residue to ensure that only the newly introduced cavity cysteine was available for labeling. The Cys-232 → Ser mutation has no effect on the inhibitory activity or polymerization of recombinant α1-antitrypsin (15, 29, 30).

Fluorophore Labeling of Recombinant Wild Type and Mutant α1-Antitrypsin—The cysteine cavity mutants were labeled with a 20-fold molar excess of either tetramethylrhodamine-5-iodoacetamide (5-TMRIA), 5-iodoacetamidofluorescein (5-IAF), N-4,4-difluoro-5,5-dimeth-
lysate that lacked the histidine tag (data not shown). All the mutations introduced into strand 2A elevated the $T_m$ of the protein. In particular, Gly-117 \( \rightarrow \) Phe $\alpha_1$-antitrypsin had a melting temperature that was more than 8 °C higher than the wild type protein. Helix D mutations had differing effects according to their size. Leu-100 \( \rightarrow \) Phe lowered the melting temperature by 3.5 °C, whereas a cysteine residue at the same position did not significantly alter the $T_m$ when compared with the wild type control. Furthermore, the addition of a second phenylalanine residue within the cavity at position 114 (Leu-100 \( \rightarrow \) Phe/Thr-114 \( \rightarrow \) Phe) resulted in an increase in thermal stability, reversing the effect of Leu-100 \( \rightarrow \) Phe mutation alone (Table I). Thermal stability was also assessed by incubating recombinant wild type or cavity mutants of $\alpha_1$-antitrypsin between 30 and 100 °C, at increments of 10 °C for 15 min, and assessing the samples by 7.5% (w/v) non-denaturing PAGE. Leu-100 \( \rightarrow \) Cys/Cys-232 \( \rightarrow \) Ser and Leu-100 \( \rightarrow \) Phe $\alpha_1$-antitrypsin had thermal stabilities similar to that of wild type protein. However, Gly-117 \( \rightarrow \) Phe $\alpha_1$-antitrypsin was the most thermostable as it remained monomeric following incubation at 60 °C for 15 min, which was 10 °C higher than wild type $\alpha_1$-antitrypsin. Thr-114 \( \rightarrow \) Cys/Cys-232 \( \rightarrow \) Ser, Thr-114 \( \rightarrow \) Phe, and Leu-100 \( \rightarrow \) Phe/Thr-114 \( \rightarrow \) Phe $\alpha_1$-antitrypsin all had intermediate thermal stabilities (data not shown). Thus, the differences in melting temperatures were mirrored in the thermal stability of wild type and mutant $\alpha_1$-antitrypsin when assessed by heating and separation on non-denaturing PAGE.

**Polymerization of Recombinant $\alpha_1$-Antitrypsin Variants**—Polymerization was assessed at 0.1 mg/ml and 52 °C for up to 7 days as these conditions led to polymer formation of histidine-tagged recombinant $\alpha_1$-antitrypsin that could be visualized by non-denaturing PAGE. The rate of polymer formation was calculated from the loss of intensity of the monomeric protein band using densitometry scanning (Table I). Wild type $\alpha_1$-antitrypsin almost completely polymerized within 24 h when heated at 52 °C (Fig. 3a). Leu-100 \( \rightarrow \) Phe $\alpha_1$-antitrypsin accelerated polymer formation in keeping with its lower melting temperature (Fig. 3b). However, replacing Leu-100 with a cysteine residue (Fig. 3c) or introducing another bulky phenylalanine residue at position 114 (Fig. 3d) within the cavity reversed this effect as these mutants polymerized at a rate similar to wild type $\alpha_1$-antitrypsin (Table I). Interestingly, all the mutations introduced onto s2A, independent of size, slowed polymer formation as would be predicted from their melting temperatures (Fig. 3, e–g) (Table I). The most thermostable mutant Gly-117 \( \rightarrow \) Phe $\alpha_1$-antitrypsin dramatically impeded polymer formation, as polymers were evident only after incubating at 52 °C for 72 h.

