
Surfactant Protein B: Structure and Function

Sam Hawgood

Department of Pediatrics and Cardiovascular Research Institute, University of California San Francisco,
San Francisco, Calif., USA

Key Words

Surfactant protein B · SP-B · Lamellar bodies ·
Membrane fusion

Abstract

Pulmonary surfactant is a mixture of phospholipids, neutral lipids, and associated proteins. A specific phospholipid, dipalmitoylphosphatidylcholine, is predominantly responsible for the modulation of surface tension at the alveolar air-liquid interface, but other surfactant lipid and protein components play important roles in surfactant function and metabolism. This review will focus on just one of the apoproteins, surfactant protein B, with a description of protein structure and the actions of surfactant protein B on surfactant lipid membranes.

Copyright © 2004 S. Karger AG, Basel

Following the first description of hydrophobic surfactant proteins by King et al. [1] in 1973, Phizackerley et al. [2] identified the protein now called surfactant protein B (SP-B) as a major component of lamellar bodies, the organelle in alveolar epithelial cells that stores pulmonary surfactant. More definitive descriptions and codification of the hydrophobic surfactant apoproteins came 6 years later in 1985 when several groups were able to clearly separate the two hydrophobic surfactant proteins, one from

the other [3]. The cDNA and gene for SP-B were cloned quickly thereafter [4–6]. Although there has been significant progress in the last 17 years [reviewed in detail in 7], much remains to be learnt about the structure and properties of this protein whose functions are essential for post-natal survival.

Structure of SP-B

SP-B isolated from surfactant obtained by lung lavage is a 79-amino-acid homodimer of approximately 18 kDa [5]. As illustrated in figure 1, the SP-B gene is first translated into a significantly larger monomeric preproprotein of approximately 42 kDa. The repeating periodicity of cysteines divides the proprotein into three tandem repeats, each 80–90 amino acids in length. These repeats define the amino- and carboxy-terminal flanking domains and the mature protein itself. The amino- and carboxy-terminal domains are both anionic with net charges of –6 and –2 respectively, in contrast to the strongly cationic and more hydrophobic profile of mature SP-B. In type II alveolar epithelial cells these flanking arms are cleaved in at least two steps to release the mature form of SP-B [8–10]. The proteases have not been definitively identified, but recent work suggests that both cysteine and aspartic proteases possibly including pepsinogen C and napsin A are involved [11–13]. In type II cells, SP-B processing

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
0006–3126/04/0854–0285\$21.00/0

Accessible online at:
www.karger.com/bon

Sam Hawgood, MB, BS
Suite 150, University of California San Francisco
3333 California Street
San Francisco, CA 94118-1245 (USA)
E-Mail Hawgood@itsa.ucsf.edu

