Eur Respir J, 1994, 7, 372–391
DOI: 10.1183/09031936.94.07020372
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European Respiratory Journal
ISSN 0903 - 1936

REVIEW

The proteins of the surfactant system

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ABSTRACT: The structural and functional integrity of pulmonary surfactant depends on several specific proteins. Two of these, SP-A and SP-D, are large and water-soluble, while SP-B and SP-C are small and very hydrophobic. SP-A is an 18-mer of 26 kDa polypeptide chains and contains N-linked oligosaccharides. Structurally, it can be characterized as a collagen/lectin hybrid. Together with SP-B, SP-A is required for conversion of secreted endogenous surfactant to tubular myelin in the alveolar lining. It also regulates surfactant secretion and reuptake of surfactant lipids by type II cells; these functions are probably receptor mediated.

SP-D, a 12-mer of 39 kDa polypeptide chains, is a collagenous glycoprotein with structural similarities to C-type lectins. Both SP-A and SP-D stimulate alveolar macrophages.

SP-B is a 79-residue polypeptide that contains three intrachain disulphide bridges. It exists mainly as a homodimer, which is strongly positively charged and may selectively remove anionic and unsaturated lipid species from the alveolar surface film, thereby increasing surface pressure.

SP-C is a mainly α-helical, extraordinarily hydrophobic polypeptide containing 35 amino acid residues and covalently linked palmitoyl groups. Its α-helical portion is inserted into surfactant lipid bilayers. SP-C accelerates the adsorption of lipid bilayers to an interfacial monolayer. In babies with respiratory distress syndrome, the clinical response to treatment with surfactant containing SP-B and SP-C is much faster than in babies treated with protein-free synthetic surfactant.

We speculate that, in the near future, surfactant preparations based on recombinant hydrophobic proteins will be available for clinical use.

Eur Respir J., 1994, 7, 372–391.

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Keywords: Biophysical activity
pulmonary surfactant
replacement therapy
structural analysis
surfactant proteins

Received: July 30 1993
Accepted for publication August 8 1993

This work was supported by the Swedish Medical Research Council (project Nos 3351 and 10371), the Swedish Society for Medical Research, Oscar II:s Jubileumsfond, Axel Tielmans Minnesfond and the General Maternity Hospital Foundation.

About 60 yrs ago, VON NEERGAARD [1] showed that a substantial part of the resistance to lung expansion is due to alveolar surface tension. However, these results passed without notice until the 1950s, when PATTLE [2] and CLEMENTS [3], independently, rediscovered the presence of a factor which lowered surface tension in the lung. They suggested that the surface active material, surfactant, prevented pulmonary oedema and atelectasis. PATTLE [2] also showed that treatment of the material with trypsin reduced the surface activity, indicating that protein may be an important component of the system. In 1959, AVERY and MEAD [4] showed that respiratory failure in premature infants is due to lack of surfactant. The corresponding disease, which is a major cause of neonatal morbidity and mortality [5], was initially referred to as hyaline membrane disease, from the appearance of dense membranous structures in the collapsed alveoli [6]. Today, this disease is called respiratory distress syndrome (RDS).

Specific treatment of surfactant deficiency goes back to the report by FUJIWARA *et al.* [7], who showed that babies suffering from RDS can be effectively treated by instillation of heterologous surfactant preparations in the airways. Since then, replacement therapy has been widely used in RDS [8]. Phospholipids and hydrophobic proteins are common components of all surfactant preparations from mammalian sources [7, 9–11], indicating that these specific surfactant proteins, present in low amounts and initially considered as merely contaminants [12], are essential components of surfactant preparations for replacement therapy. During the last decade, much information about the structure and the functional properties of the surfactant proteins has been obtained.

Physiological role of pulmonary surfactant

The alveoli are lined by a liquid layer containing surfactant. Without the presence of surfactant, the surface tension would cause the alveoli to collapse, and the main function of the surfactant system is, undoubtedly, to reduce the surface tension at the alveolar air/liquid interface. Under normal conditions, the alveolar surface tension at equilibrium is about 25 $mN \cdot m^{-1}$. According to theoretical considerations [13], this must be reduced to near 0 $mN \cdot m^{-1}$ at the end of expiration, in order to oppose the forces created by decreased alveolar radius. Elegant *in vivo* measurements in rat lung [14] confirmed that the surface tension is reduced to low values at the end of expiration.

Reduction of surface tension is accomplished by the presence of a monolayer consisting mainly of phospholipids at the air/liquid interface. The hydrophobic acyl chains are oriented towards the air, and the polar head groups interact with the liquid surface. During surface compression, this monolayer forms a rigid structure, consisting mainly of dipalmitoylphosphatidylcholine (DPPC), which reduces the surface tension to near zero. Before the phospholipids reach the monolayer, they are transported between several morphological entities. Surfactant is produced by the alveolar type II cell [15, 16], as originally suggested more than 30 yrs ago [17]. The surfactant components are stored intracellularly in dense, multilayered membrane structures, the lamellar bodies [15]. The lamellar body content is excreted into the alveoli, and is there converted [18–20] to a lattice-like structure of tubular lipid double-layers, called tubular myelin [21, 22]. Tubular myelin is the main intra-alveolar reservoir of surfactant [23], from which the monolayer at the air/liquid interface is eventually formed [24]. It is possible that the surface film is also formed from other intra-alveolar membrane structures. For formation of these structures and for transitions between them to occur, nonlipid surfactant components, especially the surfactant proteins, are needed [25, 26]. After use, the surfactant components are constantly removed from the surface film and taken up by the type II cells [27], or degraded by alveolar macrophages [28, 29].

In addition to its ability to reduce surface tension, surfactant interacts with the pulmonary defence system. Surfactant components stimulate phagocytic [30–32], intracellular degradational [33, 34], and migrational [35] capabilities of alveolar macrophages. Furthermore, surfactant components may be directly antibacterial [36], and SP-A (see below) apparently binds with high affinity to a 120 kDa surface glycoprotein of *Pneumocystis carinii* [37], and to cells infected with herpes simplex virus type 1 [38]. Other studies [39, 40] have suggested that SP-A alone enhances phagocytosis, and that it acts specifically on alveolar macrophages. The stimulating effect of SP-A on macrophages seems to depend on bacterial species and growth phase [40]. The functional importance *in vivo* of these findings is unclear. However, because a part of the excreted surfactant material is engulfed by the alveolar macrophages in the removal process, a regulatory role of surfactant in this context is possible. Defective surfactant removal by macrophages is associated with alveolar proteinosis [41, 42], a disease in which the alveoli become overfilled with surfactant material. Finally, a depressive effect by pulmonary surfactant on the defence system may also exist, since surfactant material decreases the stimulatory effects of antigens on pulmonary and blood lymphocytes [43, 44]. This activity is associated mainly with the surfactant lipids [44, 45], and may protect the lungs from inappropriate immune reactions.

Composition of surfactant

The first successful attempts to obtain surfactant material pure enough for chemical characterization were made in the early 1970s [46, 47]. Surfactant from bronchoalveolar lavage fluid, *i.e.* an unresolved mixture of the material lining the alveolar lumen, is composed of 85–90% lipids, about 10% proteins and 2% carbohydrates [25]. The surfactant lipid composition is essentially the same in several mammalian species [48]. The phospholipids, constituting 80–90% of the total lipid weight, consist of about 75% phosphatidylcholine (PC), 10% phosphatidylglycerol (PG), 5% phosphatidylethanolamine (PE), 5% phosphatidylserine (PS) plus phosphatidylinositol (PI), and less than 5% sphingomyelin. Cholesterol is the dominating neutral lipid, and constitutes 6–8% of the total lipids. The lipid composition of isolated lamellar bodies is very similar to that of alveolar surfactant [49], but clearly different from that of plasma membranes [50]. Nearly half of the PC content is DPPC, and this molecule, which is the major single component of surfactant, is also the principal surface tension reducing compound [51]. The high percentage of disaturated PC, especially DPPC, in surfactant is unique, as compared to the composition of PC from other sources [52]. Up to about half of the intra-alveolar content of DPPC is present in the monolayer at the air/liquid interface [53]. The content of DPPC in different animal species correlates both with total alveolar surface area [54], and with the ventilatory rate [55].

