Molecular Medicine 4: 266-275, 1998

Molecular Medicine


# Human Lung Surfactant Protein A Exists in Several Different Oligomeric States: Oligomer Size Distribution Varies between Patient Groups

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Communicated by L. Hood. Accepted February 25, 1998.

## Abstract

**Background:** Lung surfactant protein A (SP-A) is a complex molecule composed of up to 18 polypeptide chains. In vivo, SP-A probably binds to a wide range of inhaled materials via the interaction of surface carbohydrates with the lectin domains of SP-A and mediates their interaction with cells as part of a natural defense system. Multiplicity of lectin domains gives high-affinity binding to carbohydrate-bearing surfaces.
**Materials and Methods:** Gel filtration analyses were performed on bronchoalveolar lavage (BAL) fluid samples from three patient groups: pulmonary alveolar proteinosis ($n$ = 12), birch pollen allergy ($n$ = 11), and healthy volunteers ($n$ = 4). Sucrose density gradient centrifugation was employed to determine molecular weights of SP-A oligomers. SP-A was solubilized from the lipid phase to compare oligomeric state with that of water soluble SP-A.
**Results:** SP-A exists as fully assembled complexes with 18 polypeptide chains, but it is also consistently found in smaller oligomeric forms. This is true for both the water- and lipid-soluble fractions of SP-A.
**Conclusion:** The three patient groups analyzed show a shift towards lower oligomeric forms of SP-A in the following sequence: healthy–pulmonary alveolar proteinosis–pollen allergy. Depolymerization would be expected to lead to loss of binding affinity for carbohydrate-rich surfaces, with loss or alteration of biological function. While there are many complex factors involved in the establishment of an allergy, it is possible that reduced participation of SP-A in clearing a potential allergen from the lungs could be an early step in the chain of events.

## Introduction

Lung surfactant protein A (SP-A) is a member of the collectin family of mammalian proteins. Collectins contain both globular domains, which are calcium-dependent carbohydrate-binding domains (C-type lectins) and extended collagen-like regions. Other members of the collectin family are lung surfactant protein D (SP-D) and the plasma proteins mannose-binding lectin (MBL), conglutinin, and CL-43 (1–3). SP-A is regarded as having central roles in the structure (4,5), metabolism (6), and function (7,8) of surfactant as well as in host defense (9).

SP-A is a complex molecule composed of up to 18 polypeptide chains, each about 30 kD (Fig. 1). Human SP-A is composed of two similar, but distinct types of polypeptide chains, designated $\alpha$2 and $\alpha$3 (10,11). The $\alpha$3 chain differs in amino acid sequence from the $\alpha$2 chain in five places (approximately 2% of residues); most notable is the presence of an additional cysteine

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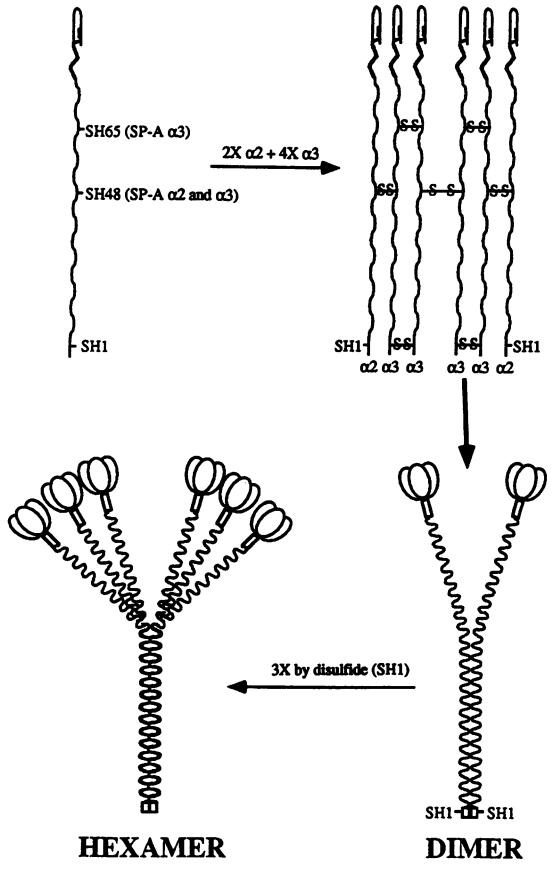


**Fig. 1. The proposed disulfide pattern, oligomerization, and overall morphology of SP-A.** SH denotes free cysteine residues, followed by a number to show their position in the polypeptide, except for SH1, which represents all the cysteine residues in the N-terminal segment. The disulphide pattern is based on the presence of both the α2 and α3 chains in each SP-A molecule, which allows the optimum pairing of all the cysteine residues involved. Based on Lu and Sim (13).

