The Brichos Domain-containing C-terminal Part of Pro-surfactant Protein C Binds to an Unfolded Poly-Val Transmembrane Segment*

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Native lung surfactant protein C (SP-C) is a 4.2-kDa acylpeptide that associates with alveolar surfactant phospholipids via a transmembrane α-helix. This helix contains mainly Val, although poly-Val is inefficient in helix formation, and helical SP-C can spontaneously convert to β-sheet aggregates and amyloid-like fibrils. SP-C is cleaved out from a 21-kDa integral membrane protein, proSP-C, in the alveolar type II cell. Recently several mutations localized in the endoplasmic reticulum-luminal (C-terminal) part of proSP-C (CTproSP-C) have been associated with intracellular accumulation of toxic forms of proSP-C, low levels of mature SP-C, and development of interstitial lung disease. CTproSP-C contains a ~100-residue Brichos domain of unknown function that is also found in other membrane proteins associated with amyloid formation, dementia, and cancer. Here we find that recombinant CTproSP-C binds lipid-associated SP-C, which is in β-strand conformation, and that this interaction results in an increased helical content. In contrast, CTproSP-C does not bind α-helical SP-C. Recombinant CTproSP-C(L188Q), a mutation associated with interstitial lung disease, shows secondary and quaternary structures similar to those of wild type CTproSP-C but is unable to bind lipid-associated β-strand SP-C. Transfection of CTproSP-C into HEK293 cells that express proSP-C(L188Q) increases the amount of proSP-C protein, whereas no effect is seen on cells expressing wild type proSP-C. These findings suggest that CTproSP-C binds nonhelical SP-C and thereby prevents β-sheet aggregation and that mutations in CTproSP-C can interfere with this function.

Human surfactant protein C (SP-C)2 is synthesized as a 197-residue proprotein (proSP-C) that is processed to a 35-residue mature transmembrane peptide corresponding to residues 24–58 of proSP-C. ProSP-C is exclusively expressed by the alveolar type II cells and is an integral membrane protein with a type II orientation in the endoplasmic reticulum (ER) membrane (C-terminal in the ER lumen) (1–6). SP-C together with SP-B and phospholipids are responsible for lowering the surface tension at the air/liquid interface and by doing so prevent alveolar collapse, (see Ref. 7 for a review). SP-C is a highly conserved and very hydrophobic protein that lacks known structural homologues. The transmembrane part of SP-C is an α-helix made up of almost only valines. Valines are rare in α-helices and overrepresented in β-strands, and the SP-C α-helix is metastable and can convert to β-sheet aggregates and form amyloid-like fibrils (8–10). Such fibrils of SP-C have been isolated from patients suffering from pulmonary alveolar proteinosis, a lung disease characterized by alveolar accumulation of proteinaceous material. Expression of mature SP-C in the absence of the N- and C-terminal propeptide results in aggregation of SP-C in the secretory pathway and severe lung malformation during embryonic development, indicating that the propeptide is necessary for correct SP-C maturation (1, 11). Mutations in the gene encoding proSP-C are associated with familial and sporadic cases of interstitial lung disease in which the mutations cause the protein to misfold and form toxic intracellular aggregates (12–14). The so far characterized mutations have been identified on only one allele but can result in almost complete absence of mature SP-C. This suggests that mutations in the proSP-C gene generate a dominant-negative toxic gain of function. A mutation that causes exclusion of exon 4 by alternative splicing (SP-C<sup>AE<sub>Exon 4</sub></sup>) leading to a 37-amino acid C-terminally shortened proprotein results in total absence of mature SP-C (12). The exchange of glutamine for leucine at position 188 gives abnormal-appearing lamellar bodies, slowed cell growth, and signs of cytotoxicity (14). Expression of proSP-C with mutations in positions 66 or 73 (E66K and I73T) give rise to detectable amounts of mature SP-C (15, 16). Thus it appears that the effect on proSP-C processing depends on where the mutation is located in the proprotein. The C-terminal part of proSP-C stretches from residues 59 to 197, and it has an unknown structure and function. In this region, a novel domain called Brichos (residues 94–197 in proSP-C) is located (17). Most of the mutations found in the proSP-C gene are located in the Brichos part of proSP-C (18). The Brichos domain contains about 100 amino acids and is also found in other previously unrelated proteins, such as BRI associated with amyloid formation and dementia, ChM-I associated with chondrosarcoma,
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and CA11 associated with stomach cancer (17). All of these proteins have a transmembrane sequence in the N-terminal region, and proSP-C and Bri are posttranslationally processed by proteases. One suggested function for the Brihos domain was a chaperone-like activity, which would prevent misfolding and aggregation of the parent protein; consistent with this hypothesis is the observation that the Brihos domain structurally matches the apical domain of GroEL (17). These findings provided the incentive to investigate the function of the C-terminal part of proSP-C in the relation to folding of the mature peptide.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—A region from nucleotide 175 (in the codon for His59) to nucleotide 991 (in the codon for Ile197) of the proSP-C cDNA sequence (i.e. coding for CTproSP-C) was amplified from FirstChoice PCR-Ready human lung cDNA (Ambion, Cambridgeshire, UK). For the PCR amplification two primers (DNA Technology AIS, Aarhus, Denmark) were used: 5’-GGTGCGCATATCAATGACCAAGACAACACGGCGCTTG-3’ (forward primer) and 5’-GTCAGAGGATCCGGATCCCTAGATGTAGTAGAGCGG-3’ (reverse primer); the underlined sequences are BamHI and Ncol cleavage sites, respectively. The amplified cDNA fragment was digested with BamHI and Ncol and ligated into the expression vector pET-32c (Novagen, Madison, WI) for cloning for thioredoxin, hexahistidine, and S tags upstream of the insertion site. Thrombin and enterokinase cleavage sites allow proteolytic removal of the tags from the fusion protein. The mutant CTproSP-C (L118Q) was created from the wild type cDNA using the forward primer 5’-AGGCACAGATGCTGCGCATTTTAA-3’ and the reverse primer 5’-GCCACACTGTTGCTACGCGCACTCC-3’; the mutation sites are underlined.