**Binary Complex Formation between Recombinant Wild Type and Mutant $\alpha_1$-Antitrypsin and Exogenous Reactive Loop Peptides**—A 12-mer peptide corresponding to the reactive center loop of antithrombin (P14–23) was used to assess the patency of $\beta$-sheet A. Binary complexes were formed by incubating recombinant $\alpha_1$-antitrypsin (0.5 mg/ml) with a 50–100-fold molar excess of amino-terminal acetylated 12-mer peptide at 37 °C for 48 h. Samples were examined on 7.5% (w/v) non-denaturing PAGE containing 8 M urea. Recombinant wild type $\alpha_1$-antitrypsin and the cavity mutants all formed a binary complex with the 12-mer peptide with a 1:1 stoichiometry (Fig. 4, a–g). Gly-117 \( \rightarrow \) Phe $\alpha_1$-antitrypsin formed a binary complex with the peptide at a rate faster than wild type $\alpha_1$-antitrypsin (Table II and Fig. 4b), whereas binary complex formation was significantly retarded by Leu-100 \( \rightarrow \) Phe $\alpha_1$-antitrypsin (Fig. 4c). The other cavity mutations all similarly slowed annealing of the peptide to $\beta$-sheet A. Neither recombinant wild type nor Leu-100 \( \rightarrow \) Phe $\alpha_1$-antitrypsin was able to form a binary complex with the 6-mer peptide, corresponding to P7–2 of the reactive loop of $\alpha_1$-antitrypsin, under the same conditions at 24 h (data not shown).

**Secretion of Recombinant $\alpha_1$-Antitrypsin from the Xenopus Oocyte Expression System**—The effects of the mutants were then assessed on the polymerization of the Z variant of $\alpha_1$-antitrypsin. This mutant is too unstable to be expressed as a recombinant protein, and the mutants were therefore assessed for their effect on the secretion of Z $\alpha_1$-antitrypsin in vivo. 62% (S.E. ± 4%) of the wild type protein was secreted from the
Hydrophobic Cavity of α1-Antitrypsin as a Therapeutic Target

The inhibitory activity, melting temperatures, and rate of polymer formation of recombinant wild type and mutant α1-antitrypsin

| α1-Antitrypsin mutant | Inhibitory activity | Melting temperature | Polymer formation |
|-----------------------|---------------------|---------------------|------------------|
| Wild type             | 66.8 ± 0.4          | 65.4 ± 0.7          | 5.6 ± 0.8        |
| Leu-100 → Phe         | 76.7 ± 1.6          | 61.9 ± 0.2          | 2.6 ± 0.4        |
| Thr-114 → Phe         | 64.8 ± 1.2          | 68.4 ± 0.1          | 56.2 ± 8.01      |
| Gly-117 → Phe         | 62.6 ± 1.1          | 73.9 ± 0.4          | 96.3 ± 0.02      |
| Leu-100 → Phe/Thr-114 → Phe | 46.6 ± 0.5    | 67.8 ± 0.4          | 8.6 ± 1.43       |
| Leu-100 → Cys/Cys-232 → Ser | 35.1 ± 0.8    | 66.3 ± 0.8          | 5.9 ± 0.72       |
| Leu-100 → Cys/Cys-232 → Serα | 27.0 ± 1.6    | 63.8 ± 0.2          | 6.4 ± 0.01       |
| Thr-114 → Cys/Cys-232 → Ser | 59.0 ± 1.5    | 67.8 ± 0.1          | 85.6 ± 1.43      |
| Thr-114 → Cys/Cys-232 → Serα | 46.0 ± 2.1    | 67.5 ± 0.7          | 53.8 ± 7.13      |

This represents the cysteine variants maximally labeled with 5-IAF in 50 mM Tris, pH 8.5, at 37 °C for 2 h. The inhibitory activity was determined against bovine α-chymotrypsin, and melting temperature was calculated from circular dichroic spectrum analysis at 222 nm. Polymer formation was assessed by incubating each α1-antitrypsin variant at 0.1 mg/ml and 52 °C for 24 h. The t\textsubscript{90} was calculated from the loss of intensity of the monomeric protein band using densitometry scanning. The results are the mean ± S.D. of three measurements.