residue in the collagen-like region. Subunits of the protein are each built up by the association of three polypeptide chains, the collagenous regions of which intertwine to form a collagen triple helix. The noncollagenous regions form a globular "head" consisting of three lectin domains. A total of six such subunits may associate to form the characteristic "bunch of tulips" structure seen in electron microscopy of isolated SP-A. In this structure, the globular heads form the "flowers" and the collagen helices form the "stalks" (2,3,12). The range of carbohydrate-bearing targets that the lectin domain of SP-A recognizes has not been extensively explored. The current hypothesis is that SP-A is likely to bind to a wide range of inhaled materials via the interaction of surface mannose or glucosamine with the lectin domains of SP-A, and to mediate their interaction with cells (phagocytes or specialised epithelium) as part of a natural defense system (1–3,13,14). SP-A has been shown to play a role in host defense mechanisms; uptake of SP-A-coated *Staphylococcus aureus* by monocytes via collectin receptor has been demonstrated (15). In addition, SP-A is known to bind to influenza virus (16), pollen grains (17), *Mycobacterium tuberculosis* (18), and *Klebsiella pneumoniae* (19). The importance of SP-A in host defense has been shown in knockout mice, which are susceptible to infection by group B streptococcus (20). Further evidence from knockout studies suggests that the major role of SP-A is not the metabolism of surfactant, as had previously been thought; lack of SP-A has minimal effects on the overall metabolism of other surfactant components (21).

The binding affinity of a single lectin domain for carbohydrate is very low, as shown for MBL (22). Three lectin domains, held together as a single subunit of the collectins, have a higher affinity for carbohydrate-rich surfaces, as shown for CL-43 by Holmskov and colleagues (23). However, the greater multiplicity of lectin domains found in higher-order multimers of SP-A and MBL (up to 18 lectin domains) or SP-D and conglutinin (up to 12 lectin domains) is required to give high-affinity binding to carbohydrate-bearing surfaces. Isolated SP-A has been regarded as being mainly "hexameric" in structure, i.e., 6 subunits each with 3 polypeptides. Oxidation of isolated SP-A leads to depolymerization and loss of binding to whole pollen grains (24). From this observation it was hypothesized that the quaternary structural breakdown was due to oxidative cleavage of disulfide bonds. The disulfide bonding pattern in SP-A is not established, but a pattern based on available sequence information and similarity to C1q has previously been proposed (13).

MBL has been reported to exist as a mixture of oligomers (25,26). Here we present evidence that SP-A exists not only as hexamers (18 lectin heads) but also as tetramers (12), dimers (6), and polypeptides (1,2). The ratio of polymers differs between samples and may have implications for disease. Approximately 90% of SP-A is lipid associated, especially within tubular myelin, whereas only 10% is in the fluid phase (27). It was therefore important to investigate whether the differential state of oligomerization applies to both water-soluble and lipid-associated SP-A.

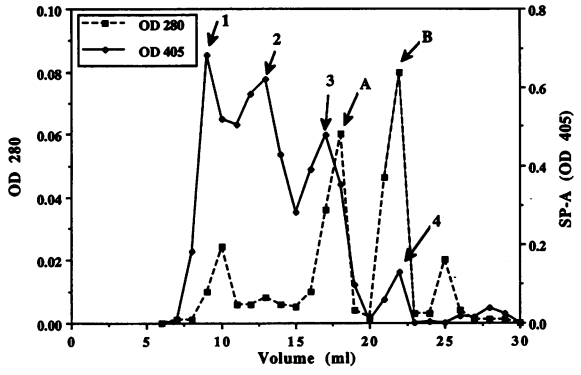


**Fig. 2. Gel filtration analysis of bronchoalveolar lavage (BAL) fluid from a pulmonary alveolar proteinosis (PAP) patient.** An aliquot (250 μl) of BAL from a PAP patient was passed through a Superose 6 gel filtration column. The running buffer was PBS, 5 mM EDTA (pH 7.3). Fractions (1 ml) were analyzed by ELISA to detect SP-A (OD 405). The numbered peaks correspond to the different molecular weight forms of SP-A: 1) hexamers, 2) tetramers, 3) dimers, and 4) apparent single polypeptides. The OD 280 profile shows the elution positions of (A) IgG and (B) albumin.

## Materials and Methods

SP-A is the most abundant lung surfactant protein. The most readily available source of human SP-A is the lung lavage fluid from pulmonary alveolar proteinosis patients. The condition causes a build-up of proteins within the lungs which requires regular removal by lavage.

Bronchoalveolar lavage (BAL) fluid was obtained from three sources: pulmonary alveolar proteinosis (PAP) patients (Dr. Cliff Morgan, Royal Brompton Hospital, London); pollen allergy patients (Dr. Shahin Sanjar, Glaxo Wellcome, Stevenage, U.K.); and healthy volunteers (Dr. T. Tetley, Charing Cross Medical School, London).