For expression of wild type and mutant CTproSP-C, transformed Escherichia coli strain Origami (DE3) pLysS (Novagen, Madison, WI) were grown at 30 °C in LB medium (1 liter of LB medium contains 10 g of tryptone, 5 g of yeast, and 10 g of NaCl) containing 100 μg/ml ampicillin for 16 h with constant stirring. Expression was induced at an A600 around 1.2 by the addition of isopropyl β-D-thiogalactopyranoside to 0.5 mM, the temperature was lowered to 25 °C, and the bacteria were grown for another 4 h. The cells were then harvested by centrifugation at 6000 × g for 20 min at 4 °C and stored at −70 °C. The cells were lysed by lysozyme (1 mg/ml) treatment for 30 min on ice, followed by sonication for 10 min. The cell lysate was centrifuged at 6500 × g for 20 min, and the pellet was suspended in 2 M urea in buffer A (20 mM Tris, 0.5 mM NaCl) containing 5 mM imidazole, pH 8, and sonicated for 5 min. After centrifugation at 6000 × g for 30 min at 4 °C, the supernatant was filtered through a 0.45-μm filter, then mixed with 5 ml of nickel-nitrilotriacetic acid-agarose (Qiagen Ltd., West Sussex, UK), and poured into a column. The column was washed with 100 ml of 2 M urea in buffer A, 5 mM imidazole, pH 8, and then with 100 ml 1 M urea in the same solution and finally with 100 ml of buffer A with 5 mM imidazole only. The fusion protein was then eluted with 300 mM imidazole in buffer A, pH 8. The eluted protein was dialyzed against 20 mM Tris, 50 mM NaCl, pH 7.4. The thioredoxin and His tags were removed by cleavage with thrombin (kind gift from Prof. Steven Olson, Chicago, IL) at an enzyme/substrate weight ratio of 0.002 for 5–16 h at 4 °C. After cleavage, the solution was reapplied to a Ni2+ column to remove the released thioredoxin-His tag fragment and uncleaved fusion protein. After elution from the Ni2+ column, the protein was applied on an anion exchange column (5 ml of HiTrap QFF from Amersham Biosciences) equilibrated with 20 mM Tris, 20 mM NaCl, pH 7.4. The protein eluted as a single peak using a linear gradient from 20 mM to 1 M NaCl and was dialyzed against buffer B (20 mM NaH2PO4, 5 mM NaCl), pH 7.4. The protein concentration was determined from the absorbance at 280 nm using an extinction coefficient of 1.15 μM−1 cm−1. The protein purity was checked by SDS-PAGE under nonreducing conditions and by nondenaturing PAGE.