Oocytes compared with 10% (±2%) of Z α1-antitrypsin (p = 0.0001, Student’s t test with Welch correction). Gly-117 → Phe and Leu-100 → Phe had little effect on the secretion of Z α1-antitrypsin (17 ± 5 and 18 ± 5%, respectively). However Thr-114 → Phe more than doubled the secretion of Z α1-antitrypsin to 23 ± 4% (p = 0.0018 compared with Z α1-antitrypsin). The results are the mean of 5–9 separate experiments.

Fluorophore Labeling of Recombinant α1-Antitrypsin Cysteine Cavity Mutants—The accessibility of the cavity was examined by labeling the cysteine variants Leu-100 → Cys/Cys-232 → Ser and Thr-114 → Cys/Cys-232 → Ser α1-antitrypsin with a number of fluorophores having different length side chains (Table III). The labeling reactions were performed in the dark at either 20 or 37 °C for up to 72 h. Incubation of Leu-100 → Cys/Cys-232 → Ser α1-antitrypsin with 5-TMRIA at 20 °C resulted in 14, 24, 20, and 20% labeling when incubated for 12, 24, 48, and 72 h, respectively. Likewise, incubation of Thr-114 → Cys/Cys-232 → Ser α1-antitrypsin with 5-TMRIA at 20 °C resulted in 15, 20, 26, and 24% labeling when incubated for 12, 24, 48, and 72 h, respectively. These results imply that maximal labeling of the cavity cysteine residues with 5-TMRIA was achieved within 24 h. Other fluorescent probes were assessed for their ability to label the cysteine variants in an attempt to improve the labeling efficiency (Table III). The addition of the reducing agent tris-(2-carboxyethyl)phosphine and raising the reaction temperature both increased the amount of protein labeled with 5-IAF but promoted labeling of other susceptible residues (histidines, methionines, and lysines) as evidenced by multiple bands on non-denaturing PAGE (data not shown). This experimental artifact could be overcome by raising the pH to 8.5 and limiting the reaction time to 2 h at 37 °C. With this method 35% of Leu-100 → Cys/Cys-232 → Ser α1-antitrypsin and 28% of Thr-114 → Cys/Cys-232 → Ser α1-antitrypsin were labeled with 5-IAF (Table III). As only approx...
Hydrophobic Cavity of α₁-Antitrypsin as a Therapeutic Target

Binary complexes were formed by incubating 100-fold molar excess of antithrombin 12-mer peptide with recombinant M α₁-antitrypsin at 20 or 37 °C between 2 and 16 h in the dark. Labeled protein was separated from excess fluorophore by gel filtration, and the labeling efficiency was calculated spectrophotometrically, ND, not done. The abbreviations used are as follows: FL IA, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl-N′-iodoacetylethlenediamine; FL C₁ IA, N′(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N′-iodoacetylethlenediamine; FL C₁ IA, N′(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N′-iodoacetylethlenediamine; FL C₁ IA, N′(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N′-iodoacetylethlenediamine; FL C₁ IA, N′(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N′-iodoacetylethlenediamine; TCEP, tris-(2-carboxyethyl)phosphine.

Fluorophore Duration | Leu-100 → Cys/Cys-232 | Thr-114 → Cys/Cys-232
---|---|---
| h | % labeled | % labeled |
| FL IA | 16 | 10 | 5 | 10 | 4 |
| FL C₁ IA | 16 | 10 | 7 | 9 | 8 |
| 5-TMRIA | 16 | 24 | 25 | 20 | 13 |
| 5-IAF | 4 | 14 | 28 | 27 | 36 |
| 5-IAF and TCEP | 16 | 27 | 35 | 28 | 29 |
| 5-IAF and TCEP | 16 | 12 | 36 | 33 | 43 |
| 5-IAF, pH 8.5 | 2 | ND | 36 | ND | 28 |

