Biochemical and Biophysical Research Communications 445 (2014) 191–195

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Retarded protein folding of the human Z-type α_1-antitrypsin variant is suppressed by Cpr2p

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ARTICLE INFO

Article history:
Received 21 January 2014
Available online 3 February 2014

Keywords:
α_1-Antitrypsin
Folding disease
Peptidyl-prolyl isomerase
Protein folding
Protein interaction

ABSTRACT

The human Z-type α_1-antitrypsin variant has a strong tendency to accumulate folding intermediates due to extremely slow protein folding within the endoplasmic reticulum (ER) of hepatocytes. Human α_1-antitrypsin has 17 peptidyl-prolyl bonds per molecule; thus, the effect of peptidyl-prolyl isomerases on Z-type α_1-antitrypsin protein folding was analyzed in this study. The protein level of Cpr2p, a yeast ER peptidyl-prolyl isomerase, increased more than two-fold in Z-type α_1-antitrypsin-expressing yeast cells compared to that in wild-type α_1-antitrypsin-expressing cells. When *CPR2* was deleted from the yeast genome, the cytotoxicity of Z-type α_1-antitrypsin increased significantly. The interaction between Z-type α_1-antitrypsin and Cpr2p was confirmed by co-immunoprecipitation. *In vitro* folding assays showed that Cpr2p facilitated Z-type α_1-antitrypsin folding into the native state. Furthermore, Cpr2p overexpression significantly increased the extracellular secretion of Z-type α_1-antitrypsin. Our results indicate that ER peptidyl-prolyl isomerases may rescue Z-type α_1-antitrypsin molecules from retarded folding and eventually relieve clinical symptoms caused by this pathological α_1-antitrypsin.

1. Introduction

Human α_1-antitrypsin (α_1-AT) is synthesized in the liver and is secreted into the blood to protect tissues against indiscriminate proteolytic attacks from neutrophil elastases [1]. Among the more than 90 α_1-AT genetic variants reported, Z-type α_1-AT is most frequently found in patients with serious clinical problems such as liver cirrhosis and emphysema [2]. The structural feature of most deficient α_1-AT variants is conformational instability [3], leading to rapid clearance by endoplasmic reticulum (ER)-associated degradation [4]. However, some variants such as D256V, L41P, and Z-type (E342K) α_1-AT exhibit extremely retarded protein folding compared to that of the wild-type molecule [3]. Once folded, the stability and inhibitory activity of these variant proteins are comparable to those of wild-type α_1-AT. Retarded folding leads to the accumulation of folding intermediates, which are prone to forming intermolecular loop-sheet polymers in the ER of hepatocytes and which can cause liver cirrhosis. Indeed, only ~15% of newly synthesized Z-type AT molecules reach the extracellular compartment [5], and loop-sheet polymers of Z-type α_1-AT have been reported [6].

The folding of newly synthesized polypeptide chains is facilitated by folding-assistant proteins. For example, chaperonins (a major family of chaperones) facilitate the folding of limited numbers of client polypeptides [7]; protein disulfide isomerases catalyze the formation and exchange of disulfide bonds; and peptidyl-prolyl isomerases (PPIases) accelerate the rate-determining proline *cis–trans* isomerization step during protein folding [8]. As Z-type α_1-AT has no disulfide bonds and exhibits retarded folding, the possibility of folding assistance by an ER PPIase was evaluated in this study. PPIases are expressed in all organisms and are classified into three classes: cyclophilins, FK506-binding proteins, and parvulins. Cyclophilins are a major PPIase family that was originally known to form complexes with the immunosuppressant drug cyclosporin A and to block calcineurin-mediated immune responses involved in the rejection of transplanted organs [9]. Peptide bonds in native protein structures exist preferentially in the *trans* configuration, but approximately 7% of X-Pro peptide bonds are in the *cis* configuration [10,11]. The particular configuration of the protein must be acquired during folding; however, the required peptidyl-prolyl isomerization is a slow reaction because it involves rotation around a partial double bond [12]. Human

Abbreviations: PPIase, peptidyl-prolyl isomerase; α_1-AT, α_1-antitrypsin; YPD, 1% yeast extract, 2% peptone, and 2% glucose; YPGal, 1% yeast extract, 2% peptone, and 2% galactose.