Protein-Peptide and Protein-Lipid Interactions—Phospholipids were isolated from the modified natural surfactant preparation, Curosurf, which contains 98% (w/w) phospholipids and about 1% each of SP-B and SP-C (Chiesi Farmaceutici, Parma, Italy). Curosurf paste was dissolved in MeOH/CHCl3 (4:1, v/v), and phospholipids were separated from SP-B and SP-C by reversed phase liquid chromatography using a Lipidex 5000 column (40 × 6.5 cm; Amersham Biosciences) (19). Synthetic porcine SP-C (LRIPCCPNLKRLLVVLKVVLVVLVGVALLML) was made and purified as described (20), and native SP-C was purified from pig lung homogenates as described (21). Synthetic SP-C forms β-sheet aggregates that can only be dissolved in neat acids, e.g. formic acid (20). For reconstitution of peptides and phospholipids, surfactant phospholipids were dissolved in MeOH/CHCl3 (1:1, v/v) and peptides, synthetic SP-C, or native SP-C dissolved in formic acid or in MeOH/CHCl3 (1:1 v/v), respectively, were added, and the solutions were incubated at 37 °C until the solvents were evaporated. Lipids and peptides were then resuspended in buffer B, pH 7.4, by vortexing and sonication, giving a final concentration of 5 mg/ml phospholipids and 20 μM peptide. Phospholipids without peptide and Curosurf paste were resuspended in the same manner. Wild type or mutant CTproSP-C was then added to the phospholipids or phospholipid/peptide mixtures to a concentration of 50 μg/ml. The mixtures were incubated at 37 °C for 1 h and then centrifuged at 40 000 × g for 30 min at 4 °C. The pellets and supernatants obtained were analyzed by SDS-PAGE using a 15% gel run under nonreducing conditions. After transfer to a nitrocellulose membrane, CTproSP-C was visualized by Western blot using a horseradish peroxidase-conjugated anti-S tag antibody (Novagen, Madison, WI).

For analysis of interactions between CTproSP-C and water-soluble polypeptide or proteins, the peptides KKVVVVVVVKK (K7V1K5) and KKVVVKVK (K7V1K5) were purchased from Thermo Electron GmbH, Germany. The Lys residues at the N and C termini were introduced to make the peptides soluble in water. CTproSP-C (10 μM) and peptides (10, 50, or 100 μM) were covalently incubated in buffer B, pH 7.4, at 37 °C for 1 h, and thereafter the mixtures were resolved by nondenaturing PAGE using a 12% gel.

CD Experiments—CD spectra in the far-UV region (190–260 nm) were recorded at 22 °C with a Jasco J-810–1505 spectropolarimeter (Jasco, Tokyo, Japan) using a bandwidth of 1 nm and a
response spectrum shown is the average of three consecutive recordings. Spectra were recorded of wild type or mutant CTproSP-C (10 μM) in (i) buffer B, pH 7.4, or in (ii) 2% (w/v) SDS micelles in buffer B and of (iii) synthetic or native SP-C (10 μM) in 2% (w/v) SDS micelles in buffer B and (iv) combinations of CTproSP-C and SP-C peptides in 2% (w/v) SDS micelles. For incorporation of peptides in SDS micelles, SDS was dissolved in MeOH, and synthetic or native SP-C (dissolved in formic acid or MeOH/CHCl₃, respectively) was added, and the solutions were incubated at 37 °C until the solvents were evaporated. SDS micelles, with or without peptides, were then prepared by resuspension in buffer B, pH 7.4. For analysis of CTproSP-C in the presence of SDS micelles, the micelles were prepared first, and then the CTproSP-C was added. Estimation of secondary structure contents from the CD spectra were performed by deconvolution into four simple components (22) or by using the residual molar ellipticity values for 208 and 222 nm (23). Recombinant cystatin A (kind gift from Prof. Ingemar Björk, Uppsala, Sweden) was used instead of CTproSP-C in control experiments.