The existence of hydrophilic surfactant proteins was recognized early [25], but it was not until 1979 that PHIZACKERLEY *et al.* [56] purified hydrophobic surfactant

Table 1. – Major characteristics of the surfactant-associated proteins

	Polypeptide chain MW kDa	Quaternary structure	Special properties	[Ref]
SP-A	26	18-mer	Glycosylated collagen/lectin hybrid	[57–61]
SP-B	8.7	dimer	Many basic residues	[62–65]
SP-C	3.7	monomer	Very hydrophobic palmitoylated	[65–68]
SP-D	39	12-mer(?)	Glycosylated collagen/lectin hybrid	[69–71]

SP: surfactant protein; MW: molecular weight; [Ref]: references.

proteins from ether/ethanol extracts of porcine surfactant. Four surfactant-specific proteins, with different structural properties, have been identified (table 1). Also, low amounts of serum proteins, such as albumin and immunoglobulin G have been observed in surfactant [47, 72]. The surfactant-specific proteins are called surfactant protein (SP) followed by a letter reflecting their order of discovery [73]. The major surfactant protein, SP-A, and a recently discovered protein, SP-D, are water soluble, while SP-B and SP-C are strictly hydrophobic, requiring organic solvents for solubilization. These two hydrophobic proteins constitute about 1% of the total surfactant mass [74]. Direct attempts to purify SP-B and/or SP-C or related polypeptides from extrapulmonary sources were unsuccessful [75]. In transgenic mice, using SP-C/diphtheria toxin A or SP-C/chloramphenicol acetyltransferase gene hybrids, SP-C was found not to be expressed in any other major organ than the lung [76, 77], indicating that at least this surfactant protein is lung-specific. This might not be the case for all surfactant proteins, since intestinal brush-border excretes particles, similar in structure to lamellar bodies, and SP-B and SP-D have been identified in this tissue by immunological means [78]. A surfactant protein, probably SP-A, has also been found in the middle ear [79].

Surfactant metabolism

Synthesis and secretion

Both surfactant lipids and proteins are produced in the alveolar type II cells [16], which occupy about 5% of the alveolar surface area [80]. The principal lipid component, DPPC, is produced in the endoplasmic reticulum [16], with glucose as dominating precursor [81]. The palmitoyl groups of DPPC originate mainly from palmitoyl-coenzyme A [82]. The lipids are transferred from the endoplasmic reticulum *via* the Golgi complex to the lamellar bodies, and finally appear in intra-alveolar tubular myelin [16]. Surfactant proteins are also produced in the endoplasmic reticulum, but they are transferred to the lamellar bodies *via* so-called multivesicular bodies [16]. A similar picture of the protein routing has emerged from immunological studies with antibodies directed to SP-A [29, 83–85], or SP-B [86, 87]. The lamellar bodies contain both SP-A [84, 85, 88], and hydrophobic proteins [56, 89, 90], but not SP-D [91]. In contrast to alveolar surfactant, where SP-A is the major protein, the lamellar bodies seem to contain mainly SP-B and SP-C [90], indicating that a relatively small amount of secreted SP-A is released with lamellar bodies [92]. The explanation for this is not known, but it might be related to the supposed intra-alveolar functions of the proteins (*cf.* below). Thus, the lamellar body is apparently the site of fusion of surfactant lipid and protein components. The reasons for the apparently divergent intracellular routes for the surfactant lipid and protein components are unknown. There is immunocytochemical evidence that SP-A [84, 85], and SP-B [93] are also synthesized in nonciliated bronchiolar cells, *i.e.* Clara cells. Whether this synthesis contributes significant amounts of surfactant protein to the alveolar surfactant system is not known.

Excretion of surfactant components is accomplished by exocytosis of the lamellar body content [18]. Both synthesis and excretion of surfactant is regulated by several compounds. However, synthesis and secretion are probably independently regulated processes [94]. Increased ventilation augments surfactant secretion, and the effect is apparently mediated by acetylcholine [95], β-adrenergic agents [96], and prostaglandins [96]. Furthermore, β-adrenergic compounds have been reported to increase the excretion of surfactant *via* cyclic adenosine monophosphate (cAMP)-dependent processes [97], protein kinase C-mediated mechanisms [98], leukotrienes [99, 100], and increased intracellular Ca^{2+} levels [101]. Perhaps of greater interest is that this process seems to be regulated in a feed-back fashion, since SP-A has been observed to inhibit surfactant secretion [102, 103]. For inhibition to occur, SP-A must bind to a high affinity site on type II cells. This binding requires Ca^{2+} [104], but the carbohydrate moieties of SP-A (*cf.* below) are not needed [105, 106]. Also, lectins other than SP-A have been shown to inhibit phospholipid secretion from type II cells [107].

Actin filaments are present in human bronchoalveolar lavage [108], and have been shown to be involved in the movement and exocytosis of lamellar bodies [109]. Such actin-dependent mechanisms might be under regulatory control, since in type II cells actin is phosphorylated by a cAMP-dependent mechanism [110].

Regulation of surfactant protein synthesis

The synthesis of surfactant proteins is both developmentally and multihormonally regulated [111–113]. SP-B and SP-C seem to be expressed at an earlier stage of gestation than SP-A. Messenger ribonucleic acids (mRNA) for human SP-B and SP-C are detected from the second trimester [114], whereas, from the third trimester the levels of SP-A mRNA [115] and protein [116] increase in parallel with the surfactant phospholipids. Interestingly, the formation of lamellar bodies precedes the expression of SP-A in mouse embryos [117]. The intracellular processing and secretion of SP-A is dependent on proline hydroxylation, but not on addition of N-linked carbohydrate [118]. Furthermore, SP-B and SP-C are probably independently regulated, since there is no obvious relationship between their respective mRNA levels in individual lungs [114].

The hormonal regulation of the surfactant proteins is complex. SP-B and SP-C levels in foetal cell cultures are increased after exposure to glucocorticoids [114, 119, 120]. However, induction of SP-B is reported to include an increase in both transcription rate and mRNA stability, whilst induction of SP-C requires ongoing protein synthesis, involving an increase in the rate of gene transcription, with no change in mRNA stability [120]. SP-A synthesis has been reported to be both stimulated [115], and inhibited [121, 122], by glucocorticoids. The explanation to this apparent inconsistency is probably that SP-A exhibits a

biphasic, dose-dependent response to glucocorticoid treatment [123, 124], where the stimulatory and inhibitory effects are due to increased transcription and reduced mRNA stability, respectively [125]. Furthermore, in an adenocarcinoma cell-line, glucocorticoid enhanced pro SP-B expression, whilst the expression of SP-A was reduced [126]. Also, cAMP takes part in the regulation of surfactant protein synthesis. In foetal rabbit lung, SP-A mRNA was increased by a cAMP analogue *via* a protein factor [127], and there are indications that the effects of glucocorticoids and cAMP, at least on SP-A and SP-B, are additive [123, 128]. Finally, epidermal growth factor increases, and transforming growth factor-β decreases, the synthesis of SP-A [129].