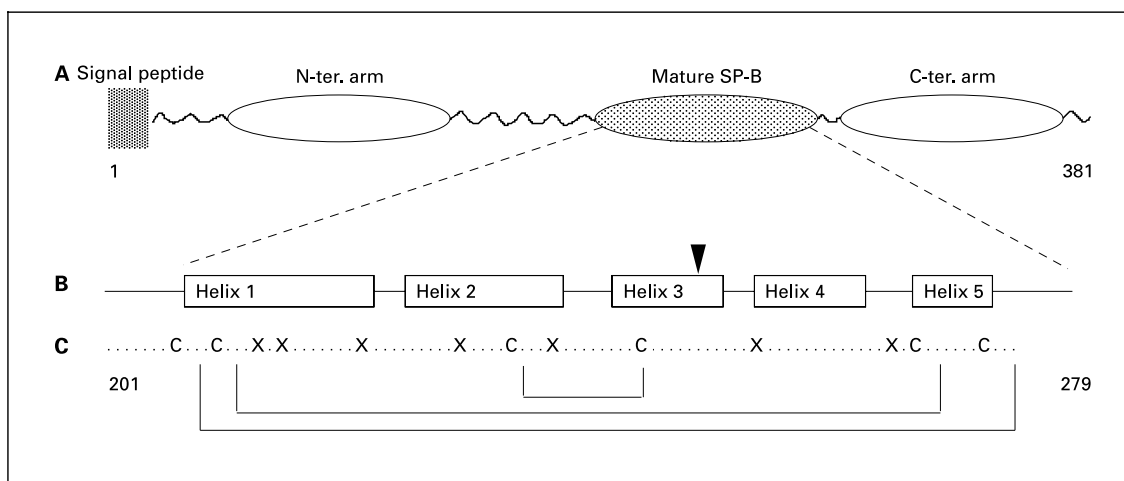


Fig. 1. A schematic representation of the SP-B preproprotein and mature SP-B. **A** The SP-B preproprotein (residues 1–381) with the signal peptide as a shaded box and the three saposin-like domains of the proprotein as ovals. **B** The mature form of SP-B (residues 201–279 of the proprotein) with the five predicted helices placed as per the NK-lysin structure [18]. **C** The consensus sequence defining the saposin-motif showing the periodicity of the 6 conserved cysteines and the location of the conserved hydrophobic residues (X). The disulfide bonds are as described for SP-B [15] and NK-lysin [18]. The location of the intermolecular disulfide unique to mature SP-B is marked by an arrowhead in **B** [from 7 with permission].

occurs in the multivesicular body and is completed before or soon after fusion of this compartment with lamellar bodies [14]. Each mature SP-B monomer contains three intramolecular disulfide bridges that link cys8–cys77, cys11–cys71, and cys35–cys46 [15]. The remaining cysteine in SP-B at position 48 forms the intermolecular bond responsible for dimerization. The disulfide linkages presumably constrain the protein's flexibility and contribute to the remarkable thermal stability of the secondary structure [16] and the unusual resistance to both acid and proteolytic degradation.

Sequence alignments have uncovered a family of proteins that have in common with SP-B the same periodicity of the 6 cysteine residues paired as disulfides and an additional set of 7 hydrophobic residues [reviewed in 17, 18]. This family of saposin-like proteins takes its name from the lysosomal proteins, saposins A–D, the first proteins of this family characterized. In addition to the four saposins (all cleaved from a common precursor, similar to the SP-B proprotein) and SP-B, other proteins sharing the saposin motif include the T cell cytolytic protein, NK-lysin or its human homolog granulysin, the *Entamoeba histolytica* pore-forming proteins, amoebopores, and domains of human acid sphingomyelinase and acyloxyacylhydrolase. The NMR structure for NK-lysin has recently been determined [18]. The strict conservation of key structural resi-

dues in the saposin-like family suggests the general features of the NK-lysin fold will be common to all members of this family including SP-B. Caution must obviously be exercised in transposing the water-soluble NK-lysin structure to SP-B, particularly as the charge distribution in NK-lysin is not shared by SP-B and SP-B stands out from the other saposin-like proteins in being both water-insoluble and dimeric. Nevertheless, the NK-lysin structure significantly extends our understanding of the folding topology adopted by this family of membrane-associated proteins. The loops between helices and solvent-exposed residues are far less conserved between saposin-like members. These more variable residues probably confer binding and functional specificity.

The Actions of SP-B on Membranes – Role in Lamellar Body Organogenesis

There is a growing body of work describing the actions of SP-B, either alone or in cooperation with other surfactant proteins, on membranes. These activities include: membrane binding, membrane lysis, membrane fusion, promotion of lipid adsorption to air-liquid surfaces, stabilization of monomolecular surface films, and respreading of films from collapse phases (fig. 2). As an amphipathic