Human Embryonic Kidney (HEK) 293 Cell Experiments—HEK293 cells stably transfected with human wild type proSP-C (24), proSP-C(L188Q), or empty vector (pTRE2-Hyg; BD Biosciences) were grown in Eagle’s minimum essential medium (Sigma) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml PeSt (SVA, Uppsala, Sweden), and 75 μg/ml hygromycin B (Duchefa, Haarlem, The Netherlands), at 37 °C in 5% CO₂. For testing the ability of CTproSP-C to rescue misfolded proSP-C(L188Q), 1 × 10⁶ of each of the stably transfected cells were plated into 6-well plates and then grown in the same medium as described above, except for the omission of hygromycin B. After 24 h the cells were transfected with ~1 μg/well of a vector (pIRES2) encoding human SP-B₁–₂₃–CTproSP-C (SP-B₁–₂₃ encodes a signal peptide) and enhanced green fluorescent protein. For each transfection 250 μl of OPTI-MEM (Invitrogen) was mixed with 4 μl of Lipofectamine 2000 (Invitrogen), and the mixture was left at room temperature for 5 min. The vector DNA was mixed with 250 μl of OPTI-MEM and then mixed with the Lipofectamine solution. Finally, the DNA/Lipofectamine solution was added dropwise to the cells, and the cells were incubated at 37 °C with 5% CO₂ for 24 h. The cells were then harvested in Laemmli buffer and sonicated for 5 min. The samples were analyzed by SDS-PAGE/ Western blotting with antibodies against the N- or C-terminal prokaryotic proSP-C, and an antibody against enhanced green fluorescent protein.

RESULTS

CTproSP-C Expression and Characterization—A fragment covering residues 59–197 of human proSP-C was expressed in E. coli and purified to homogeneity. This fragment starts just C-terminally of the part corresponding to the mature SP-C peptide and ends with the proSP-C C terminus. CTproSP-C was expressed as a fusion protein with thioredoxin, His, and S tags at the N-terminal end. Solubilization of the pellet obtained after cell lysis with 2 M urea followed by removal of urea, affinity chromatography, and cleavage of the thioredoxin and His tags, resulted in a yield of about 4 mg of S tag-CTproSP-C/liter of culture. The S tag was kept to allow Western blot analysis using commercially available anti-S tag antibodies. Analysis by nonreducing SDS-PAGE (Fig. 1A) shows that >90% of the purified protein corresponds to monomeric CTproSP-C. Western blot analysis (Fig. 1C) indicates that the minor bands with higher molecular mass correspond to oligomeric forms of CTproSP-C. Nondenaturing PAGE (Fig. 1B) likewise shows one major band, with a minor band migrating just anodally. Comparison of the migration of CTproSP-C upon nondenaturing PAGE with that of proteins with similar calculated sizes and isoelectric points indicate that CTproSP-C forms an oligomer, and gel filtration shows that the main form is a tetramer (data not shown). CD spectroscopy of CTproSP-C (Fig. 2) shows a broad minimum
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from 205 to 225 nm, indicating that the protein is folded and contains a mixture of helical and sheet structure. The rather low residual molar ellipticity suggests that CTproSP-C also contains unordered structure. From the spectrum in Fig. 2, a helical content of 20–30% and a $\beta$-sheet content of 30% is estimated. This is in reasonable agreement with the secondary structure content of CTproSP-C predicted using the ExPASy server (www.expasy.org), which yields about 30% helix and 20% $\beta$-sheet. In the presence of SDS micelles (see Fig. 5A) or phospholipid vesicles (data not shown), the amplitudes of the CD spectra of CTproSP-C were increased compared with the values in buffer (Fig. 2), indicating that CTproSP-C is more structurally ordered in the presence of detergents or phospholipids. The structure and stability of CTproSP-C under different conditions is currently under further investigation.