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http://dx.doi.org/10.1016/j.bbrc.2014.01.156
0006-291X/

α_1-AT has 17 X-Pro peptide bonds of 393 peptidyl bonds, which may limit the folding rate of the protein.

Yeast is an ideal model system to investigate the contribution of cyclophilins due to the availability of high-throughput functional genomics methods [14]. Humans have seven major cyclophilins (hCypA, hCypB, hCypC, hCypD, hCypE, hCyp40, and hCypNK), whereas *Saccharomyces cerevisiae* possesses eight different cyclophilins (Cpr1–Cpr8) [13]. Yeast Cpr2p is localized to the ER and is induced by tunicamycin, an inhibitor of glycosylation, and heat shock [15,16], suggesting that this protein plays a role in the folding of secreted proteins [16]. Here, the contribution of Cpr2p to the folding of human Z-type α_1-AT was investigated in several biochemical and cellular analyses, including co-immunoprecipitation, *in vitro* folding assays, knockout studies, and secretion analyses.

2. Materials and methods

2.1. Materials

Rabbit anti-human α_1-AT antibody and goat anti-rabbit IgG antibody conjugated to peroxidase were purchased from Sigma (St. Louis, MO, USA). Protein A/G PLUS-Agarose was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Rat anti-Cpr2p antibody was from Aprogen Co. (Daejeon, Korea). Q-sepharose™ Fast Flow column, Hybond™ ECL™ nitrocellulose membrane, and PD-10 desalting column were purchased from Amersham Bioscience Co. (Piscataway, NJ, USA). Ni^{2+}-NTA (nitrilo-tri-acetic acid) agarose was from Peptron Co. (Daejeon, Korea). Curix CP-BU, a medical X-ray film, was purchased from Agfa Co. (Ridgefield Park, NJ, USA).

2.2. Yeast strains and transformation

Human wild-type and Z-type α_1-AT were overexpressed in *S. cerevisiae* BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) (Open Biosystems Inc., Huntsville, AL, USA). pYInu-AT, containing an inulinase (Inu) signal sequence under the control of the yeast *GAL10p* promoter, was used to express human α_1-AT in *S. cerevisiae* [17]. The cDNA coding for the Z-type α_1-AT variant replaced the wild-type α_1-AT gene on pYInu-AT; the resulting plasmid was named pYInu-ATZ. Wild-type BY4741 and the derived *cpr2Δ* yeast strain were transformed with pYInu-AT or pYInu-ATZ using the standard lithium acetate method [18]. Transformants were selected by growing cells in drop-out medium lacking uracil at 30 °C for 3 days.

2.3. Monitoring cell growth by a spotting assay

The *CPR2* knockout yeast strain transformed with pYInu-AT or pYInu-ATZ was cultured in YPD (1% yeast extract, 2% peptone, and 2% glucose) liquid medium at 30 °C overnight. Cultured cells were harvested and serially diluted to reach adequate cell densities. Ten microliter of each dilution was spotted on both YPD plates (which did not induce α_1-AT expression) and YPGal (1% yeast extract, 2% peptone, and 2% galactose) plates (which induced α_1-AT expression). The plates were further incubated at 30 °C for 2–3 days, and cell growth was observed.

2.4. Complementation analysis

The *CPR2* gene was amplified from *S. cerevisiae* Y2805 (*MATa pep4::HIS3 prb1-d can1 GAL2 his3 ura3-52*) genomic DNA by polymerase chain reaction (PCR) using *Pfu* polymerase (Promega Co., Madison, WI, USA). The forward primer was 5′-CTCCAAGCTTATGAAATTCAGTGGCTTGTGGTGTTGGTTG-3′ and the reverse primer was 5′-TCCAAAGCTTTCAAGAAGAGAGCTCAGGCGTCCACTCA-3′. The PCR products were digested using *Hind*III and cloned into the *Hind*III sites of a yeast expression vector, pACT2 AD (Clontech Laboratories Inc., Mountain View, CA, USA). The resulting plasmid was named pACT2-CPR2. The *cpr2Δ* yeast strain was co-transformed with pYInu-ATZ and pACT2-CPR2, and the co-transformants were selected in drop-out medium lacking leucine and uracil at 30 °C for 3–4 days. Cell growth was monitored by a spotting assay upon the expression of Z-type α_1-AT.