*Anti-SP-A Antibodies*

IgG was purified from rabbit polyclonal anti-human SP-A (gift from Dr. P. Eggleton, MRC Immunochemistry Unit), by triple precipitation with sodium sulfate. The IgG pool was preabsorbed on total human serum proteins linked to CNBr-activated Sepharose. The adsorbed IgG fraction was specific for SP-A. On Western blotting the antibodies showed nonspecific recognition of IgG heavy chain (see Fig. 4b) but did not recognize IgG in enzyme-linked immunosorbant assay (ELISA) (see Fig. 2).

*SDS-PAGE*

Proteins were separated by SDS-PAGE as described by Laemmli (28). The resolving gel [10% (w/v) acrylamide, 0.025% (w/v) SDS, 0.375 M Tris-HCl, pH 8.8, polymerized with 0.05% (w/v) ammonium persulfate and 0.05% (v/v) N, N, N′, N′-tetramethyl ethylenediamine] was overlaid with the stacking gel [5% (w/v) acrylamide, 0.025% (w/v) SDS, 125 mM Tris, pH 6.8, polymerized as before]. Protein samples were prepared reduced by the addition of an equal volume of dithiothreitol [30 mg/ml in 0.2 M Tris-HCl, 2% (w/v) SDS, 8 M Urea, pH 8.2] (29). Electrophoresis was performed at 150 V in 192 mM glycine, 0.1% (w/v) SDS, 25 mM Tris, pH 8.2, using 7 × 9 cm minigel format (mini-Protean II apparatus, Bio-Rad). Proteins on gels were detected by silver staining (Bio-Rad) or by Western blot analysis.

*Western Blot Analysis (30)*

Electrophoretic transfer of proteins from SDS-PAGE gels to Immobilon-P membranes (Millipore) was carried out using a Biometra Fast-Blot semi-dry horizontal blotter using transfer buffer 0.039 M glycine, 0.048 M Tris, 0.0375% (w/v) SDS. The transfer membrane was briefly soaked in methanol prior to transfer. A constant current of 0.8 mA/cm$^2$ was applied for 1.5 hr. After transfer, the membrane was blocked by overnight incubation at 4°C with phosphate-buffered saline (PBS), 5 mM EDTA, 0.05% Tween-20, pH 7.3 (PBS-Tween). Antibodies at their appropriate concentration in PBS-Tween were applied and incubated with the blot for 2 hr at room temperature. After extensive washing with PBS-Tween, goat anti-rabbit IgG alkaline phosphatase (Sigma) was added and the blot developed with 5-bromo-4-chloro-3-indolyl phosphate–nitrobluetetrazolium (BCIP-NBT) tablets (Sigma).

*Gel Filtration*

BAL fluid samples were filtered (0.2 μm) before being fractionated on a Superose 6 column (300 × 10 mm) equilibrated in PBS, 5 mM EDTA pH 7.3, (PBS-EDTA), using the fast protein liquid chromatography (FPLC) system (Pharmacia). The column was calibrated using a range of standards [blue dextran, thyroglobulin (bovine), apoferritin, β-amylase, alcohol dehydrogenase, bovine serum albumin (BSA) and carbonic anhydrase] and operated at a flow rate of 0.4 ml/min. The void volume of the column was 7.0 ml.

Aliquots (250 μl) of BAL were loaded onto the column; 1-ml fractions were collected and 100 μl of each analyzed by ELISA.

Standard protein Stokes radius values were calculated as described by Ackers (31) from diffusion coefficients taken from Smith (32):

$$a = KT/6\pi ZD$$

where $a$ is the Stokes radius, $K$ is the Boltzman constant, $T$ is the temperature in °K, $Z$ is the viscosity of water at 20°C, and $D$ is the diffusion coefficient in water at 20°C.

The Stoke's radii of SP-A oligomers was determined by their elution from the gel filtration column relative to the protein standards.

*Sucrose Density Gradient Centrifugation*

Linear sucrose density gradients (12 ml) of 5–40% (w/v) sucrose in 10 mM Tris-HCl, pH 7.4 were prepared as described by Martin and Ames (33). Aliquots (1.5 ml) of sucrose solutions (40% to 5% in 5% intervals) were carefully layered into centrifuge tubes and the linear gradients generated by centrifugation at 16,000 rpm for 16 hr at 4°C (Beckman SW40Ti rotor). Protein samples (500 μl), in 10 mM Tris-HCl, pH 7.4, were loaded onto the gradients and centrifuged at 37,000 rpm for 14 hr at 4°C.