Association of CTproSP-C with Phospholipid Membranes and SP-C—In sproSP-C, the region corresponding to CTproSP-C is localized close to lipid membranes because the SP-C part forms a transmembrane region. We therefore investigated whether recombinant CTproSP-C binds to phospholipid membranes. CTproSP-C was incubated with surfactant phospholipids with and without incorporated SP-C peptides for 1 h at 37 °C, and the membrane fraction was collected by centrifugation at 40,000 × g. The CD spectra in the pellets and supernatants were analyzed by SDS-PAGE and Western blot (Fig. 3). Virtually no detectable CTproSP-C associates with the peptide-free phospholipid membranes. The presence of synthetic SP-C, which is nonhelical (see below) in the phospholipid membranes, however, leads to a significant portion of membrane-associated CTproSP-C (Fig. 3, lane 9), whereas CTproSP-C does not bind to membranes containing native, $\alpha$-helical, SP-C (lane 5). Incubation of phospholipid membranes containing both SP-B and SP-C resulted in detectable CTproSP-C exclusively in the supernatant after centrifugation, showing that CTproSP-C does not bind to SP-B and that the presence of SP-B does not result in binding to helical SP-C. Centrifugation of CTproSP-C in the absence of phospholipid membranes leads to no detectable pellet protein (data not shown). These experiments show that CTproSP-C binds to phospholipid membranes harboring synthetic (nonhelical) SP-C.

CTproSP-C Interactions with Soluble Poly-Val Peptides—The apparent interaction between CTproSP-C and membrane-bound nonhelical SP-C prompted us to investigate whether CTproSP-C can bind a poly-Val peptide in the absence of lipids. CTproSP-C and the peptide $K_2V_7K_2$ were coincubated for 1 h, and then the mixture was resolved by nondenaturing PAGE (Fig. 4). This resulted in the formation of a CTproSP-C-peptide complex when the peptide was present in excess. CTproSP-C incubated with peptides containing no or shorter poly-Val segments ($K_2V_7K_2$ or peptides corresponding to the N-terminal 12 or 17 residues of SP-C; see “Experimental Procedures” for the amino acid sequence of SP-C) did not result in detectable complexes.

CD Experiments of CTproSP-C-Peptide Mixtures in Detergent Micelles—Experiments were carried out to investigate whether interactions between CTproSP-C and SP-C peptides with helical (native SP-C; see CD spectrum in Fig. 5C) or nonhelical (synthetic SP-C; see CD spectrum in Fig. 5A) structure result in a change in conformation. The strictly hydrophobic nature of SP-C necessitated the use of SDS micelles for these experiments. The far-UV CD spectra of CTproSP-C or synthetic SP-C in SDS micelles and the mixture thereof are shown in Fig. 5A. Fig. 5A also shows the calculated spectrum of the mixture of CTproSP-C and synthetic SP-C, obtained by combining the individual spectra of the two components. Evidently the experimental and calculated spectra of CTproSP-C-synthetic SP-C mixtures correspond to a mixture of helical (CTproSP-C) and random coil (synthetic SP-C) structure, as expected for a complex of random coil and helical proteins. On the other hand, the CD spectrum of the mixture of CTproSP-C and native SP-C is that of a mixture of helical and random coil proteins, which is also what we expect for the mixture of helical and random coil proteins.
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Figure 5. A, recorded far-UV CD spectra of wild type CTproSP-C (wt), synthetic SP-C, and the mixture thereof (denoted exp) in SDS micelles. The combination of the spectra for CTproSP-C and the synthetic SP-C is also shown (denoted calc). B, difference spectra obtained by subtraction of the experimentally measured and calculated spectra for mixtures of CTproSP-C and synthetic SP-C (solid line) and for mixtures of CTproSP-C and native SP-C (dashed line). C, corresponding spectra as in A, but with native SP-C instead of synthetic SP-C. The residual molar ellipticity (θ) is expressed in kdeg × cm² × dmol⁻¹.

The 23-residue N-terminal flanking domain of proSP-C is apparently involved in directing proSP-C through the secretory pathway (1, 25), and its presence increases the stability of the SP-C apparent bridge(s) that increase stability. However, CTproSP-C(L188Q) in contrast to the wild type counterpart does not change its structure in the presence of SDS micelles (compare Figs. 2 and 6A). From this we conclude that CTproSP-C(L188Q) can form a soluble protein with an overall secondary structure in water that is similar to that of the wild type protein but that the two proteins differ in the way they are influenced by the presence of detergent micelles. Mixing CTproSP-C(L188Q) with phospholipids containing synthetic SP-C result in significantly lower membrane binding than for the wild type protein (Fig. 3, lanes 9 and 11). Likewise, mixing CTproSP-C(L188Q) with synthetic SP-C in SDS micelles does not result in a change in conformation (Fig. 6). Like wild type CTproSP-C, the L188Q mutant does not bind pure membranes or membranes containing α-helical SP-C (Fig. 3, lanes 3 and 7), nor does mixing CTproSP-C(L188Q) with helical SP-C result in a conformational change (Fig. 6, B and C).