Intra-alveolar metabolism

During each inspiration, spreading of phospholipids from tubular myelin (or other intra-alveolar membrane structures) to the monolayer at the air/liquid interface has to occur in less than 1 s, in order to cover the expanding liquid surface. Although the directly surface active monolayer consists mostly of DPPC, several other surfactant components seem to be of vital importance. The importance of the surfactant proteins in this context will be discussed below, in light of their structural properties.

The surfactant phospholipid composition, as such, is of importance for rapid adsorption of the monolayer. Unsaturated PC and PG lower the solid to liquid transition temperature of the lipids below body temperature, and thereby facilitate the spreading from tubular myelin to the monolayer. Furthermore, PG and other negatively charged phospholipids may be important for "spontaneous" disruption of those lipid bilayers that touch the air/liquid interface [130]. The surface film first appearing after spreading of the lipids, containing unsaturated species for increased fluidity, is probably refined by physical squeezing out of unsaturated phospholipids, leaving only a stiff DPPC layer behind [131, 132]. Yu and Possmayer [133] and Mathialagan and Possmayer [134] reported that the removal of PG from the monolayer is facilitated by SP-B, and may be further enhanced by SP-A in the presence of SP-B. SP-C did not seem to have any major impact on the refinement process.

The monolayer, presumably after refinement, has a turnover time of 3–11 h [135]. Surfactant lipids [28], and proteins [29, 85, 86] are both removed by alveolar macrophages. However, a more important route of removal [27] and reutilization [136] of surfactant is reuptake by the type II cells. SP-C is removed from the alveoli at a faster rate than PC [137, 138]. Several investigations indicate that surfactant lipids at least are recycled between lamellar bodies and the alveoli [135, 139, 140], although not to the same extent for all lipid species [141–143]. Whether the phospholipids are reutilized as intact molecules, or degraded and used in *de novo* synthesis is unclear [81]. What signals, if any, direct the surfactant components to macrophages or type II cells are not known, but some clues to this riddle have been obtained. Lectins bind to [144] and are taken up by type II cells, and transferred to lamellar bodies [145]. This implies that the lectin-like SP-A and SP-D may stimulate surfactant reuptake. In fact, SP-A has been observed to enhance surfactant uptake by type II cells, and protect PC from degradation [146, 147], and also other, nonsurfactant lectins can stimulate PC uptake by type II cells [148]. SP-A is probably internalized by type II cells *via* receptor-mediated endocytosis [149]. Thus, the regulation of surfactant turnover might involve multiple receptor-dependent functions of SP-A, since it both decreases secretion (*cf*. above), and stimulates reuptake of surfactant components. The higher content of SP-A than SP-B/SP-C in alveoli, and the opposite relationship in lamellar bodies, might be due to a longer intra-alveolar lifetime of SP-A than of the hydrophobic surfactant polypeptides [90], or different secretion pathways for surfactant proteins [92].

A hydrophobic ether/ethanol soluble surfactant protein has been claimed to stimulate the uptake of liposomal phospholipids by type II cells [150]. A negative linkage between synthesis and reuptake might, therefore, exist as a hydrophobic protein inhibits phospholipid synthesis in type II cells [151]. The increased uptake caused by the hydrophobic protein could, however, be entirely accounted for by phospholipid components in the protein fraction [152]. Furthermore, SP-B and SP-C increased the uptake of PC in both type II cells and lung fibroblasts by nonreceptor mediated mechanisms, whilst SP-A specifically enhanced the uptake by type II cells [153].

Structure and molecular biology of surfactant proteins

Hydrophilic proteins

SP-A is the most abundant surfactant protein in the alveoli, constituting about 50% of the total surfactant protein [154, 155]. It actually constitutes a family of molecules with both different sizes (26–38 kDa in the reduced state) [155–163], and different charges (isoelectric points pH 4–5) [72, 159, 164]. The differences are due to a multitude of post-translational modifications. SP-A has been reported to be modified *via* N-linked glycosylation [159, 165], sialylation [72, 158, 162, 164], acetylation [57, 166], sulphation [167], hydroxylation [168], and also by vitamin K-dependent carboxylation of glutamic acid (Glu) residues [169]. However, the proposed presence of γ-carboxyglutamic acid in SP-A has been challenged in other reports [170, 171]. The amino acid sequence of the polypeptide chain from human [57, 58], canine [59], rat [60], and rabbit [172] origins has been deduced from the corresponding complementary deoxyribonucleic acid (cDNA) sequences. The primary translation product is a 247–248 residue-long polypeptide, depending on species, and containing a 20 residue-long signal peptide. The corresponding human gene has been localized to chromosome 10 [173, 174].

Structurally, the SP-A polypeptide chain consists of two different sections. The N-terminal portion is sensitive

to collagenase treatment [165], and has a collagen-like amino acid sequence [58–60, 160, 172], with repeating glycine (Gly)-X-Y triplets (where X and Y are any amino acid residues), and a high content of hydroxyproline. This collagen-like domain has been claimed to be important for interactions between SP-A and phospholipids [175]. The C-terminal domain, on the other hand, is collagenase-resistant [176], and exhibits structural [177], and functional [178, 179] resemblance to lectins. The C-terminal domain, furthermore, contains two intrachain disulphide bridges [180, 181], and the asparagine (Asn) residue that binds carbohydrate. Protein isolated from patients with alveolar proteinosis consists mainly of oligomeric forms of SP-A [182, 183], at least partly formed by interchain disulphides between the C-terminal domains [183]. This indicates that interchain disulphide bridges can occur in this domain, at least in this disease state. A mannose binding protein, also a lectin, from rat liver has a similar molecular architecture, and exhibits structural homology to SP-A [178]. The exon structures and the chromosome localization of the human mannose binding protein gene and the SP-A gene are also similar [184]. Mannose binding protein specifically recognizes bacterial oligosaccharides [185, 186], and the structural resemblance between SP-A and mannose binding protein, thus, further strengthens the probability that SP-A plays a role in host defence mechanisms.

In its native state, SP-A is arranged as a hexamer, with subunits consisting of trimers of polypeptide chains [61], thus, in total forming an 18-mer. The six trimeric molecules are held together by the N-terminal, collagen-like parts, that are arranged in a disulphide-dependent [59, 176], and triple helical [187], fashion. Six triple helices are, thus, held together in a stem, whilst the C-terminal parts are arranged like a floral bouquet (fig. 1) [61, 180, 188], similar to the structure of complement factor C1q [32]. Minor residue heterogeneities between the different SP-A polypeptide chains in a trimer are necessary for further polymerization to occur [189]. By using deletion mutants, SPISSINGER *et al.* [190] studied the influence of the collagenous and lectin-like part, respectively, on the folding and secretion of SP-A in SV40 transformed simian (COS) cells. Apparently, the lectin part folded correctly on its own, *i.e.* without the presence of the collagenous part, whilst the isolated collagenous part did not fold properly. This might indicate that trimers of the lectin part serve as folding nucleation sites, and that the folding then proceeds in a C-terminal to N-terminal direction [190]. The three-dimensional structure of the lectin part of the mannose binding protein has been determined by X-ray crystallography [191], and it indicates that each monomer might contribute one face of the carbohydrate binding site, and that the trimeric structure is, thus, necessary to accommodate high affinity binding. This structure is probably also grossly valid for the SP-A lectin part, considering their similar amino acid sequences.