Table II

| α₁-Antitrypsin mutant | Binary complex formation (t₀, h) |
---|---|
| Wild type | 2.6 ± 0.27 |
| Leu-100 → Phe | 7.0 ± 1.78 |
| Thr-114 → Phe | 4.0 ± 0.57 |
| Gly-117 → Phe | 1.3 ± 0.43 |
| Leu-100 → Phe/Thr-114 → Phe | 3.4 ± 0.29 |
| Leu-100 → Cys/Cys-232 → Serα | 4.3 ± 1.39 |
| Thr-114 → Cys/Cys-232 → Serα | 8.6 ± 1.43 |
| Thr-114 → Cys/Cys-232 → Serα | 9.6 ± 0.01 |

* This represents the cysteine cavity mutants maximally labeled with 5-IAF in 50 mM Tris, pH 8.5, at 37 °C for 2 h. The results are the mean ± S.D. of three experiments.

FIG. 4. Binary complexes were formed by incubating 100-fold molar excess of antithrombin 12-mer peptide with recombinant wild type and mutant α₁-antitrypsin with 100-fold molar excess of the 12-mer peptide corresponding to the reactive loop of antithrombin. The half-life (t₁/₂) was calculated from the loss of intensity of the monomeric protein band using densitometry scanning.

Table III

| Fluorophore Duration | Leu-100 → Cys/Cys-232 | Thr-114 → Cys/Cys-232 |
|---|---|---|
| h | % labeled | % labeled |
| FL IA | 16 | 10 | 5 | 10 | 4 |
| FL C₁ IA | 16 | 10 | 7 | 9 | 8 |
| 5-TMRIA | 16 | 24 | 25 | 20 | 13 |
| 5-IAF | 4 | 14 | 28 | 27 | 36 |
| 5-IAF and TCEP | 16 | 27 | 35 | 28 | 29 |
| 5-IAF and TCEP | 16 | 12 | 36 | 33 | 43 |
| 5-IAF, pH 8.5 | 2 | ND | 36 | ND | 28 |

s2A, hD, and hE that was present in the monomeric structure but predicted to reduce in size by >70% during polymer formation (24). As such this cavity provides a target for rational drug design to prevent polymerization and ameliorate the associated disease. In order to explore the role of the cavity in polymer formation, three residues, whose side chains border the cavity, were selected for site-directed mutagenesis (Fig. 1B). Introducing large phenylalanine residues at position 114 and Gly-117 on s2A is likely to fill the cavity. A detailed assessment has been undertaken to determine the effect of these mutations on polymer formation in order to determine whether the hydrophobic cavity would be a suitable target for rational drug design.

All the cavity mutants had a normal far UV circular dichroic spectrum, were active as proteinase inhibitors, and formed SDS-stable complexes. They varied in specific activity when assessed against bovine α-chymotrypsin indicating differing stoichiometry of inhibition, but overall the data show that the point mutations did not lead to a significant change in the structure of α₁-antitrypsin. All of the mutations introduced onto s2A elevated melting temperature and significantly slowed the rate at which α₁-antitrypsin formed loop-sheet polymers, particularly the introduction of bulky phenylalanine residues at either position 114 or 117. These observations are in keeping with our previous studies (14). Moreover, the addition of a phenylalanine at position 114 restored the rate of Leu-100 → Phe α₁-antitrypsin polymerization to that of the wild type protein. Furthermore, the s2A mutations all increased thermal stability of α₁-antitrypsin which is in accordance with their effect on polymerization. These data provide strong evidence that filling the cavity with mutants on s2A stabilizes α₁-antitrypsin and retards polymer formation without compromising inhibitory function in vitro.