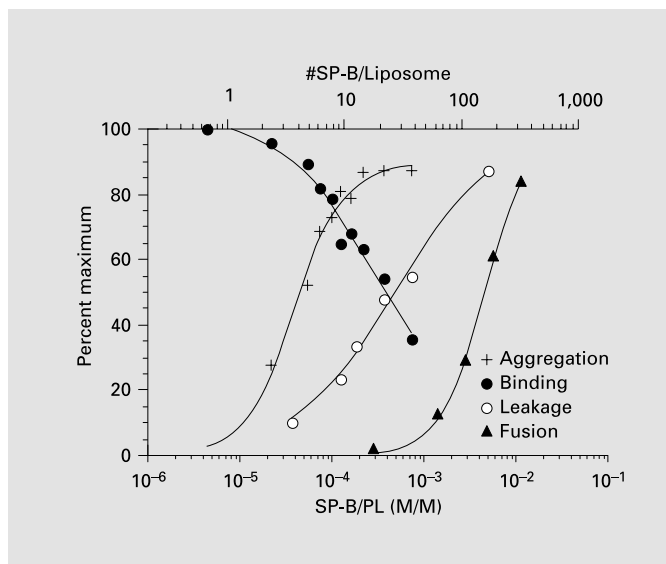


Fig. 2. SP-B binding, surface aggregation, vesicle permeabilization and vesicle fusion as a function of the SP-B/phospholipid molar ratio. The phospholipids were a 7:3 mixture of DPPC and PG prepared as large unilamellar liposomes. Binding, SP-B aggregation, permeabilization, and fusion were measured by fluorescent techniques [21]. Filled circles, SP-B binding expressed as number of SP-B dimers bound per vesicle (top x-axis). Crosses, aggregation of the membrane-bound SP-B. Open circles, permeabilization of vesicles as determined by leakage of soluble vesicle contents. Filled triangles, membrane fusion as determined by energy transfer between membrane-associated probes [adapted from 21 with permission].

cationic small protein, SP-B has, not surprisingly, a significant ‘detergent-like’ effect on preformed phospholipid liposomes. We have broken down these effects into discrete steps in an effort to model the complex processes occurring after SP-B is released from the proprotein in near proximity to lipid vesicles in the environment of the multivesicular body [19, 20]. An important but unproved assumption here is that the mature form of SP-B does not assemble with lipids until this cleavage event occurs. For purposes of description and analysis we have modeled the process of membrane association as first involving a rapid binding of SP-B to a lipid vesicle in the multivesicular body to form a lipoprotein complex, followed by reversible aggregation of the complexes, and finally reorganization of the aggregated complexes into an irreversible fused product (lamellae of the lamellar bodies) [19, 20].

SP-B binds rapidly to preformed large unilamellar vesicles made from dipalmitoylphosphatidylcholine (DPPC) and varied amounts of phosphatidylglycerol (PG) [21]. The apparent binding affinity increases linearly with the

amount of PG present. SP-B binds vesicles containing 30% PG, a composition shown to sustain approximate morphological and functional reconstitution of surfactant, with an apparent surface partition coefficient of $2.7 \times 10^7 M^{-1}$. Fluorescence self-quenching by the bound SP-B, evident when only approximately 3% of the roughly 600 potential binding sites per vesicle are occupied, suggests that essentially irreversible aggregation or oligomerization of SP-B may rapidly occur in a lipid environment [21]. This lipid-driven aggregation is not obviously associated with a large change in secondary structure as the overall content of α -helix, β -sheet and turns is similar in organic solvents [22], detergent micelles [16], lipid bilayers [23], and lipid monolayers [24]. The tertiary conformation adopted by SP-B in a lipid membrane is not known. Membrane binding is followed by a graded dose-dependent loss of vesicle contents, suggesting increasing loss of vesicle integrity [19–21]. We found no evidence for pore formation in these experiments even at low SP-B concentrations. The destabilizing effects on vesicle membranes are again markedly sensitive to the PG content. The permeabilization of membranes by SP-B is associated with a dramatic morphological rearrangement of the vesicular structure [25, 26]. At relatively high SP-B contents (0.5–1 mol%) the vesicle membranes are broken down into small discs that rapidly fuse [19, 27] to form large multilayered stacks with minimal interbilayer space [28]. It is possible, as suggested by Johansson and Curstedt [29], that the dimeric nature of SP-B may allow it to bring two lipid bilayers in close proximity. These events may be involved in the organogenesis of the lamellar body. Certainly, mice lacking SP-B seem unable to normally convert multivesicular body vesicles to organized lamellae [30].