Gradients were fractionated into approximately 20 fractions by peristaltic pumping from the base of the gradient. Individual fractions were analyzed by SDS-PAGE and by measuring OD 280. SP-A was detected in fractions by ELISA.

Sedimentation coefficients ($S_{20,w}$) were estimated by comparison of their mobility to those of standard proteins (Sigma) run simultaneously on a separate gradient under identical conditions. Sedimentation coefficients for these standard proteins [bovine thyroglobulin (19.2S), bovine liver catalase (11.2S), BSA (4.2S), equine skeletal muscle myoglobin (2.0S)] were taken from Smith (32).

*Estimation of Molecular Weight under Nondenaturing Conditions (34)*

The Stoke's radius and sedimentation coefficient values obtained were used to calculate the molecular weights of various SP-A species, under nondenaturing conditions, from the equation:

$$M_r = (6\pi zNas)/(1 - vr)$$

where $M_r$ is the relative molecular mass, $z$ is the viscosity of water at 20°C, $N$ is Avogadro's number, $a$ is the Stoke's radius, $r$ is the density of water at 20°C, and $v$ is the partial specific volume of the protein [taken as 0.712 $cm^3/g$ for SP-A, calculated from the known amino acid sequence (23)]. This calculation does not take into account the carbohydrate content of SP-A.

*Screening ELISA for SP-A*

Initially, 96-well microtiter plates (Polysorp™, Nunc) were coated with 100 μl/well of each sample which was left in contact with the plate for 2 hr at room temperature. The plates were then washed four times with 200 μl/well of PBS-Tween before the nonspecific protein binding sites on the plastic were blocked by incubation with PBS-Tween (300 μl/well) overnight at 4°C. Rabbit anti-human SP-A antibody in PBS-Tween was added to each well (total 100 μl). Incubations with primary antibody were carried out for 2 hr at room temperature. The plates were washed four times in PBS-Tween and alkaline-phosphatase-conjugated goat antibodies to rabbit IgG (Sigma; diluted to recommended concentration in PBS-Tween) were added and left for 2 hr at room temperature. After four further washes with 200 μl/well PBS-Tween, 100 μl/well of *p*-nitrophenyl phosphate (*p*NPP; 1 mg/ml; Sigma) in 25 mM Tris-HCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, pH 7.4 was added and incubated for 30 min or until sufficient color developed. The plates were read at 405 nm.

*Extraction of SP-A from Lipid*

BAL was centrifuged at 10,000 × *g* for 1 hr at 4°C to pellet the lipid-associated material. Solubilization of SP-A was carried out, by repetitive pipetting and vortexing, with the following three solutions (20 ml of each was used to solubilize the pellet from 300 ml of BAL fluid):

*(1)* 6 M Urea in 10 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.4;
*(2)* 10 mM CHAPS (3-((3-Cholamidopropyl)dimethylammonio)-1-propane sulphonate) in 10 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.4; and
*(3)* 1% v/v Triton X-100 (t-Octylphenoxypolyethoxyethanol) in 10 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.4.

Following solubilization, the solutions were again centrifuged at 10,000 × *g* for 1 hr at 4°C to pellet any insoluble material. The supernatants were dialysed against the gel filtration running