CTproSP-C Effects on Wild Type and Mutant proSP-C Levels in HEK293 Cells—The results described above suggest that CTproSP-C can bind β-sheet SP-C and thereby prevent aggregation (see “Discussion”). To investigate whether CTproSP-C can influence proSP-C in a cell, we used HEK293 cells stably transfected with either wild type proSP-C or proSP-C(L188Q). In these cells about 60–70% of the proSP-C(L188Q) protein is rapidly degraded and only a small fraction escapes to the secretory pathway. Transient transfection of these cells with a CTproSP-C construct containing a signal peptide results in about 70% increase in the amounts of proSP-C (Fig. 7). These results indicate that in cells expressing proSP-C(L188Q), which is expected to lack ability to bind β-sheet SP-C, expression of CTproSP-C in trans can substitute for the mutant, resulting in stabilization and decreased degradation of proSP-C.

**DISCUSSION**

The 23-residue N-terminal flanking domain of proSP-C is apparently involved in directing proSP-C through the secretory pathway (1, 25), and its presence increases the stability of the SP-C α-helix in vitro (25). For CTproSP-C, in contrast, no function has so far been described. CTproSP-C produced in E. coli forms aggre-

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3. H. Johansson, K. Nordling, and J. Johansson, unpublished data.
4. T. E. Weaver, unpublished data.
gates, which can be dissolved by low concentrations of urea and thereafter isolated via His tag affinity chromatography. This yields a homogeneous, water-soluble, and folded protein (Figs. 1 and 2).

CTproSP-C does not associate in a stable manner with phospholipid membranes, but CTproSP-C association with phospholipid membranes becomes significant when they contain nonhelical SP-C peptide. In contrast, when native, α-helical SP-C is bound to the phospholipids, no increase in CTproSP-C binding is observed (Fig. 3). CTproSP-C also binds water-soluble poly-Val peptides, provided that they contain more than five consecutive valines (Fig. 4). CD experiments showed that mixing CTproSP-C with micelle-bound nonhelical SP-C results in a conformational change, indicating that the two polypeptides interact, whereas mixing CTproSP-C with helical SP-C results in no change in conformation (Fig. 5). These data show that CTproSP-C is able to bind to membrane-associated nonhelical SP-C peptides. The association of CTproSP-C with nonhelical, but not helical, SP-C could be a consequence of aggregation of nonhelical SP-C forms that are partly exposed to the membrane exterior, whereas helical SP-C is embedded in the membrane. Protein exposure outside the membrane cannot, however, be the only determinant for CTproSP-C binding because SP-B, with a supposedly more superficial localization, does not bind CTproSP-C. Likewise, electrostatic interactions between CTproSP-C (with an isoelectric point of 5.9) and cationic SP-C cannot entirely account for the observed binding, because C-terminally truncated versions of SP-C ending at positions 12 or 17 (thus lacking all or most of the poly-Val part but retaining all positive charges) do not bind to CT-proSP-C.

The ability to form a compact (possibly α-helical) conformation in the ER translocon seems to reflect the helical propensities measured in aqueous solution (27). In vitro translation experiments suggest that a poly-Val segment is less efficient in helix formation in the ER than a poly-Leu segment; poly-Val segments can be found in two states, one that is helical and membrane-integrated and one that is not, whereas poly-Leu segments only appear in a helical transmembrane state (27). This implies that the poly-Val segment of proSP-C, because of its very low helical propensity and high strand propensity, poses a greater challenge to helix formation than most transmembrane segments. CTproSP-C binding to nonhelical forms of SP-C in the ER can be a way to prevent aggregation.
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The suggestion that the SP-C segment is inefficient in helix formation and that CTproSP-C acts as a scavenger of nonhelical SP-C, thereby possibly preventing aggregation-mediated cytotoxicity, is consistent with literature data. Overexpression of the mature SP-C peptide, i.e. without both the N- and C-terminal propeptides, in transgenic mice results in aggregation of SP-C in the ER and Golgi compartments, leading to cytotoxicity and altered lung development (28). Expression of C-terminally truncated proSP-C causes ER stress, intracellular protein formation, aggregation of aggresomes, cytotoxicity, and disruption of lung morphogenesis in a dose-dependent manner (29–32). Mature SP-C peptide expressed in proSP-C knock-out mice via intratracheal adenovirus injection does not reach the lamellar bodies or the alveolar space, but in wild type mice SP-C secretion is obtained (1). Expression of the mature SP-C part alone, but not SP-C linked to CTproSP-C, in cells in culture apparently results in formation of aggresomes (4).