A protein with a molecular mass of about 10 kDa, immunologically related to SP-A, has been observed [192]. This polypeptide is probably identical to a hydrophobic but ether/ethanol insoluble fragment isolated by KING and co-workers [47]. The genesis of this fragment is not clear, but it might be a metabolic product of SP-A [193, 194].

Recently, another hydrophilic surfactant protein, SP-D, has been identified. It is synthesized by isolated rat type II pulmonary cells [69, 195], but has also been found in Clara cells [91, 195, 196], and alveolar macrophages [91]. SP-D is also present in rat bronchoalveolar lavage [197], and human amniotic fluid [70]. However, lamellar bodies do not contain SP-D [91]. The native molecule is probably made up of four subunits, each of them composed of three apparently identical disulphide-linked glycosylated polypeptides of about 43 kDa in molecular mass [69, 70]. The amino acid sequence, deduced from the human [70], and rat [71], cDNA sequence, indicates that the SP-D polypeptide is 355 residues long, with a short N-terminal section of 25 residues, followed by a

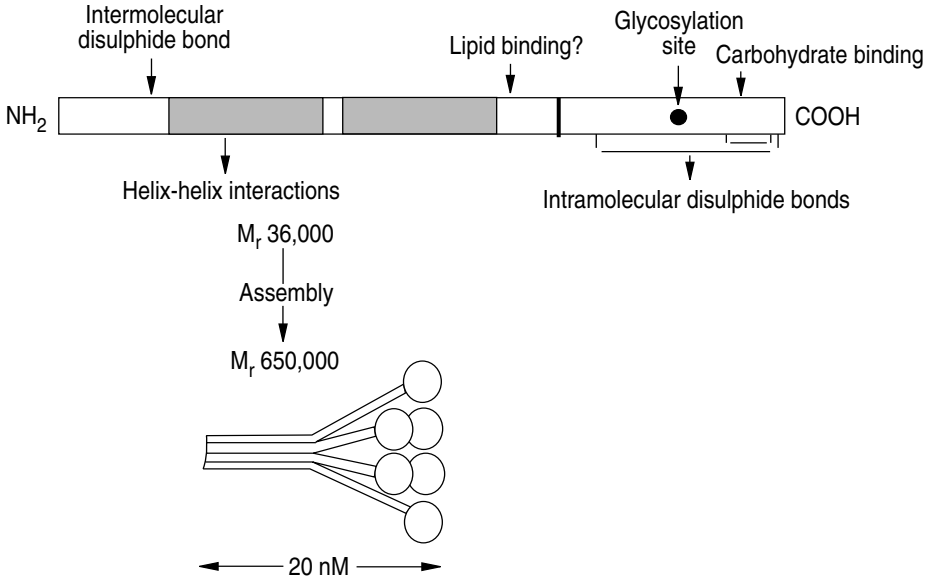

Fig. 1. – Diagrammatic representation of surfactant protein A (SP-A) linear domain structure and assembly into the native 18-meric form. (From [188] with permission).

collagen-like region of 177 residues, and a C-terminal C-type lectin domain of 153 residues. SP-D exhibits considerable sequence similarity to the C-type lectins. Thus, human, rat and bovine SP-D show more than 60% identity in sequence with bovine conglutinin [70, 71, 198]. Furthermore, a newly identified bovine serum lectin (CL-43) also has structural similarities to bovine conglutinin and SP-D [199]. The human gene for SP-D is localized to the long arm of chromosome 10, close to the genes for two other C-type lectins, SP-A and mannose-binding protein [200]. SP-D apparently does not contribute to the surface active properties of pulmonary surfactant [201]. The protein is probably not important for the structural organization of the surfactant phospholipids, since SP-A and SP-D distribute differently in rat bronchoalveolar lavage fluid [202]. However, SP-D is co-purified with lipids [202], and binds PI specifically and in a Ca^{2+}-dependent manner [203, 204]. The functional properties of SP-D in the surfactant system have not been fully elucidated, but rat SP-D enhances the production of oxygen radicals by alveolar macrophages, and may play a role in the host-defence system of the lung [205].

Hydrophobic proteins

PHIZACKERLEY *et al.* [56] first described the presence of protein in organic solvent extracts both of extracellular surfactant and lamellar bodies. These proteins, constituting only a minor part of the surfactant content, have unusual properties, which make them very difficult to purify and characterize. In fact, even the mere existence of surfactant lipoproteins has been denied, after serious but unsuccessful attempts to purify them [206]. Initially, this resulted in a low interest in the hydrophobic surfactant proteins, compared to the more abundant and water-soluble SP-A. However, when it was realized that the hydrophobic proteins are vital components of surfactant preparations for replacement therapy, they received considerable attention. Since then, it has been shown for several species that pulmonary surfactant contains at least two water-insoluble proteins [74, 207, 208], designated SP-B and SP-C. Together they constitute 1–2% of the surfactant weight, and the SP-B/SP-C molar ratio is about 1:2 [74]. Estimates of the molecular masses of SP-B and SP-C by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) have given varying results, probably due to heterogeneous preparations, and to the great hydrophobicity of the proteins. Values of 15–18 kDa and about 7 kDa have been obtained for SP-B in nonreduced and reduced form, respectively [207–209]. Larger, disulphide-dependent polymers of SP-B, with molecular masses of about 20 and 26 kDa, have also been observed [210]. The corresponding values observed for SP-C are about 10 kDa (nonreduced) and about 5 kDa (reduced) [207–209].

Characterization of the airway proteins, SP-B and SP-C was hampered for a long time, mainly because of unusual properties of the polypeptides. Incomplete amino acid sequences of bovine SP-C [208, 211–213], bovine SP-B [208, 212, 213], and porcine SP-B and SP-C [74], have been published. For successful determination of primary structures and post-translational modifications of SP-B and SP-C they had to be purified to apparent homogeneity. Several approaches to the purification of the hydrophobic surfactant proteins have been described [74, 207, 210, 211, 214–219]. CURSTEDT *et al.* [74] introduced repeated chromatography of the surfactant phospholipid fraction on Sephadex LH-60 in chloroform/methanol, 1:1 (v/v), containing 5% 0.1 M HCl. By this procedure, removal of phospholipids and separation of the two hydrophobic surfactant proteins were achieved, resulting in apparently homogeneous fractions of SP-B and SP-C.

Sequence analysis revealed that SP-B is a 79-residue polypeptide, with an exact molecular mass of 8.7 kDa [62]. SP-B is, furthermore, a disulphide-dependent homodimer, and the cysteine residue responsible for dimerization, as well as the intrachain disulphides have been determined for human and porcine SP-B (fig. 2) [63, 64]. Somewhat unexpectedly from its hydrophobic nature, SP-B exhibits a fairly conventional primary structure, with all types of amino acid residues represented, absence of covalently linked fatty acyl groups [65], and with no extremely hydrophobic segment, except for short stretches of hydrophobic residues at positions 37–42 and 54–58. However, the monomer is probably tightly folded, due to the three intrachain disulphide bridges that connect distant parts of the polypeptide chain, and overall there is an excess of aliphatic residues in SP-B. These features may explain its hydrophobic properties.