2.5. Co-immunoprecipitation of Z-type α_1-AT and Cpr2p from cell extracts

The *cpr2Δ* yeast strain harboring pYInu-ATZ and/or pACT2-CPR2 was cultured in YPGal liquid medium at 30 °C for 48 h. The cultured cells were harvested and resuspended in 1 ml of lysis buffer (50 mM HEPES, pH 7.0, 1% Triton X-100, 1 mM PMSF, and 1 μM aprotinin). Cells were lysed by vigorous vortexing with glass beads (425–600 μm in diameter), and the cell extracts were precleared with Protein A/G PLUS-Agarose beads at 4 °C for 2 h. Protein concentrations were determined using a Bio-Rad DC protein assay kit (Hercules, CA, USA). Crude lysates were then incubated with polyclonal rat anti-Cpr2p antibodies (1:100 dilution) at 4 °C overnight. Immune complexes were incubated with Protein A/G PLUS-Agarose beads for 2 h then collected by centrifugation. The immunoprecipitates were washed 4 times with IP wash buffer (50 mM HEPES, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 1 mM PMSF), and the pellet was resuspended in sodium dodecyl sulfate (SDS) sample buffer. The proteins were resolved by 10% SDS–polyacrylamide gel electrophoresis (PAGE) then transferred to a nitrocellulose membrane. The blots were probed with rabbit anti-human α_1-AT antibodies, diluted 1:1000 in PBS containing 0.03% Tween 20 (PBST), and then with horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies diluted 1:10,000 in PBST. Bound antibodies were visualized by enhanced chemiluminescence on X-ray film using luminol as the substrate.

2.6. Purification of Cpr2p expressed in Escherichia coli

To express Cpr2p in *E. coli*, the *CPR2* gene without a signal sequence was amplified from *S. cerevisiae* Y2805 genomic DNA by PCR. The forward primer was 5′-CTCAGAATTCTCTGATGTG GGTGAGTTGATT GATCAGGAC-3′ and the reverse primer was 5′-TCCACTCGAGTCAAGAAGAGAGCTCAGGCGTCCA CTCA-3′. After digestion with *Eco*RI and *Xho*I, the *CPR2*-containing fragment was subcloned into the *Eco*RI and *Xho*I sites of the pET28a vector containing a polyhistidine-tag at the 5′ end of the coding region, generating pET28a-CPR2. *E. coli* BL21 (DE3) cells (Novagen Inc., Madison, WI, USA) were transformed with pET28a-CPR2 and grown at 37 °C in LB medium containing 50 μg/ml kanamycin to an optical density at 600 nm of approximately 0.6. Cpr2p expression was then induced by adding 0.1 mM isopropyl β-D-thiogalactoside followed by a 3-h incubation at 37 °C. The cells were harvested and disrupted in buffer (0.1 mM PMSF, 50 mM Tris–Cl, 250 mM NaCl, and 8 mM imidazole, pH 7.9) using a Bandelin sonicator at 43% power and 90% pulse for 2.5 min. The cell lysates were cleared by centrifugation at 14,000 rpm for 30 min in a Hanil Micro 17R+ centrifuge (Hanil Science Industrial Co., Seoul, Korea). The supernatants were loaded on a Ni^{2+}-NTA agarose column pre-equilibrated with binding buffer (50 mM Tris–Cl, 250 mM NaCl, and 8 mM imidazole, pH 7.9) and eluted by a linear gradient of 30–1000 mM imidazole in 50 ml of binding buffer. Purified Cpr2p protein was buffer-exchanged to IP wash buffer using a PD-10 desalting column. The concentrations of the purified proteins were measured with a Bio-Rad DC protein assay kit using bovine serum albumin as the standard.

2.7. PPIase activity assay

A traditional chymotrypsin-coupled assay was used to measure PPIase activity [19]. The chromogenic peptide *N*-succinyl-Ala-Leu-Pro-Phe-*p*-nitroanilide (Bachem, Bubendorf, Switzerland) was freshly dissolved in 0.45 M LiCl in trifluoroethanol [20]. *Cis–trans* isomerization of the Leu–Pro bond, coupled with chymotryptic cleavage of the *trans* peptide was followed by an increase in absorbance at 390 nm of the liberated *p*-nitroaniline on a Beckman DU650 spectrophotometer [21].