SP-B and Extracellular Surfactant Activity

SP-B clearly enhances the rate of adsorption of phospholipids from an aqueous subphase to an air-water interface [3, 5, 31, 32]. Many of the membrane actions of SP-B discussed already may be relevant to this step as the addition of lipid to a preformed monolayer probably involves the fusion of subphase bilayer structures to the surface monolayer [24, 33] (fig. 3). SP-B is also necessary but not sufficient protein for the formation of tubular myelin, the complex three-dimensional tubular structure formed after secretion of the lamellar body contents into the alveolar space [25, 26]. The function of this fascinating structure is uncertain. Indeed, its biological significance has been

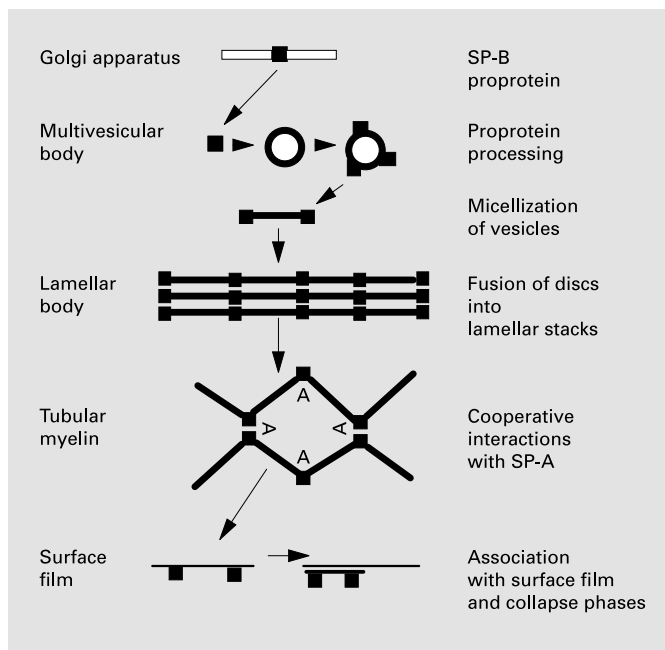


Fig. 3. A model for the actions of SP-B on surfactant lipids. The top panel depicts intracellular events in type II cells and the bottom panel depicts extracellular events. The potential sites of SP-B action are indicated to the left of the cartoon. Mature SP-B is represented by the small filled squares. Bilayers are depicted as thick circles or lines and the surface monolayer as a thin line. The letter A represents SP-A in tubular myelin [from 7 with permission].

questioned after discovering that genetically modified mice lacking tubular myelin have apparently normal lung function [34]. Nonetheless, the observation that tubular myelin formation and/or stability is dependent on interactions between SP-B and SP-A both in vitro [25, 26] and

in vivo [30, 34] emphasizes the potential for interactions between SP-B and components other than phospholipids. The roles for SP-B in alveolar surfactant dynamics might therefore include the initial formation of an interfacial film from secreted surfactant complexes and the replenishment of a preformed film during respiration by either enhanced adsorption or respreading from collapse phases. Whether for these or other reasons, SP-B is a critical component of most commercial surfactant replacement therapies.

Other Potential Roles of SP-B

The work described above related predominantly to the form of SP-B released with surfactant lipids from the alveolar epithelial type II cell. SP-B is also expressed at quite high levels in non-ciliated cells lining the small airways. What little work that has been done on SP-B in airway epithelial cells suggests SP-B processing and possibly function are quite different in the airways. Flanking domains and larger precursor forms of SP-B may be released into the airway lining fluid [unpubl. observations]. We have recently demonstrated that these soluble flanking domains have potent antimicrobial activity against gram-negative organisms in vitro [Poulain and Hawgood, unpubl. observations]. The physiological importance of this activity and other functions of SP-B in the airways have yet to be defined.

Acknowledgment

The authors acknowledge the support of the NHLBI through grant HL-24075.