If CTproSP-C scavenges SP-C that has failed to reach the helical transmembrane state, mutations could affect this function. CTproSP-C(L188Q) was expressed and isolated like the wild type protein, and the CD spectra of the two proteins in water are virtually identical (Figs. 1 and 2). However, in contrast to the wild type protein, CTproSP-C(L188Q) does not bind to nonhelical SP-C associated with phospholipid membranes or detergent micelles (Figs. 3 and 6). These features of CTproSP-C(L188Q) are compatible with the hypothesis that the disease and in vitro cytotoxicity associated with expression of proSP-C(L188Q) (14) are in part mediated by intracellular aggregates of SP-C, formed as a consequence of a reduced capacity to bind nonhelical SP-C. Whether the apparent inability of CTproSP-C(L188Q) to bind nonhelical SP-C is related to reduced interaction with the lipid membrane as such and/or to lack of lipid-induced conformational changes (see Figs. 3 and 6) seen for wild type CTproSP-C (Figs. 2 and 5) remains to be determined.

As noted above, there is a distant structural relationship between the Brichos domain and the apical domain of the chaperone GroEL (17). The amino acid sequence alignment between the human proSP-C Brichos domain (proSP-C positions 94–197) and the apical domain of GroEL that resulted from a structure prediction using a threading approach (17) is shown in Fig. 8. The alignment shown in Fig. 8 is thus the result of a search for an experimentally determined three-dimensional structure that can accommodate the Brichos polypeptide, resulting in a best hit that has no significant amino acid sequence similarity. However, the secondary structure prediction and hydrophathy profile of the Brichos domain fit the structure of the apical domain of GroEL (Protein Data Bank code 1kid) (17). Moreover, conserved residues between the Brichos domain and 1kid map to the hydrophobic core of the structure, and the most conserved region of Brichos when mapped on the 1kid structure correspond exactly with the 1kid (GroEL) peptide-binding site (17). Plotting the so far 20 described mutations in the proSP-C Brichos domain associated with lung disease (13, 18) in this alignment shows that five of the mutations map within one of the two peptide-binding regions of GroEL (33), and another six of the mutations are sequentially close to these regions (Fig. 8).

From this observation and the loss of SP-C binding of CTproSP-C(L188Q), we hypothesize that one mechanism by which mutations in the proSP-C Brichos domain cause interstitial lung disease is by interfering with the ability to scavenge nonhelical (pro)SP-C.

The Brichos domain was recently described (17), and apart from proSP-C, also Bri, chondromodulin, and CA11 were found to harbor Brichos domains. These four proteins contain known or predicted transmembrane regions, but proSP-C is the only example where the transmembrane region also constitutes the mature peptide and shows a long stretch of experimentally verified helical residues with high β-strand propensities. Recently a fifth protein (TFIZ1) containing a Brichos domain was identified: TFIZ1 is expressed and secreted in normal gastric mucosa and binds to a gastric tumor suppressor protein (34). TFIZ1 is homologous to proSP-C but apparently lacks a transmembrane segment. The structural and functional properties of the Brichos-containing proteins so far described are quite different, and the degree of sequence conservation within the Brichos family is comparatively low (17). It is therefore likely that the individual Brichos domains have adopted specific functions, but both CTproSP-C (this work) and TFIZ1 (34) binds to other polypeptide segments. This together with the distant similarity between Brichos domains and GroEL (17) suggest that the Brichos domain may be a versatile polypeptide-binding motif.

5 L. M. Nogee, personal communication.
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