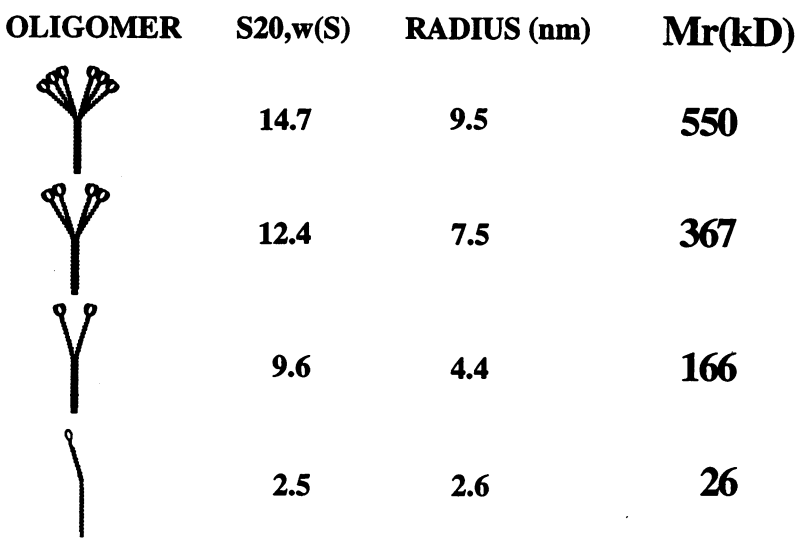


**Fig. 3. Calculated experimental molecular weight values for the SP-A peaks observed by gel filtration.** The samples analyzed were fractions from gel filtration of BAL from a PAP patient. Pooled fractions 8–11, 11–15, 15–19, and 19+ were analyzed by sucrose density gradient centrifugation, fractions were analyzed by ELISA, and the sedimentation coefficient ($S_{20,w}$) of the major SP-A peak was calculated as in Materials and Methods. Values for the molecular weights of each gel filtration peak were calculated from the sedimentation coefficient and Stoke's radius of each sample. The fractions corresponding to the oligomeric forms shown in the figure were fractions 8–11 for hexamers; 11–15 for tetramers; 15–19 for dimers; and 19+ for polypeptides. The expected sizes of SP-A oligomers are: hexamers, 540 kD; tetramers, 360 kD; dimers, 180 kD, and polypeptides, 30 kD. This is based on the molecular weight of a single polypeptide being approximately 24 kD with up to 6 kD of oligosaccharides.

buffer (PBS, 5 mM EDTA). An aliquot (10 ml) of the Triton X-100 solubilized material was added to 1 ml of Bio-Beads SM-2 (Bio-Rad) to remove the Triton X-100. Solubilized material was run on gel filtration and the fractions (1 ml) were analyzed for SP-A content by ELISA. Filtered BAL fluid from the same patient was also run on gel filtration to allow a comparison between the water and lipid soluble portions of SP-A.

## Results

*Size Analysis of SP-A Molecules*

Gel filtration of aqueous phase BAL (250 μl) produces four peaks containing SP-A (as determined by ELISA). The result of a typical gel filtration of the aqueous phase of BAL from a patient with pulmonary alveolar proteinosis (PAP) is shown in Figure 2. A total of 12 PAP patient samples were analyzed by gel filtration. The OD 405 peaks correspond to SP-A: *(1)* hexamers; *(2)* tetramers; *(3)* dimers; and *(4)* polypeptides, as shown by the Stoke's radius, calculated from gel filtration of the standard proteins, and by sucrose density gradient centrifugation. A summary of this information is shown in Figure 3. The complexity of the protein components of whole BAL is illustrated in Figure 4A and the specificity of rabbit anti-human SP-A antibodies is shown in Figure 4B. The 30 and 60 kD bands of SP-A that are characteristic in PAP patients (35) are evident. Weaker diffuse staining of IgG heavy chain is also evident in BAL and serum. The relative intensity of 30 and 60 kD bands is variable between samples of SP-A (see, e.g., Fig. 4C). The 60 kD band is likely to represent a dimer, but it is not known whether the dimer occurs naturally or is an artefact of denaturation. Fractions from the different peaks of SP-A were analyzed by Western blot to show that there is no apparent proteolytic degradation of polypeptide chains (Fig. 4C).

BAL fluid samples from four healthy patients were also analyzed by gel filtration in 250-μl aliquots. The SP-A distribution (OD 405) in these cases was shifted towards a lower elution volume, indicating that an increased proportion of high-molecular-weight oligomers was present. An example of the elution profile is shown in Figure 5A.

Samples of BAL from 11 patients with birch pollen allergy were taken during February (low season) and between May and July (high sea-