References

- 1 King RJ, Klass DJ, Gikas EG, Clements JA: Isolation of apoproteins from canine surface active material. *Am J Physiol* 1973;224:788-795.
- 2 Phizackerley PJR, Town MH, Newman GE: Hydrophobic proteins of lamellated osmiophilic bodies isolated from pig lung. *Biochem J* 1979;183:731-736.
- 3 Curstedt T, Jörnvall H, Robertson B, Bergman T, Berggren P: Two hydrophobic low-molecular-mass protein fractions of pulmonary surfactant. Characterization and biophysical activity. *Eur J Biochem* 1987;168:255-262.
- 4 Jacobs KA, Phelps DS, Steinbrink R, Fisch J, Kriz R, Mitscock L, Dougherty JP, Tausch HW, Floros J: Isolation of a cDNA clone encoding a high molecular weight precursor to a 6-kDa pulmonary surfactant-associated protein. *J Biol Chem* 1987;262:9808-9811.
- 5 Hawgood S, Benson BJ, Schilling J, Damm D, Clements JA, White RT: Nucleotide and amino acid sequences of pulmonary surfactant protein SP 18 and evidence for cooperation between SP 18 and SP 28-36 in surfactant lipid adsorption. *Proc Natl Acad Sci USA* 1987;84:66-70.
- 6 Glasser SW, Korfhagen TR, Weaver T, Pilot-Matias T, Fox JL, Whitsett JA: cDNA and deduced amino acid sequence of human pulmonary surfactant-associated proteolipid SPL(Phe). *Proc Natl Acad Sci USA* 1987;84:4007-4011.
- 7 Hawgood S, Derrick M, Poulain F: Structure and properties of surfactant protein B. *Biochim Biophys Acta* 1998;1408:150-160.
- 8 Hawgood S, Damm D, Wright JR, Latham D, Benson B, White RT: Expression and partial processing of recombinant human SP-B by Chinese hamster. *Am Rev Respir Dis* 1990;141:A693.