SP-C contains, dependent on species, 33–35 amino acid residues [66–68], and two juxtapositioned cysteines in the N-terminal part contain one thioester-linked palmitoyl group each (fig. 3) [65]. The total molecular mass of SP-C is 4.2 kDa. Canine SP-C has only one palmitoylcysteine, the second one being replaced by phenylalanine [68]. The SP-C polypeptide sequence is highly unusual, lacking, dependent on species, 8–10 of the 20 common types of amino acid residues. Between positions 13 and 28, it contains only aliphatic branched-chain residues (*i.e.* valine, leucine or isoleucine) and up to seven consecutive valines (fig. 3). This extremely hydrophobic part of the molecule is highly conserved between the species analysed. On the other hand, the N-terminal third of the molecule exhibits some hydrophilicity, having two or three positively charged residues, and also varies between species. This gives the polypeptide chain a detergent-like appearance. However, this overall arrangement is less obvious, considering the entire molecule, since the thioester-linked palmitoyl groups are bound to the N-terminal part, making this part of the molecule hydrophobic also.

Both SP-B and SP-C have also been studied at the cDNA level. Such analyses have revealed that the airway proteins must be derived from larger precursor forms, that are apparently not present in the alveoli. Thus, SP-B is derived from a 40–42 kDa precursor [219–223], pro SP-B (fig. 2), that contains one conserved signal for N-linked glycosylation in the C-terminal region. It is probable that pro SP-B is actually glycosylated, because it is sensitive to endoglycosidase F treatment [119, 224].

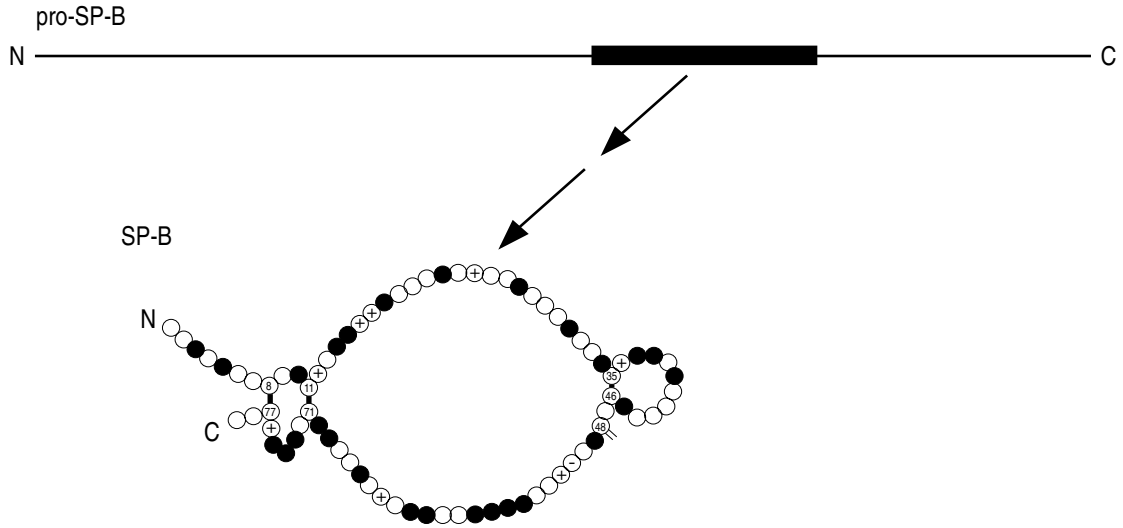

Fig. 2. – Schematic presentation of the surfactant protein B (SP-B) covalent structure and the processing of pro SP-B to SP-B. The thick line in pro SP-B represents the SP-B portion. In SP-B each circle represents one amino acid. Black circles symbolize aliphatic, branched-chain residues; circles with a plus or a minus sign represent residues with basic and acidic side chains, respectively. Filled lines connecting numbered residues symbolize intrachain disulphide bridges, and the open line from cysteine 48 represents the interchain disulphide that links two polypeptide chains into a homodimer.

In an adenocarcinoma cell-line, pro SP-B was processed in several steps, as observed by SDS-PAGE analysis [225]. Firstly a leader-like N-terminal peptide was removed, followed by apparent cleavage of pro SP-B in the vicinity of the residue corresponding to the SP-B N-terminus. Further processing was not observed, and the intra- or extracellular location of the processing was not clarified. SP-C, likewise, emanates from a 20 kDa proform [226–228], pro SP-C (fig. 3), lacking potential glycosylation sites. In contrast to pro SP-B, pro SP-C lacks an N-terminal signal sequence, but the internal SP-C part, as such, has been shown to be important for directing pro SP-C to lipid bilayers; and pro SP-C behaves as a type II transmembrane protein [229]. The SP-B gene is localized to chromosome 2 [230, 231], while two SP-C genes are situated on chromosome 8 [232, 233].

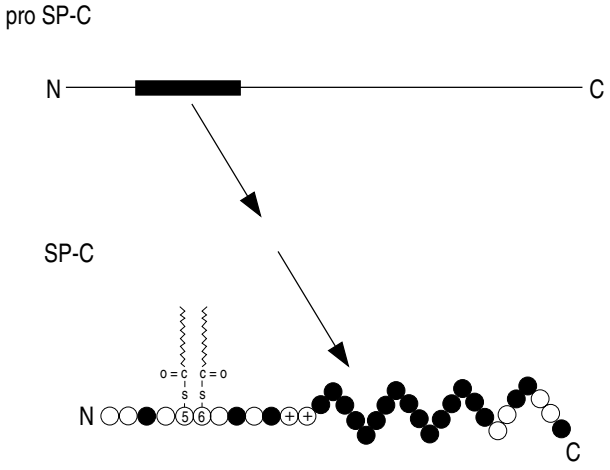

Fig. 3. – Illustration of the processing of pro SP-C to SP-C, and the SP-C structure. Symbols are analogous to those used in figure 2. Cysteines 5 and 6 each have one thioester-linked palmitoyl group. SP: surfactant protein.

Preparations of both SP-B and SP-C exhibit truncated polypeptides, lacking 1 or 2 of the N-terminal residues compared to the longest form [62, 65–68]. The ratio of the different truncated forms is 10 to 30% of the full-length forms, and varies both between animal species and between adult and foetal forms of human SP-C [67]. The biological significance of N-terminal truncations in general is unknown, but it appears to occur in widely different proteins, and to varying extent [234]. Since both SP-B and SP-C are probably derived from the corresponding proforms by proteolysis, the truncated forms may be due to low specificity of the liberating enzyme(s). Later aminopeptidase-like activity cannot, however, be ruled out as a cause for the truncated polypeptides. Artifactual origin seem less probable, because of constant occurrence and species-dependent pattern. Some internal positions in both SP-B and SP-C exhibit polymorphism [62–64, 67]. The explanation behind this phenomenon is not obvious. However, two human genes for SP-C have been identified [232], but only one for human SP-B [231]. Both for the N-terminal truncations and for the internal residue polymorphism, it is difficult to visualize any major structural deviation between the different forms and, therefore, the corresponding functional implications are probably minor, if any.

The overall secondary structures of SP-B and SP-C, solubilized in phospholipid bilayers containing DPPC and PG as major components, have been determined using Fourier transform infra-red spectroscopy [235–238]. SP-B contains about 27–45% α-helical structures [237–239], and about 22% β-sheets [237]. The α-helices interact preferentially with superficial parts of the lipid bilayers, and the basic residues of SP-B (fig. 3) are thought to be important due to interaction with anionic phospholipids [237]. SP-C is predominantly α-helical. The reported α-helical content of SP-C, however, varies between 46% [238], and 90% [236]. This difference is probably not caused by the fact that SP-C of different origin was used

(bovine and porcine, respectively), but may be due to the fact that, in the study by VANDENBUSSCHE *et al*. [236], the SP-C/phospholipid mixtures were centrifuged over a sucrose gradient before analysis. This might remove non-α-helical forms of SP-C, thereby explaining the higher α-helical content obtained. In agreement with this, PASTRANA *et al*. [235] also found that at higher SP-C concentrations (1.5 mol %) β-sheet structures were formed. This was interpreted to be a result of peptide aggregation. In the phospholipid bilayers, the α-helix region of SP-C is oriented parallel to the lipid acyl chains [235, 236], strongly indicating that the hydrophobic part of SP-C is transmembranous. Interestingly, removal of the palmitoyl groups of SP-C, apparently, also reduces the α-helical content significantly when the polypeptide is incorporated into phospholipid bilayers [236].