2.8. Refolding assay for Z-type α_1-AT

pFEAT30-ATZ was used for Z-type α_1-AT expression in *E. coli* [22]. Recombinant Z-type α_1-AT was expressed as inclusion bodies in *E. coli* BL21 (DE3) and refolded as described previously [3]. In brief, the inclusion bodies were dissolved in 6 M urea and Z-type α_1-AT was quickly purified at 4 °C on a Q-Sepharose™ Fast Flow column equilibrated with refolding buffer (10 mM phosphate, pH 6.5, 50 mM NaCl, and 1 mM EDTA). To achieve the native conformation of α_1-AT, 20 μg of α_1-AT protein was incubated at 30 °C for 1 h with/without an equimolar amount of purified Cpr2p. α_1-AT folding was monitored by 10% nondenaturing gel electrophoresis in a Tris–glycine buffer system.

2.9. Secretion of Z-type α_1-AT proteins

Yeast strains expressing wild-type α_1-AT, Z-type α_1-AT, or Cpr2p in addition to Z-type α_1-AT were cultured in YPGal liquid medium at 30 °C for 48 h. A total of 20 μl of culture supernatant was collected and boiled for 5 min in SDS sample buffer. The proteins were separated by SDS–PAGE and immunoblotted with rabbit anti-human α_1-AT antibodies as described above.

3. Results

3.1. The knockout of CPR2 aggravates the cytotoxicity of Z-type α_1-AT expression in yeast

The role of Cpr2p, an ER PPIase, on the folding of Z-type α_1-AT molecules was examined. Human wild-type and Z-type α_1-AT proteins were expressed in *S. cerevisiae* using the yeast expression plasmids pYInu-AT and pYInu-ATZ, respectively. When differential protein expression between the wild-type and Z-type α_1-AT-expressing yeast cells was analyzed using two-dimensional differential gel electrophoresis and matrix-assisted laser desorption ionization-time of flight tandem mass spectroscopy, Cpr2p was 2.3-fold more abundant in Z-type α_1-AT-expressing cells (Shin, unpublished data). If Cpr2p is critical for the response to a stressful condition of misfolded Z-type α_1-AT protein accumulation in the ER, knocking out the coding gene would exacerbate the cytotoxicity of Z-type α_1-AT. To test this hypothesis, the effect of a *CPR2* knockout on the viability of Z-type α_1-AT-overexpressing cells was studied. The *CPR2*-deleted yeast strain (Open Biosystems Inc.) was transformed with either pYInu-AT or pYInu-ATZ. α_1-AT expression was induced on YPGal plates, and cell growth was monitored visually. Overexpression of Z-type α_1-AT caused slightly reduced cell growth in wild-type yeast (Fig. 1, wt), whereas it exacerbated the cytotoxicity in *CPR2*-deleted cells (Fig. 1, *cpr2Δ*). To confirm that the increased cytotoxicity in the *cpr2Δ* strain was due to loss of the *CPR2* gene, *CPR2* was cloned and reintroduced into the *cpr2Δ* strain. *CPR2* was cloned in pACT2 AD to allow constitutive expression of Cpr2p under control of the yeast *ADH1* promoter. The resulting pACT2-CPR2 construct was co-transformed with pYInu-ATZ into *cpr2Δ* cells, and the expression of both proteins was confirmed by Western blot analysis. Next, whether the strain recovered from the exacerbated Z-type α_1-AT-induced cytotoxicity was monitored. As expected, reintroducing the *CPR2* gene rescued the *cpr2Δ* strain from the aggravated cytotoxicity of Z-type α_1-AT (Supplementary Fig. 1). These results suggest that *CPR2* is involved in the cellular response to misfolded Z-type α_1-AT accumulation.