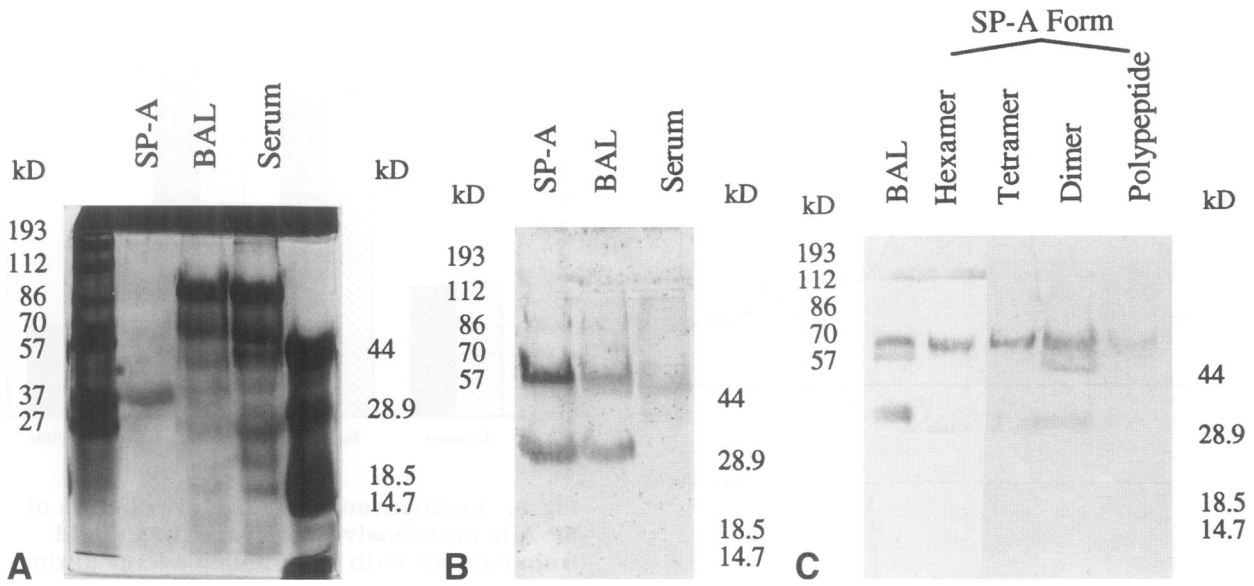


**Fig. 4. Detection of SP-A: (A) The complexity of BAL; (B) polyclonal anti-human SP-A detects two bands of reduced SP-A; (C) detection of SP-A from gel filtration peaks.** (A) Reduced partially purified SP-A (5 μg), BAL (30 μl), and human serum (1 μl) were run on an SDS-PAGE gel (10% w/v acrylamide). Proteins on the gel were detected by silver stain (Bio-Rad). Partially purified SP-A was a gift from Mr. P. Strong (MRC Immunochemistry Unit). (B) IgG was purified from polyclonal rabbit anti-human SP-A by sodium sulfate precipitation and preabsorbed against "aged" serum, and was shown to be specific for SP-A. Reduced SP-A, BAL, and serum were run on an SDS-PAGE gel (10% w/v acrylamide) before blotting to PVDF membrane. The blot was blocked with PBS-EDTA, 0.05% Tween-20 (PBS-Tween), and probed with the anti-human SP-A antibodies (30 μg/ml in PBS-Tween). Bound antibody was detected with goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma; diluted to recommended concentration in PBS-Tween). (C) Representative fractions from within the four peaks of SP-A seen on gel filtration (hexamer, fraction 10; tetramer, 13; dimer, 17; and polypeptide, 21) were run reduced on an SDS-PAGE gel (10% w/v acrylamide) before blotting to Immobilon P membrane (Millipore). A representative sample of BAL was compared on the same blot. The blot was blocked and analyzed as above. Background staining of IgG heavy chain is seen where IgG is present (BAL and dimer fraction). The 30 and 60 kD bands of SP-A are visible in the hexamer–polypeptide fractions, showing that there is no apparent proteolytic degradation. The protein standards used for all the above gels were Sigma high-molecular weight (Cat. No. MW-SDS-BLUE) and Gibco BRL low molecular weight (Cat. No. 16040-016).

son). Aliquots (250 μl) of these 22 samples were analyzed on Superose 6 gel filtration under the same conditions as above. The analysis of SP-A size distribution in each sample was carried out by ELISA of the fractions. A typical SP-A distribution profile from these lavage samples is shown in Figure 5B. The SP-A size distribution in these samples was analyzed by assessing the mean percentage of the major forms of SP-A pooled as stated in the legend to Figure 3. The results of this assessment are shown in Figure 6. There appear to be no major differences between the size distribution of SP-A in the low and high seasons.

A comparison of the overall results from the three groups of patients studied is shown, as percentage of total SP-A, in Figure 7. The BAL fluid from healthy subjects contained the largest proportion of fully assembled, hexameric SP-A, with the pollen allergy patients' BAL fluid containing the smallest proportion of hexameric SP-A. At the other end of the size scale, the proportion of SP-A found in the dimer/polypeptide form was greatest in BAL fluid samples from the pollen allergy patients, where it accounted for nearly half the total amount of SP-A, and was lowest in samples from healthy subjects. Overall, the amount of higher-molecular-weight forms of SP-A decreased across the patient groups in the following order: healthy, pulmonary alveolar proteinosis, and birch pollen allergy.

*Extraction of SP-A from Lipid*

The three methods for extracting SP-A from the lipid phase of BAL fluid, involving solubilization