In conclusion, in spite of their overall hydrophobic nature, SP-B and SP-C are structurally entirely different. The polypeptide chains differ in length and their amino acid sequences are unrelated. The post-translational modification of each polypeptide is also unique, as well as the secondary and quaternary structures of the molecules. The amino acid sequences surrounding the sites necessary to cleave in pro SP-B and pro SP-C to generate the mature airway peptides are also unrelated, indicating that generation of SP-B and SP-C from the corresponding proforms is carried out by different enzymes. The structural differences between SP-B and SP-C, of course, make it tempting to speculate that they fulfil quite separate functions in the surfactant system.

Functional correlations

SP-A, SP-B and SP-C are all present in alveolar surfactant. However, only the hydrophobic surfactant polypeptides, SP-B and SP-C, appear to be essential components in surfactant preparations for replacement therapy (see below), indicating that they have unique functional roles in the formation of the surface active monolayer. Surfactant proteins are required both for the transition between lamellar bodies and tubular myelin, and for the spreading of tubular myelin components to the surface film. Besides lipids extracted from pulmonary surfactant, SP-A and calcium ions are essential for the transition between lamellar bodies and tubular myelin [240–244]. Particles structurally similar to tubular myelin can also be formed by recombining SP-A, SP-B and calcium ions with a mixture of DPPC and PG [245, 246]. Thus, all results indicate that both SP-A and SP-B are essential proteins in the formation of tubular myelin. SP-A has been shown to bind specifically 2–3 Ca^{2+} per molecule [171]. An attractive explanation for the requirement of both SP-A and calcium is that Ca^{2+} neutralizes SP-A carboxylate ions at physiological pH [244].

Although observations clearly indicate specific functions for SP-A in the surfactant system, it seems that SP-A is not required for the creation of the surface active monolayer. Hence, a surfactant preparation containing only lipids and 1% of proteins soluble in organic solvents exhibited surface properties similar to those of natural surfactant [247]. SP-B and/or SP-C, in contrast to SP-A, are required for a rapid adsorption of phospholipids to an air/liquid interface [74, 133, 207, 214–216, 239, 248, 249]. Apparently, both SP-B and SP-C are required for optimal activity, but after addition of either SP-B or SP-C to phospholipids, surface properties that are superior to those of phospholipids alone have been observed [74, 133]. There is also evidence for a co-operative function of the hydrophilic and hydrophobic surfactant proteins in the formation of a surface active monolayer [219, 250]. In a model system, the presence of SP-B and SP-C in the interphase phospholipid monolayer further accelerates the adsorption of additional phospholipids from the subphase [251]. This effect occurs independently of lipid class, but is dependent on the protein concentration, and SP-B seems to be more effective than SP-C in this respect [251, 252]. Furthermore, a monoclonal antibody directed against SP-B [89] inhibited rapid adsorption of surface active material and caused increased surface tension *in vitro* [86]. Mice inoculated with hybridomas producing the antibody developed severe pulmonary damage [253, 254], and neonatal respiratory distress could be induced by instilling the anti-SP-B antibody into the airways of newborn rabbits [255]. None of these effects could be obtained with nonspecific immune sera, indicating that the hydrophobic surfactant proteins, at least SP-B, carry out essential functions in the surfactant system.

Synthetic peptides corresponding to some parts of native SP-B, when associated with phospholipids, possess some biophysical and biological activity [239, 256–260], but are clearly inferior to native SP-B in both respects. Also, synthetic peptides with simplified sequences designed to mimic specific parts of the SP-B polypeptide [258, 260], and amphipathic α-helical peptides based on SP-A [261], exhibit some surface activity in combination with phospholipids. However, although encouraging, such results should perhaps be taken somewhat cautiously, since an amphipathic α-helical decapeptide with a sequence unrelated to SP-B [262], or simple amino acid homopolymers [263] combined with phospholipids also exhibit biophysical activities that are superior to phospholipids alone, but inferior to natural pulmonary surfactant.

Plasma proteins leaking into the airspaces inhibit surfactant and raise the alveolar surface tension, a mechanism that might be of pathophysiological importance in adult respiratory distress syndrome (ARDS) [264]. Fibrin monomer is a particularly potent inhibitor [265]. SP-A has been shown to reverse such an inhibition of surfactant caused by an excess of plasma proteins in the alveoli [266], whilst a recombinant SP-C polypeptide (*i.e.* lacking the covalently linked palmitoyl groups) seems to be less effective in this respect [267]. However, the palmitoyl groups of SP-C (fig. 3) and/or other additional factors are probably important in this context, since an organic-solvent extract of surfactant used for replacement therapy is more resistant to inhibition than a synthetic mixture of phospholipids and hydrophobic proteins [267], and since natural surfactant in high concentration is almost resistant to inhibition by albumin [268]. The overall structural and functional implications of the palmitoyl

groups in SP-C are, however, incompletely understood. SP-C is soluble only in organic solvents even after release of the palmitoyl groups. This is in contrast to several other palmitoylated proteins, where the palmitoyl groups are linked to an otherwise hydrophilic polypeptide chain [269–271]. However, depalmitoylated SP-C and synthetic SP-C without palmitoyl groups are much more difficult to handle than the intact molecule, probably, at least partly, because of formation of oligomeric forms. The disulphide-dependent dimeric forms of SP-C observed upon SDS-PAGE [207–209, 238], may be artifacts formed by inadvertent cleavage of the thioester linkages and subsequent formation of intermolecular disulphides. However, BAATZ *et al*. [238] reported that about 15–20% of bovine SP-C is probably a true dimer. In a model membrane containing DPPC/egg PG/palmitic acid (PA) 68:22:9 (by weight) dimeric SP-C seems to be located in a hydrophobic environment, exhibiting almost exclusively β-sheet structures [238]. The different properties of monomers and oligomers might indicate that the palmitoyl moieties serve as thiol-blocking groups, thereby preventing oligomerization. This is compatible with the facts that canine SP-C only has one palmitoylated cysteine (Cys) [68], and that the molar ratio between palmitoyl groups and Cys residues is close to 1.0 in all species analysed. However, removal of the palmitoyl groups causes conformational changes of the polypeptide chain, without apparent aggregation [236]. There is no support for other fatty acyl groups thioester-linked to SP-C than palmitoyl groups [65, 68], indicating that palmitoyl moieties are of special importance. This suggests that this modification fulfils other functional properties than blocking free -SH groups. Palmitoylation/depalmitoylation has, for example, in another context, been suggested to regulate protein activity [272], perhaps in a manner similar to phosphorylation/dephosphorylation, and protein bound palmitoyl groups apparently have a significantly faster turnover rate than the protein itself [273]. Furthermore, in SP-C the sequence flanking the Cys residue(s) is remarkable, and conserved in all species so far studied. In particular, the proline (Pro) residue next to each Cys might be of importance due to its ability to prevent rotation of the polypeptide backbone. These facts strongly indicate that the thioester-linked palmitoyl groups have a specific impact on the structural and functional properties of SP-C.