3.2. Z-type α_1-AT protein interacts with Cpr2p

To examine whether the Z-type α_1-AT molecule interacts with Cpr2p in yeast cells, co-transformed yeast cells harboring pYInu-ATZ and pACT2-CPR2 were subjected to co-immunoprecipitation assays. Cell extracts were prepared from cells grown in YPGal liquid medium to induce α_1-AT expression. The cell extracts were incubated with polyclonal anti-Cpr2p antibodies, and the immune complexes were precipitated with Protein A/G PLUS-Agarose beads. The co-precipitated α_1-AT protein was detected by immunoblotting using polyclonal anti-human α_1-AT antibodies. Z-type α_1-AT was co-immunoprecipitated with Cpr2p from co-transformed yeast cells but not from single-transformed cells expressing either Cpr2p or Z-type α_1-AT alone (Fig. 2). This result shows that Z-type α_1-AT protein interacts with Cpr2p in yeast cells.

3.3. Cpr2p facilitates the folding and secretion of Z-type α_1-AT protein

The results described above suggest that Cpr2p may reduce Z-type α_1-AT-induced cytotoxicity by promoting Z-type α_1-AT protein folding. To test this possibility, Cpr2p was overexpressed in *E. coli* and purified by Ni^{2+}-NTA-agarose column chromatography. Cpr2p purification was followed by 12% SDS–PAGE (Supplementary Fig. 2). PPIase activity of the purified Cpr2p was confirmed using a protease-coupled assay, as described previously [19]. Adding Cpr2p facilitated the peptidyl-prolyl *cis–trans* isomerization of the substrate *N*-succinyl-Ala-Leu-Pro-Phe-*p*-nitroanilide and promoted proteolytic cleavage of the substrate by chymotrypsin. This result shows that purified Cpr2p is a functional PPIase.

Whether Cpr2p can rescue Z-type α_1-AT from retarded protein folding was examined using *in vitro* protein folding assays. Z-type α_1-AT was expressed in *E. coli* as inclusion bodies, and unfolded Z-type α_1-AT was quickly purified as described previously [3]. Refolding to achieve the native conformation of α_1-AT was conducted at 30 °C with/without purified Cpr2p. Wild-type α_1-AT folded immediately into the tight native conformation, whereas the Z-type α_1-AT proteins remained as folding intermediates when incubated alone, as shown previously [3]. However, adding Cpr2p to the folding assay facilitated the folding of Z-type α_1-AT into the native conformation (Fig. 3), rescuing this variant from deficient protein folding.

The increased folding of Z-type α_1-AT by Cpr2p may also permit the variant protein to progress more efficiently through the yeast cell secretory pathway to the extracellular compartment. Culture supernatants from yeast cells expressing wild-type α_1-AT, Z-type α_1-AT, or Cpr2p in addition to Z-type α_1-AT were collected and subjected to immunoblotting to monitor the amount of secreted α_1-AT protein. As expected, a very small amount of Z-type α_1-AT was secreted to the extracellular compartment (Fig. 4, lane 2) compared to wild-type α_1-AT (Fig. 4, lane 1). However, co-expression of Cpr2p significantly increased the extracellular secretion of Z-type α_1-AT protein (Fig. 4, lane 3), although not quite to wild-type levels.

4. Discussion

Many dysfunctional α_1-AT proteins are unstable and easily adopt a loop-sheet polymeric conformation. In particular, high

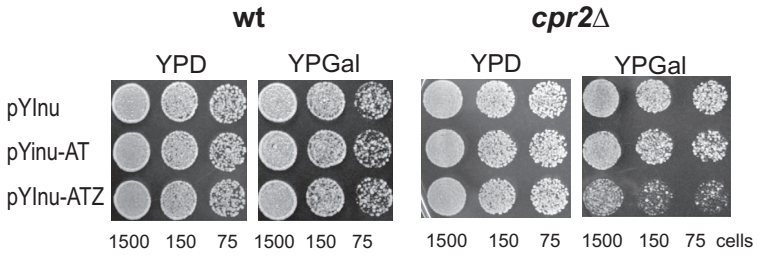

Fig. 1. Cytotoxicity of Z-type α_1-AT in *CPR2* knock-out yeast. The yeast cells containing either wild-type (pYInu-AT) or Z-type α_1-AT expression vector (pYInu-ATZ), were serially diluted, so that aliquots of 10 μl of each dilution contains approximately 1500, 150, 75 cells. Ten microliter of each dilution were spotted onto both YPD plates (not to induce α_1-AT) and YPGal plates (to induce α_1-AT). The plates were incubated at 30 °C for 2–3 days and cell growth was observed. (wt) Overexpression of Z-type α_1-AT induces mild cytotoxicity in the wild-type yeast. (*cpr2Δ*) Overexpression of Z-type α_1-AT causes increased cytotoxicity in *cpr2Δ* strain, compared to that in the wild-type yeast.