Polymerization results from the sequential insertion of the reactive center loop of one molecule into β-sheet A of another (15). Point mutations that favor polymerization are predicted to open β-sheet A to facilitate incorporation of an exogenous reactive center loop (31). The accessibility of β-sheet A was assessed in the mutants by measuring the rate at which they annealed an exogenous reactive loop peptide to form a binary complex (32). Gly-117 → Phe α₁-antitrypsin formed a binary complex with the peptide at a rate faster than wild type protein. This was unexpected as the mutant significantly retarded polymer formation. Moreover, the most polymerogenic mutant, Leu-100 → Phe α₁-antitrypsin, formed a binary complex with

immediately a third of the protein labeled with fluorophore, it was difficult to interpret the effect of these agents on the rate of polymerization.

DISCUSSION

Our high resolution crystal structure of recombinant M α₁-antitrypsin demonstrated a hydrophobic cavity bounded by

![Image](60x400 to 305x737)

![Image](59x380)
The surface cavity bounded by s2A, hD, and hE is ideal for rational drug design as it is accessible to external agents that can block polymerization without an accompanying loss of inhibitory activity. Thus inhibition of α1-antitrypsin polymerization within hepatocytes will prevent the liver disease associated with Z α1-antitrypsin. Moreover, an increase in the amount of circulating active α1-antitrypsin may offer a treatment for the associated emphysema.