- 9 Weaver TE, Whitsett JA: Processing of hydrophobic pulmonary surfactant protein B in rat type II cells. *Am J Physiol* 1989;257:L100-L108.
- 10 Weaver TE, Lin S, Bogucki B, Dey C: Processing of surfactant protein B proprotein by a cathepsin D-like protease. *Am J Physiol* 1992;263:L95-L103.
- 11 Foster C, Aktar A, Kopf D, Zhang P, Guttentag S: Pepsinogen C: A type 2 cell-specific protease. *Am J Physiol* 2004;286:L382-L387.
- 12 Guttentag S, Robinson L, Zhang P, Brasch F, Buhling F, Beers M: Cysteine protease activity is required for surfactant protein B processing and lamellar body genesis. *Am J Respir Cell Mol Biol* 2003;28:69-79.
- 13 Brasch F, Ochs M, Kahne T, Guttentag S, Schauer-Vukasinovic V, Derrick M, Johnen G, Kapp N, Muller KM, Richter J, Giller T, Hawgood S, Buhling F: Involvement of napsin A in the C- and N-terminal processing of surfactant protein B in type-II pneumocytes of the human lung. *J Biol Chem* 2003;278:49006-49014.
- 14 Voorhout WF, Veenendaal T, Haagsman HP, Weaver TE, Whitsett JA, van Golde LMG, Geuze HJ: Intracellular processing of pulmonary surfactant protein B in an endosomal/lysosomal compartment. *Am J Physiol* 1992;263:L479-L486.
- 15 Johansson J, Curstedt T, Jörnvall H: Surfactant protein B: Disulfide bridges, structural properties, and kringle similarities. *Biochemistry* 1991;30:6917-6921.
- 16 Andersson M, Curstedt T, Jörnvall H, Johansson J: An amphipathic helical motif common to tumourolytic polypeptide NK-lysin and pulmonary surfactant polypeptide SP-B. *FEBS Lett* 1995;362:328-332.
- 17 Leippe M: Ancient weapons: NK-lysin, is a mammalian homolog to pore-forming peptides of a protozoan parasite. *Cell* 1995;83:17-18.
- 18 Liepinsh E, Andersson M, Ruyschaert JM, Otting G: Saposin fold revealed by the NMR structure of NK-lysin. *Nat Struct Biol* 1997;4:793-795.
- 19 Poulain FR, Allen L, Williams MC, Hamilton RL, Hawgood S: Effects of surfactant apolipoproteins on liposome structure: Implications for tubular myelin formation. *Am J Physiol* 1992;262:L730-L739.
- 20 Poulain FR, Nir S, Hawgood S: Kinetics of phospholipid membrane fusion induced by surfactant apoproteins A and B. *Biochim Biophys Acta* 1996;1278:169-175.
- 21 Chang R, Nir S, Poulain FR: Analysis of binding and membrane destabilization of phospholipid membranes by surfactant apoprotein B. *Biochim Biophys Acta* 1998;1371:254-264.
- 22 Cruz A, Casals C, Perez-Gil J: Conformational flexibility of pulmonary surfactant proteins SP-B and SP-C, studied in aqueous organic solvents. *Biochim Biophys Acta* 1995;1255:68-76.
- 23 Vandenbussche G, Clercx A, Curstedt T, Johansson J, Jörnvall H, Ruyschaert JM: Secondary structure and orientation of the surfactant protein SP-B in a lipid environment. A Fourier transform infrared spectroscopy study. *Biochemistry* 1992;31:9169-9176.
- 24 Oosterlaken-Dijksterhuis MA, Haagsman HP, van Golde LM, Demel RA: Characterization of lipid insertion into monomolecular layers mediated by lung surfactant proteins SP-B and SP-C. *Biochemistry* 1991;30:10965-10971.
- 25 Suzuki Y, Fujita Y, Kogishi K: Reconstitution of tubular myelin from synthetic lipids and proteins associated with pig pulmonary surfactant. *Am Rev Respir Dis* 1989;140:75-81.
- 26 Williams MC, Dobbs LG: Expression of cell-specific markers for alveolar epithelium in fetal rat lung. *Am J Respir Cell Mol Biol* 1990;2:533-542.
- 27 Oosterlaken-Dijksterhuis MA, van Eijk M, van Golde LMG, Haagsman H: Lipid mixing is mediated by the hydrophobic surfactant protein SP-B but not by SP-C. *Biochim Biophys Acta* 1992;1110:45-50.
- 28 Williams MC, Hawgood S, Hamilton RL: Changes in lipid structure produced by surfactant proteins SP-A, SP-B, and SP-C. *Am J Respir Cell Mol Biol* 1991;5:41-50.
- 29 Johansson J, Curstedt T: Molecular structures and interactions of pulmonary surfactant components. *Eur J Biochem* 1997;244:675-693.
- 30 Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BR, Weaver TE, Whitsett JA: Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proc Natl Acad Sci USA* 1995;92:7794-7798.
- 31 Perez-Gil J, Tucker J, Simatos G, Keough KMW: Interfacial adsorption of simple lipid mixtures combined with hydrophobic surfactant protein from pig lung. *Biochem Cell Biol* 1991;70:332-338.
- 32 Yu SH, Possmayer F: Comparative studies on the biophysical activities of the low-molecular-weight hydrophobic proteins purified from bovine pulmonary surfactant. *Biochim Biophys Acta* 1988;961:337-350.
- 33 Oosterlaken-Dijksterhuis MA, Haagsman HP, van Golde LM, Demel RA: Interaction of lipid vesicles with monomolecular layers containing lung surfactant proteins SP-B or SP-C. *Biochemistry* 1991;30:8276-8281.
- 34 Korfhagen TR, Bruno MD, Ross GF, Huelsman KM, Ikegami M, Jobe AH, Wert SE, Stripp BR, Morris RE, Glasser SW, Bachurski CJ, Iwamoto HS, Whitsett JA: Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci USA* 1996;93:9594-9599.