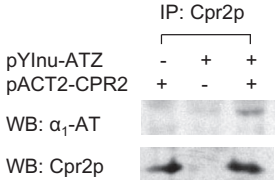

Fig. 2. Co-immunoprecipitation of CPR2 and Z-type α_1-AT. The *cpr2Δ* yeast strain containing pYInu-ATZ and/or pACT2-CPR2 were cultured in YPGal liquid medium. The cell extracts were incubated with polyclonal anti-Cpr2p antibodies (diluted 1:100), and immune complexes were precipitated using Protein A/G PLUS-Agarose beads. The precipitated proteins were resolved on 10% SDS–polyacrylamide gels and analyzed by immunoblotting using polyclonal anti-human α_1-AT antibodies.

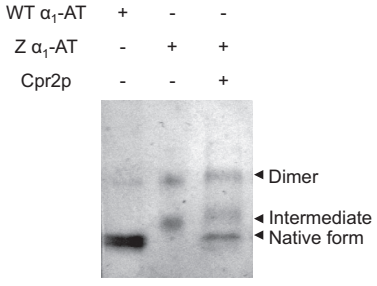

Fig. 3. Refolding assay of α_1-AT. Recombinant α_1-AT was expressed as inclusion bodies in *E. coli* BL21 (DE3) and refolded in refolding buffer (10 mM phosphate, pH 6.5, 50 mM NaCl, and 1 mM EDTA) at 30 °C for 1 h with/without the addition of Cpr2p. The α_1-AT folding was monitored on 10% nondenaturing gel. Migration position of native, intermediate, and dimeric forms are indicated by arrow heads.

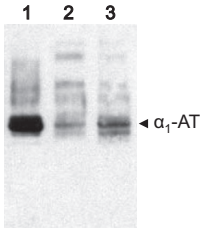

Fig. 4. Increased secretion of Z-type α_1-AT by overexpression of Cpr2p. *S. cerevisiae* expressing either wild-type or Z-type α_1-AT was cultured in YPGal at 30 °C for 48 h, and culture supernatants were collected and subjected to immunoblotting for α_1-AT. Lane 1, culture supernatant from the wild-type α_1-AT-expressing cells; lane 2, culture supernatant from the Z-type α_1-AT-expressing cells; lane 3, culture supernatant from the cells expressing both Z-type α_1-AT and Cpr2p.

body temperature during inflammation is likely to exacerbate α_1-AT polymerization and lead to the clinical symptoms of α_1-AT deficiency [23]. Z-type α_1-AT progresses to the native form very slowly [3] and accumulates intermediate forms that are prone to aggregation in its place of biosynthesis (i.e., the ER of hepatocytes). Polymerization prior to secretion also leads to a plasma deficiency of α_1-AT. However, once folded, the native form of this variant has comparable stability and inhibitory activity to that of the wild-type molecule [3]. Therefore, different approaches such as promoting protein folding might be more appropriate to overcome the molecular defects of this clinically significant Z-type α_1-AT variant.

Some polypeptide chains in the protein folding pathway fold spontaneously into their native state, whereas others require the assistance of enzymes such as chaperones, protein disulfide isomerases, and PPIases. Considering that Z-type α_1-AT has no disulfide bonds, it is reasonable to look for other chaperones that assist in Z-type α_1-AT folding. When the differential protein expression patterns between Z-type and wild-type α_1-AT-expressing yeast cells were studied, Cpr2p was among those proteins showing a greater than two-fold difference between the two cell types. To determine whether the identified Cpr2p is involved in the response to Z-type α_1-AT accumulation, the effect of knocking out *CPR2* on the cell toxicity of Z-type α_1-AT in yeast was studied (Fig. 1). Although the viability of wild-type yeast decreased slightly due to Z-type α_1-AT-induction, the deletion of *CPR2* caused more severe cytotoxicity. Co-immunoprecipitation assays showed that Cpr2p interacted with Z-type α_1-AT (Fig. 2). The purified recombinant Cpr2p protein indeed accelerated Z-type α_1-AT protein folding (Fig. 3), suggesting that Cpr2p reduces the accumulation of aggregation-prone folding intermediates and thus decreases subsequent polymerization leading to liver toxicity. The aggravated Z-type α_1-AT-induced cytotoxicity of the knockout strain might be due to a lack of facilitated protein folding of the Z-type variant by Cpr2p.