**REFERENCES**

1. Carrell, R. W., and Lomas, D. A. (1997) *Lancet* 350, 134–138
2. Carrell, R. W., and Lomas, D. A. (2002) *N. Engl. J. Med.* 346, 45–53
3. Lomas, D. A., Evans, D. L., Finch, J. T., and Carrell, R. W. (1992) *Nature* 357, 605–607
4. Peltonen, J., Korzus, E., and Travin, J. (1994) *J. Biol. Chem.* 269, 15957–15960
5. Silverman, G. A., Bird, P. I., Carrell, R. W., Church, F. C., Couhlin, P. B., Gettins, P., Irving, J., Lomas, D. A., Moyer, R. W., Pemberton, P., A. Remold O'Donnell, E., Salvesen, G. S., Travis, J., and Whisstock, J. C. (2001) *J. Biol. Chem.* 276, 32923–32926
6. Jeppsson, J.-O. (1976) *FEBS Lett.* 65, 195–197
7. Seger, T. (1998) *Acta Paediatr. Scand.* 77, 847–851
8. Eriksson, S., Carlsson, J., and Velez, R. (1986) *N. Engl. J. Med.* 314, 736–739
9. Sharp, H. L., Bridges, R. A., Krivi, W., and Freier, E. F. (1969) *J. Lab. Clin. Med.* 73, 934–939
10. Piitulainen, E., and Eriksson, S. (1999) *Eur. Respir. J.* 13, 247–251
11. Elliott, P. R., Abrahams, J.-P., and Lomas, D. A. (1998) *J. Mol. Biol.* 275, 419–425
12. Ryu, S. E., Choi, H.-J., Kwon, K.-S., Lee, K. N., and Yu, M.-H. (1996) *Structure* 4, 1181–1192
13. Kim, S.-J., Woo, J.-R., Seo, E. J., Yu, M.-H., and Ryu, S.-E. (2001) *J. Mol. Biol.* 316, 109–119
14. Dafforn, T. R., Mahadeva, R., Elliott, P. R., Sivasothy, P., and Lomas, D. A. (1999) *J. Biol. Chem.* 274, 9548–9555
15. Sivasothy, P., Dafforn, T. R., Gettins, P. G. W., and Lomas, D. A. (2000) *J. Biol. Chem.* 275, 33663–33668
16. Mahadeva, R., Dafforn, T. R., Carrell, R. W., and Lomas, D. A. (2002) *J. Biol. Chem.* 277, 6771–6774
17. Janczukiewicz, R., Dominatiere, R., Sternby, N. H., Piitulainen, E., and Eriksson, S. (2002) *J. Biol. Chem.* 277, 26540–26546
18. Lomas, D. A., Finch, J. T., Seyama, K., Nukawa, K., and Carrell, R. W. (1993) *J. Biol. Chem.* 268, 15337–15345
19. Lomas, D. A., Elliott, P. R., Siddar, S. K., Foreman, R. C., Finch, J. T. C., Cox, D. W. J., and Carrell, R. W. (1995) *J. Biol. Chem.* 270, 10664–10670
20. Elliott, P. R., Stein, P. E., Bilton, D., Carrell, R. W., and Lomas, D. A. (1996) *Nat. Struct. Biol.* 3, 910–911
21. Mahadeva, R., Chang, W.-S. W., Dafforn, T., Oxlade, D. J., Foreman, R. C., and Lomas, D. A. (1999) *J. Clin. Invest.* 103, 999–1006
22. Kim, J., Lee, K. N., Y., G.-S., and Yu, M.-H. (1995) *J. Biol. Chem.* 270, 8597–8601
23. Siddhar, S. K., Lomas, D. A., Carrell, R. W., and Foreman, R. C. (1995) *J. Biol. Chem.* 270, 8393–8396
24. Elliott, P. R., Pei, X. Y., Dafforn, T. R., and Lomas, D. A. (2000) *Protein Sci.* 9, 1274–1291
25. Stratiakos, E., and Gettins, P. G. W. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 4, 453–458
26. Zeng, A., Carrell, R. W., and Huntington, J. A. (2001) *J. Biol. Chem.* 276, 27541–27547
27. Keny, F. J., and Kaiser, E. T. (1970) *Methods Enzymol.* 19, 3–20
28. Beatty, K. B., Bith, J., and Travin, J. J. (1980) *J. Biol. Chem.* 255, 3931–3934
29. Hopkins, P. C. R., Chang, W.-S. W., Wardell, M. R., and Stone, S. R. (1997) *J. Biol. Chem.* 272, 3905–3909
30. Bottomley, S. P., Hopkins, P. C., and Whisstock, J. C. (1998) *Biochem. Biophys. Res. Commun.* 251, 1–5
31. Stein, P. E., and Carrell, R. W. (1995) *Nat. Struct. Biol.* 2, 96–113
32. Schulze, A. J., Baumann, U., Knof, S., Jaeger, E., Huber, R., and Laurell, C.-B. (1999) *Eur. J. Biochem.* 266, 51–58
33. Carr, M. C., Chaudhry, C., and Kim, P. S. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 14306–14311
34. Lie, C., Park, S. H., Lee, M.-Y., and Yu, M.-H. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 7727–7731
35. Im, H., Seo, J. E., and Yu, M.-H. (1999) *J. Biol. Chem.* 274, 11072–11077
36. Lee, C., Maeng, J.-S., Kocher, J.-P., Lee, B., and Yu, M.-H. (2003) *Protein Sci.* 12, 1446–1453
37. See, E. J., Im, H., Maeng, J.-S., Kim, K. E., and Yu, M.-H. (2000) *J. Biol. Chem.* 275, 16904–16909
38. Foreman, R. C., Judah, J. D., and Colman, A. (1984) *FEBS Lett.* **168**, 84–88
39. Burrows, J. A. J., Willis, L. K., and Perlmutter, D. H. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 1796–1801
40. Devlin, G. L., Purfrey, H., Tew, D. J., Lomas, D. A., and Bottomley, S. P. (2001) *Am. J. Respir. Cell Mol. Biol.* **24**, 727–732
41. Tjernberg, L. O., Naslund, J., Lindqvist, P., Johansson, J., Karlström, A. R., Thyberg, J., Terenius, L., and Nordstedt, C. (1996) *J. Biol. Chem.* **271**, 8545–8548
42. Soto, C., Sigurdsson, E. M., Morelli, I., Kumar, R. A., Castaño, E. M., and Frangione, B. (1998) *Nat. Med.* **4**, 822–826
43. Warrick, J. M., Chan, H. Y., Gray-Board, G. L., Chai, Y., Paulson, H. L., and Bonini, N. M. (1999) *Nat. Genet.* **23**, 425–428