Lamellar bodies contain different enzymes [274, 275], but palmitoylation of SP-C probably occurs at an earlier stage, although the exact location is unknown. The intracellular location(s) of the enzyme(s) that process pro SP-C to SP-C is not known either. Hence, it is possible that pro SP-C already is palmitoylated. This is attractive from a functional point of view, since pro SP-C is hydrophilic, except for the SP-C part [227]. Thus, palmitoylation of pro SP-C could be a means of associating the molecule with surfactant lipids before proteolytic generation of SP-C occurs. Although there is no obvious amino acid similarity in the region surrounding palmitoylated cysteines in general [276], the absence of a conserved segment in SP-C that can easily be interpreted as a recognition signal for palmitoylation [65, 68] also favours the concept that the palmitoyl groups are attached to pro SP-C.

The functions of the surfactant proteins at a molecular level are not known. A spectroscopic study [277] indicated that a high concentration of SP-A induces ordering in a mixture of DPPC/dipalmitoyl/phosphatidylglycerol (DPPG), 85:15 (w/w). Such actions are more compatible with a function of SP-A in the packing of phospholipids and/or surfactant reuptake into type II cells, than in the facilitation of rapid surface adsorption of lipid components. The same study showed that an undefined mixture of surfactant proteins other than SP-A induced disordering of the lipids, and SHIFFER *et al*. [278] showed that an unresolved mixture of the hydrophobic surfactant proteins increased permeability of lipid vesicles and caused fusion of liposomes. These effects were increased by the simultaneous presence of Ca^{2+} and PG. Other biophysical measurements [279], although not conclusive, suggested that SP-C might increase lipid adsorption, by inducing the formation of non-bilayer lipid structures. Using fluorescent phospholipid probes, HOROWITZ *et al*. [280] found that SP-C orders the bilayer surface, but disrupts the interior acyl chain packing. The latter effect may be of importance for the rapid spreading of phospholipids from bilayer structures to the interphase monolayer, while the former effect might be of importance for stabilizing the interphase monolayer once it is formed.

It is possible to correlate some of the supposed molecular mechanisms of SP-B and SP-C with their covalent structures. Both SP-B and SP-C, in contrast to the negatively charged SP-A, have an excess of positive charges. SP-B has a great excess of basic residues, which are spread over almost the entire polypeptide chain (fig. 2). It is, therefore, probably capable of making several strong electrostatic interactions with anionic phospholipids, of which PG is predominant in surfactant. A structural model has been proposed [237], whereby SP-B associates with phospholipid bilayers by a combination of electrostatic interactions between polypeptide basic side-chains and anionic phospholipids, and hydrophobic interactions between polypeptide hydrophobic α-helices and lipid acyl chains. The suggested structural relationship between SP-B and the kringle structure of complex serine proteases [63] also indicates that SP-B has binding functions, since some kringles can bind to phospholipids. Interestingly, SP-B cooperates with PG in the rapid spreading of the phospholipid film, and it might also participate in the refinement of the monolayer by removing PG species. A fluorescence anisotropy study [281] indicated that SP-B might indeed interact selectively with PG to yield a more ordered bilayer surface.

The high degree of conservation of pro SP-B outside the SP-B portion [222] may indicate that the proform and/or other proteolytic fragments than SP-B are also functionally important. The observed relationship between pro SP-B and prosaposin [282] suggests defined functional roles for pro SP-B, possibly in the surfactant system, in addition to serving as a precursor to SP-B. Prosaposins are, like pro SP-B, proteolytically processed to smaller fragments [282], and there are indications that serine protease-activity is also required for the metabolism of alveolar surfactant, since α_1-antitrypsin inhibits the conversion of tubular myelin to vesicular forms of surfactant,

while inhibitors of metallo-, thio-, and acid proteases exhibited no inhibitory effect [283]. The corresponding enzyme has been purified [284]. However, pro SP-B or any other fragments of it than SP-B have not yet been shown to be present in the alveoli, and whether pro SP-B actually gives rise to other biologically active fragments than SP-B has not yet been studied.

SP-C has a net positive charge at physiological pH because of two juxtapositioned and conserved basic residues (fig. 3). However, the clearly outstanding structural feature of SP-C is the extremely hydrophobic middle/C-terminal segment, which lacks known counterparts. The hydrophobic segment is certainly long enough to interact with PC liposomes [285], and provided that the α-helical components observed [235, 236] represent one continuous straight α-helix, it will be capable of spanning the width of a lipid bilayer. Thus, the present picture is that the hydrophobic, α-helical middle/C-terminal part of SP-C interacts with the hydrocarbon tails of the phospholipids in the interior part of a surfactant lipid bilayer, while the somewhat hydrophilic N-terminal polypeptide part, especially its charged groups, interacts with the polar phospholipid head groups. Whether the palmitoyl groups interact with the same bilayer as the polypeptide chain, or cross-link different bilayer entities, is unknown. Such interactions might destabilize the bilayers, and thereby facilitate their disruption. Once the conversion of bilayers to monolayers at the air/liquid interface has been initiated, the process might continue spontaneously in a zipper-like fashion, thus explaining that low amounts of hydrophobic surfactant protein are sufficient for optimal activity.

Role of proteins in exogenous surfactants for clinical use

The mission of an exogenous surfactant is to compensate for a defective endogenous supply (as in a preterm baby with RDS), or to overcome surfactant inhibition in clinical conditions characterized primarily by disturbance of lung permeability and accumulation of proteinaceous oedema in the airspaces (as in a patient with ARDS). Patients with pneumonia leading to surfactant dysfunction may also benefit from replacement therapy [286]. Optimal design of the exogenous surfactant preparation may vary with the clinical situation, including the degree of urgency for a therapeutic response. As mentioned above, a number of clinically relevant biophysical and physiological properties of an exogenous surfactant depend on the presence of specific proteins. These properties include speed of action, resistance to inactivation by leaking serum proteins, capacity to stimulate phagocytosis of bacteria and viruses by alveolar macrophages, and influence on bacterial proliferation. Some types of slow-acting exogenous surfactants seem mainly to upgrade the pool of alveolar phospholipids available for recycling, other preparations apparently provide material "ready for use", and therefore quickly restore stability and gas exchange in a surfactant-deficient lung. The proteins make the difference.

Surfactant currently used in clinical practice

The material administered to babies with RDS in the pioneering clinical trial of FUJIWARA *et al*. [7], Surfactant TA (Tokyo Tanabe, Tokyo, Japan), is based on natural lipids and hydrophobic proteins extracted from minced bovine lungs. Synthetic lipids are added to this surfactant to standardize composition and improve the physical and physiological properties. Surfactant TA is manufactured and marketed outside Japan, in slightly modified form, under the trade name Survanta (Abbott, North Chicago, IL, USA). Calf Lung Surfactant Extract (CLSE) [287], and its equivalent Infasurf (ONY, Buffalo, USA) [9], and Alveofact (Thomae, Biberach, Germany) [288] are other widely used bovine surfactants, isolated by lung washing and subsequent extraction of the lavage fluid with organic solvents. Curosurf (Chiesi Farmaceutici, Parma, Italy) [289], used in large European multicentre trials [11, 290, 291], is isolated from minced pig lungs, by a combination of washing, chloroform-methanol extraction and liquid-gel chromatography. This surfactant differs from those previously listed by being devoid of triglycerides, cholesterol and cholesteryl esters. The content of hydrophobic proteins (SP-B, SP-C) is of the same order in all these preparations, about 1%. The hydrophilic proteins (SP-A, SP-D) are absent [292].