The viral vector-mediated transfer of miRNA sequences targeting the α_1-AT gene has been performed to prevent liver cirrhosis caused by Z-type α_1-AT accumulation [24]. Augmentation of autophagic activity either by carbamazepine [25] or transcription factor EB gene transfer [26] increased Z-type α_1-AT polymer degradation. However, this approach also reduced α_1-AT secretion in plasma, indicating that it would not correct the loss-of-function phenotype of the variant. A 6-mer peptide that selectively anneals to Z-type α_1-AT has been used to prevent polymerization of the variant proteins [27], but this peptide would also make the protein nonfunctional. In contrast, our approach promotes Z-type α_1-AT folding into the native form, which can be secreted to mitigate the plasma deficiency of α_1-AT. As with Z-type α_1-AT, protein folding of the D256V and L41P variants was markedly retarded, but their native forms retain sufficient stability and inhibitory activity [3]. Our results indicate that the retarded folding rate of the D256V and L41P variants might also be suppressed by an ER PPIase such as Cpr2p. Because human hCypC shares 59% identity with yeast Cpr2p, it would be interesting to see whether human hCypC can also rescue Z-type α_1-AT from folding arrests in human hepatocytes. The find-

ings of this and future studies will impact the development of therapeutics for folding diseases.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2013-020636).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.156.