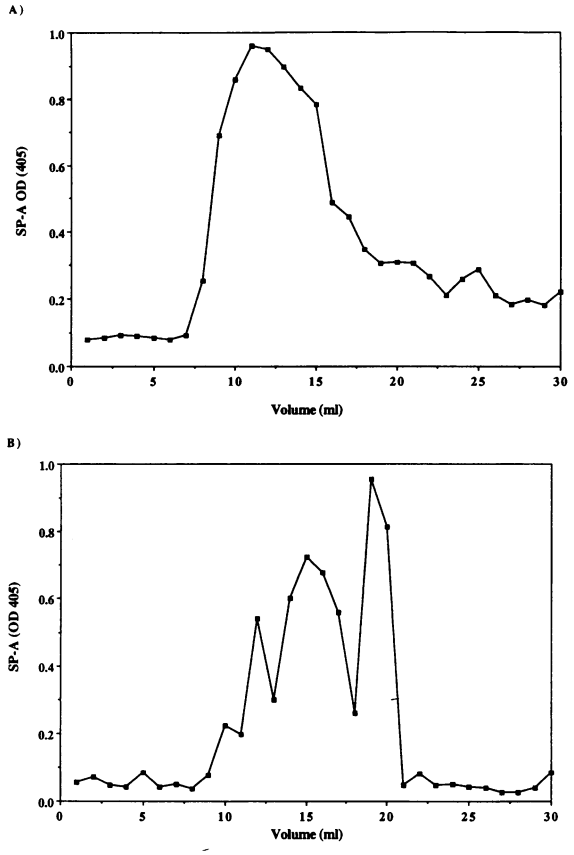


**Fig. 5. Gel filtration analysis of bronchoalveolar lavage (BAL) fluid from (A) a healthy subject and (B) a patient with an allergy to birch pollen.** Aliquots (250 μl) of BAL from healthy subjects and from patients with an allergy to birch pollen were filtered and then passed through a Superose 6 gel filtration column (Pharmacia). Typical profiles for the two groups of patients are shown; the complete analysis of the two groups is shown in Figure 6. The running buffer was PBS, 5 mM EDTA (pH 7.3). Fractions (1 ml) were analyzed by ELISA to detect SP-A (OD 405) as described in Materials and Methods.

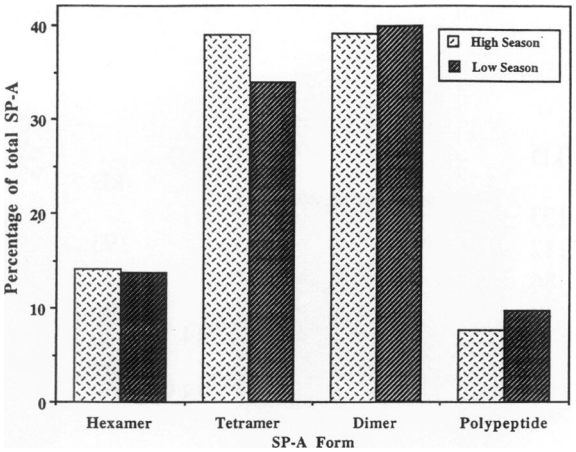


**Fig. 6. Comparison of the size distribution of SP-A in bronchoalveolar lavage (BAL) fluid from patients with birch pollen allergy during the high- and low-pollen seasons.** Samples of BAL from 11 patients with an allergy to birch pollen were taken when there was no birch pollen (low season, February) and when birch pollen was at higher levels (high season, May–July). The average relative amount of SP-A in each form, as determined by gel filtration, is shown for the two seasons. Division of the gel filtration profile into species named hexamer, tetramer, dimer, and polypeptide is as stated in the legend to Figure 2.

with urea, CHAPS, or Triton X-100, all proved successful in obtaining enough SP-A to analyse by gel filtration. All three processes gave similar results in terms of the oligomeric state of the lipid-soluble SP-A extracted. Figure 8 shows that the oligomer distribution of SP-A extracted from lipid is very similar to the distribution of the SP-A from the fluid phase of the same BAL sample.

## Discussion

In contrast with previously published results, which have been performed mainly with purified SP-A, the gel filtration and sucrose density gradient centrifugation results show that unpurified SP-A does not exist purely as fully assembled complexes with 18 polypeptide chains but is consistently found in smaller oligomeric forms. Purification of SP-A by affinity or other method may favor the isolation of only one or a limited range of the oligomers present, therefore analysis of purified SP-A does not provide a complete picture. We have observed that SP-A purified by maltose affinity chromatography is mainly hexameric, because smaller oligomers do not bind to the maltose resin (data not shown). This is the first extensive analysis of unpurified SP-A, although this cannot be said to be native, as it is diluted, or detergent extracted relative to its state in the lung. However, preliminary work with undiluted synovial fluids show a similar distribution. The size distribution is arbitrarily divided into hexameric, tetrameric, dimeric, and polypeptides for convenience in calculation, but there may be a continuous distribution of oligomers (Figs. 2 and 5) that shows equilibration between them. This possibility is being investigated. Depolymerization would be expected to lead to loss of binding affinity for carbohydrate-

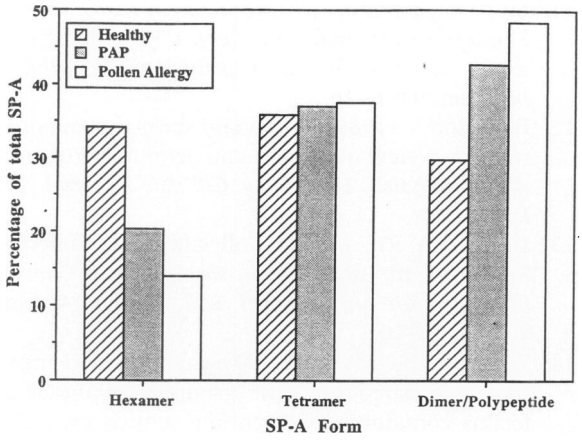


**Fig. 7. Comparison of the distributions of SP-A oligomers in BAL fluids from the three patient groups studied.** The content of SP-A as hexamers, tetramers, and lower-order structures was calculated as a percentage of total SP-A. Division between forms was done on the basis of the pooled fractions as described in Figure 2.