Human surfactant, isolated from amniotic fluid by sucrose gradient centrifugation, as described by HALLMAN *et al*. [293], has been used mainly in Finland and California. It contains, apart from lipids, approximately 6% proteins, including hydrophilic (SP-A, SP-D, and nonsurfactant proteins), and hydrophobic polypeptides (SP-B, SP-C).

All these protein-containing preparations, which can be classified as "natural" or "modified natural" surfactants [294], are characterized by rapid adsorption to an air/liquid interface, and low minimum surface tension during cyclic film compression [292]. In a baby with RDS, tracheal instillation of an adequate dose of either type of surfactant usually results in a dramatic improvement of gas exchange. In the first controlled European multicentre trial of Curosurf for treatment of severe neonatal RDS [11], there was a threefold increase in the arterial-to-alveolar oxygen tension ratio (a/AP_{O_2}) within 5 min of treatment with surfactant (200 $mg \cdot kg^{-1}$). Such a rapid effect, requiring alertness of the attending neonatologist and nearly immediate adjustment of the ventilator setting, is not observed with protein-free synthetic surfactants (*cf* below).

Two synthetic surfactants are currently used in clinical practice. Artificial Lung Expanding Compound (ALEC) (Britannia Pharmaceuticals, Redhill, Surrey, UK) is a mixture of DPPC and unsaturated PG 7:3 (w/w), suspended in cold saline [295]. The name of the drug is an abbreviation, but also happens to be the first name of one of its inventors, Alec Bangham. The composition of the drug is based on the concept that unsaturated PG promotes spreading of DPPC at an air/liquid interface. This is undoubtedly true under controlled *in vitro* conditions, at least if the mixture is applied as dry particles onto the surface [296]. Spreading may be less effective after instillation of the material as an aqueous suspension into the airways. ALEC has been evaluated in preterm newborn

animals, with conflicting results, some investigators reporting a moderate effect on lung compliance [297], others denying a physiological effect [298]. ALEC does not seem to be effective in babies with established RDS [299], but improves lung function, reduces mortality, and lowers the incidence of intraventricular haemorrhage when administered prophylactically, especially in babies with a gestational age of less than 30 weeks [295, 300]. Increased lung compliance has been reported in preterm babies treated with ALEC at birth, but this was not statistically significant until 6 h after administration of the drug [301]. The limited effects of ALEC in established RDS can probably be explained by the absence of proteins in the preparation.

Exosurf (Burroughs Wellcome, Research Triangle Park, NC, USA) is a mixture of DPPC, hexadecanol and tyloxapol 13.5:1.5:1 (by weight). The material is suspended in saline at a phospholipid concentration of 13.5 $mg \cdot ml^{-1}$. The alcohol and the detergent are added to enhance spreading of the phospholipid at the air/liquid interfaces of the lung.

According to the original description of the product [302], a film of Exosurf has a minimum surface tension close to 0 $mN \cdot m^{-1}$ during 40% surface compression in a pulsating-bubble system. This is in contrast to more recent observations [303], indicating that Exosurf adsorbs to an air/liquid interface much slower than natural surfactant, and that minimum surface tension remains at 29 $mN \cdot m^{-1}$. Minimum surface tension of an organic solvent extract of natural surfactant in the same assay system is close to 0 $mN \cdot m^{-1}$ [289, 303].

Nevertheless, Exosurf improves gas exchange and lung compliance when instilled into the airways of preterm newborn experimental animals [302, 304], but this effect is less prominent than that obtained with natural surfactant. Exosurf also improves lung function in babies with RDS, but the therapeutic response is insidious [305], and may not be significant until after several hours. Some neonatologists regard this slow effect as an advantage, as it does not require quick readjustment of ventilator settings; others are less impressed and prefer fast-acting natural surfactant preparations.

Again, these biophysical and physiological differences reflect the absence of hydrophobic proteins in the synthetic product. In fact, recent studies with Exosurf have documented that its physical properties (film adsorption rate, minimum surface tension during cyclic film compression) can be improved significantly by adding hydrophobic proteins, in amounts similar to those present in modified natural surfactant isolated by extraction with organic solvents, *i.e.* 1% [303]. Corresponding data were obtained in quasi-static pressure-volume recordings on surfactant-depleted excised rat lungs. Exosurf alone failed to restore normal stability during deflation, but with hydrophobic proteins added the effects of the synthetic preparation approached that of natural surfactant extract (CLSE) [303].

Surfactant proteins and resistance to inhibition

In addition to their direct effects on film adsorption and stability, the surfactant-associated proteins seem to influence resistance to inhibition. Surfactant preparations with admirable *in vitro* properties, including minimum surface tension close to 0 $mN \cdot m^{-1}$ during cyclic film compression, can be made from a mixture of DPPC and PA (9:1, w/w). However, such an artificial surfactant is easily inactivated by serum proteins, and fails to restore normal deflation stability in surfactant-depleted adult lungs [268]. When hydrophobic proteins are added to a suspension of DPPC and egg-PG, the product becomes more resistant to inhibition by albumin [306]. This is also the case for surfactant containing DPPC/PG/PA 68.5:22.5:9 (by weight) and 2% recombinant SP-C, compared to a protein-free suspension of the same lipids [267]. Addition of SP-A to a natural surfactant extract (containing lipids, SP-B and SP-C) also seems to increase resistance to inhibition by albumin and fibrinogen [266]. These differences between various exogenous surfactant preparations may be important in the treatment of ARDS, a disease characterized by a disturbance of lung permeability rather than primary deficiency of surfactant.

Surfactant proteins and lung defence

As mentioned above, SP-A stimulates phagocytosis of bacteria and viruses by alveolar macrophages [38–40, 307], and both SP-A and SP-D enhance the production of oxygen radicals by the same cells [205]. The presence of these proteins in an exogenous surfactant preparation may, therefore, influence the clinical response to replacement therapy in a baby with pneumonia. Studies on preterm newborn rabbits infected with aerosolized Group B *streptococci* (GBS) have indicated that the magnitude of bacterial proliferation in the lungs varies with the type of surfactant instilled into the airways. Exosurf had a bacteriostatic effect; with natural surfactant extract and human amniotic fluid, bacterial proliferation occurred but was not increased compared to inoculated control animals not receiving surfactant [308]. In our studies, treatment with Curosurf reduced 5 h proliferation of GBS inoculated into the lungs of near-term newborn rabbits, in comparison with nontreated infected controls (Herting *et al.*, unpublished). More systematic studies, confronting pneumonia caused by different micro-organisms with different types of surfactants (with or without different surfactant-associated proteins), are clearly required to evaluate the potential of surfactant treatment in infectious lung disease, and to further clarify the roles of surfactant proteins in the pulmonary defence system.

Perspectives for the future

In the near future, a new generation of artificial surfactants will probably be introduced in clinical practice. These are likely to contain surfactant proteins or analogous polypeptides that are either synthesized or produced by gene expression in bacterial or other cell culture systems, and then "reconstituted" with DPPC and, perhaps, other synthetic lipids. Artificial surfactants based on SP-B, SP-C or peptide analogues have been developed, but the

physiological properties of these various preparations have, so far, remained inferior to those of natural surfactant [66, 74, 133, 207, 209, 215, 216, 218, 238, 248, 249, 256–263, 267, 268, 309]. Certain fundamental problems in this context remain to be resolved. These relate to the three-dimensional orientation of the hydrophobic proteins in the aggregates of surfactant lipids generating the surface film. Proper understanding of the interaction between lipids and proteins in the surfactant system is a prerequisite for a rational approach to making artificial surfactant substitutes for replacement therapy.

Acknowledgements: JJ is grateful to H. Jörnvall for continuous support.

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