References

[1] R.W. Carrell, J.-O. Jeppsson, C.-B. Laurell, S.O. Brennan, M.C. Owen, L. Vaughan, D.R. Boswell, Structure and variation of human α_1-antitrypsin, Nature 298 (1982) 329–334.
[2] M. Brantly, T. Nukiwa, R.G. Crystal, Molecular basis of alpha 1-antitypsin deficiency, Am. J. Med. 84 (1988) 13–31.
[3] C.-H. Jung, Y.-R. Na, H. Im, Retarded protein folding of deficient human α_1-antitrypsin D256V and L41P variants, Protein Sci. 13 (2004) 694–702.
[4] S.E. Smith, S. Granell, L. Salcedo-Sicilla, G. Baldni, G. Egea, J.H. Teckman, G. Baldini, Activating transcription factor 6 limits intracellular accumulation of mutant α_1-antitrypsin Z and mitochondrial damage in hepatoma cells, J. Biol. Chem. 286 (2011) 41563–41577.
[5] A. Le, G.A. Ferrell, D.S. Dishon, Q.Q. Le, R.N. Sifers, Soluble aggregates of the human PiZ alpha 1-antitrypsin variant are degraded within the endoplasmic reticulum by a mechanism sensitive to inhibitors of protein synthesis, J. Biol. Chem. 267 (1992) 1072–1080.
[6] R. Mahadeva, W.S. Chang, T.R. Dafforn, D.J. Oakley, R.C. Foreman, J. Calvin, D.G. Wight, D.A. Lomas, Heteropolymerization of S, I, and Z α_1-antitrypsin and liver cirrhosis, J. Clin. Invest. 103 (1999) 999–1006.
[7] E. Jacob, A. Horovitz, R. Unger, Different mechanistic requirements for prokaryotic and eukaryotic chaperonins: a lattice study, Bioinformatics 23 (2007) 240–248.
[8] L.N. Lin, H. Hasumi, J.F. Brandts, Catalysis of proline isomerization during protein-folding reactions, Biochim. Biophys. Acta 956 (1988) 256–266.
[9] S.L. Schreiber, Chemistry and biology of the immunophilins and their immunosuppressive ligands, Science 251 (1991) 283–287.
[10] D.E. Stewart, A. Sarkar, J.E. Wampler, Occurrence and role of *cis* peptide bonds in protein structures, J. Mol. Biol. 214 (1990) 253–260.
[11] F.X. Macarthur, L.M. Mayr, M. Thornton, Influence of proline residues on protein conformation, J. Mol. Biol. 218 (1991) 397–412.
[12] F.X. Schmid, L.M. Mayr, M. Mücke, E.R. Schönbrunner, Prolyl isomerase: role in protein folding, Adv. Protein Chem. 44 (1993) 25–66.
[13] T.J. Pemberton, J.E. Kay, Identification and comparative analysis of the peptidyl-prolyl *cis*/*trans* isomerase repertoires of *H. sapiens*, *D. melanogaster*, *C. elegans*, *S. cerevisiae* and *Sz. pombe*, Comp. Funct. Genomics 6 (2005) 277–300.
[14] H. Zhu, M. Bilgin, M. Snyder, Proteomics, Annu. Rev. Biochem. 72 (2003) 783–812.
[15] K. Sykes, M. Gething, J. Sambrook, Proline isomerases function during heat shock, Proc. Natl. Acad. Sci. USA 90 (1993) 5853–5857.
[16] K. Dolinski, S. Muir, M. Cardenas, J. Heitman, All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 94 (1997) 13093–13098.
[17] B.H. Chung, S.J. Kim, H.A. Kang, M.-H. Yu, Secretory expression of human α_1-antitrypsin in *Saccharomyces cerevisiae* using galactose as a gratuitous inducer, Biotechnol. Lett. 20 (1998) 307–311.
[18] A. Adams, D.E. Gottschling, C.A. Kaiser, T. Stearns, Techniques and protocols: high-efficiency transformation of yeast, in: M.M. Dickerson (Ed.), Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, 1997, pp. 99–102.
[19] G. Fischer, H. Bang, E. Berger, A. Schellenberger, Conformational specificity of chymotrypsin toward proline-containing substrates, Biochim. Biophys. Acta 791 (1984) 87–97.
[20] J.L. Kofron, P. Kuzmic, V. Kishore, G. Gemmecker, S.W. Fesik, D.H. Rich, Lithium chloride perturbation of *cis–trans* peptide bond equilibria: effect on conformational equilibria in cyclosporine A and on time-dependent inhibition of cyclophilin, J. Am. Chem. Soc. 114 (1992) 2670–2675.
[21] C. Scholz, T. Schindler, K. Dolinski, J. Heitman, F.X. Schmid, Cyclophilin active site mutants have native prolyl isomerase activity with a protein substrate, FEBS Lett. 414 (1997) 69–73.
[22] K.S. Kwon, J. Kim, H.S. Shin, M.H. Yu, Single amino acid substitutions of alpha 1-antitrypsin that confer enhancement in thermal stability, J. Biol. Chem. 269 (1994) 9627–9631.
[23] D.A. Lomas, D.L. Evans, J.T. Finch, K. Seyama, R.W. Carrell, The mechanism of Z α_1-antitrypsin accumulation in the liver, Nature 357 (1992) 605–607.
[24] C. Mueller, Q. Tang, A. Gruntman, K. Blomenkamp, J. Teckman, L. Song, P.D. Zamore, T.R. Flotte, Sustained miRNA-mediated knockdown of mutant AAT with simultaneous augmentation of wild-type AAT has minimal effect on global liver miRNA profiles, Mol. Ther. 20 (2012) 590–600.
[25] T. Hidvegi, M. Ewing, P. Hale, C. Dippold, C. Beckett, C. Kemp, N. Maurice, A. Mukherjee, C. Goldbach, S. Watkins, G. Michalopoulos, D.H. Perlmutter, An autophagy-enhancing drug promotes degradation of mutant α_1-antitrypsin Z and reduces hepatic fibrosis, Science 329 (2010) 229–232.
[26] N. Pastore, K. Blomenkamp, F. Annunziata, et al., Gene transfer of master autophagy regulator TFEB results in clearance of toxic protein and correction of hepatic disease in α_1-antitrypsin deficiency, EMBO Mol. Med. 5 (2013) 397–412.
[27] R. Mahadeva, T.R. Dafforn, R.W. Carrell, D.A. Lomas, 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization: implication for the prevention of Z α_1-antitrypsin-related cirrhosis, J. Biol. Chem. 277 (2002) 6771–6774.