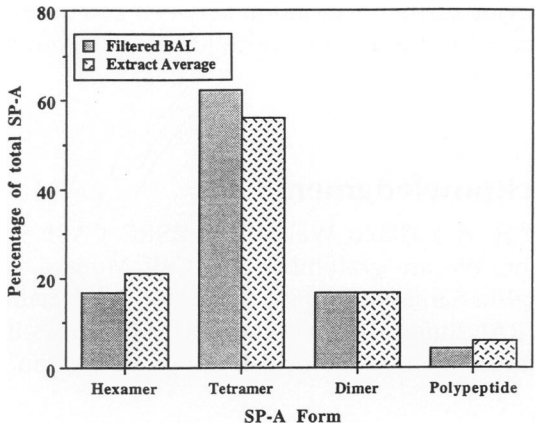


**Fig. 8. Size distribution of SP-A extracted from the lipid phase of bronchoalveolar lavage (BAL) fluid taken from an alveolar proteinosis patient.** SP-A was extracted from the lipid phase of BAL with 6 M urea, 10 mM CHAPS, or 1% Triton X-100 in 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 150 mM NaCl. Solubilized proteins and an aliquot of filtered whole BAL were passed through a Superose 6 gel filtration column (Pharmacia). The running buffer was PBS, 5 mM EDTA (pH 7.3). Fractions (1 ml) were analyzed by ELISA to detect SP-A (OD 405). The relative amounts of each oligomer of SP-A were determined. The ratios for the three extraction methods for SP-A being similar, the average results for the extracted materials were calculated.

rich surfaces (2,24) with loss or alteration of biological function. Loss of opsonic activity would be expected.

The distribution of SP-A molecules, among the oligomeric forms observed, is different in each sample of BAL fluid. However, from the three patient groups that we analyzed (healthy, pulmonary alveolar proteinosis, and birch pollen allergy), there was clearly a larger variation between groups than between individuals within the same group. Of the three groups, the patients with a birch pollen allergy have the smallest proportion of fully assembled SP-A, with less than half that of healthy subjects (Fig. 7). This could indicate that the state of the SP-A had a role in the initial susceptibility to birch pollen, although there was no significant difference in the SP-A size distribution in these patients when samples were compared from low- and high-pollen seasons (Fig. 6). While there are many complex factors involved in the establishment of an allergy, it is possible that reduced participation of SP-A in clearing a potential allergen from the lungs could be an early step in the chain of events.

The extraction of SP-A from lipid shows that it is not only the water-soluble portion that exhibits the different oligomers but all of the SP-A behaves in this way (Fig. 8). Whatever the significance of this distribution, the phenomenon is common to human SP-A from many sources, including both lung and synovial fluid samples.

The predominant subunit appears to contain a dimer of collagen triple helical units (i.e., 6 polypeptides). This supports the assembly pattern proposed for SP-A by Lu and Sim (13). Following this structure further (Fig. 1), it would appear likely that the dimers associate via free SH groups on cysteine residues in the short N-terminal portion of the polypeptide chains. Therefore, it may be possible that damage to these free SH groups, e.g., through oxidation, could result in the inability to form oligomers of SP-A higher than the intermediate dimer unit. Reduction of disulfide bridges with subsequent covalent modification of free SH groups is also a possible route of depolymerization. The factors involved in the oligomerization of SP-A are being further investigated.

There could be many factors involved in the oxidation of SP-A; as the protein ages it becomes increasingly likely that oxidation occurs. The exposure of the lungs to environmental pollutants, such as ozone, $H_2S$, $SO_2$, or nitrogen oxides, could contribute to the breakdown of SP-A. Considering the roles of SP-A in host defense (1–3,13–19) and its reduced ability to bind target carbohydrates when depolymerized (24) could

provide further association between gaseous pollutants and alterations in allergic and immune responses.

## Acknowledgments

T.P.H. is a Glaxo Wellcome/BBSRC CASE student. We are grateful to Dr. Cliff Morgan, Dr. Shahin Sanjar and Dr. T. Tetley for the donation of BAL fluids. This work arose from studies initially funded by the British Lung Foundation.

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