Hydrophobic Proteins and Their Analogues in Pulmonary Surfactant

T.	Tore	Curstedt ^a
G.	Gunhild	Nilsson ^a
B.	Bengt	Robertson ^b
J.	Jan	Johansson ^c

^aDepartment of Clinical Chemistry, Karolinska Institutet at Danderyd Hospital, Danderyd; ^bDivision of Experimental Perinatal Pathology, Karolinska Hospital, Stockholm, and ^cDepartment of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Prof. Tore Curstedt, MD, Department of Clinical Chemistry, Karolinska Institute at Danderyd Hospital, S-182 28 Danderyd (Sweden)

Respiratory distress syndrome can be effectively treated by tracheal instillation of natural surfactant preparations composed of phospholipids and small amounts of specific proteins [1]. In particular, the two hydrophobic, low molecular weight proteins, SP-B and SP-C, appear to be essential components of surfactant. These proteins increase the adsorption of the phospholipids to the air-liquid interface and prevent the alveoli from collapsing at end expiration [2]. We have systematically studied the biophysical properties of different combinations of phospholipids and polypeptides and correlated the secondary structure of the peptides to surfactant activity in order to produce an artificial preparation which can be used for replacement therapy.

The surface activity of different artificial surfactants was studied in vitro using the Wilhelmy balance technique. Porcine SP-B, porcine SP-C, synthetic SP-C analogues and low molecular weight polypeptides with high α -helical contents were recombined with 1,2-dipalmitoylphosphatidylcholine (DPPC):

1-palmitoyl-2-oleoylphosphatidylcholine: 1,2-dioleoylphosphatidylglycerol (55:35:10, w/w/w), DPPC:egg phosphatidylglycerol (7:3, w/w) or DPPC:phosphatidylglycerol:palmitic acid (68:22.9, w/w/w).

Pure lipid mixtures had low surface activity but the preparation containing DPPC:phosphatidylglycerol:palmitic acid had a faster spreading and a lower equilibrium surface tension than the other lipid mixtures. Addition of 2-5% SP-C to any of the lipid mixtures dramatically increased surface activity. This protein is a 33-35 residue polypeptide with palmitoyl groups thioester-linked to the cysteine residues in the N-terminal part of the molecule [3, 4]. SP-C mainly adopts an α -helical conformation with its transmembrane α -helix oriented parallel to the lipid acyl chains [5, 6]. The positively charged residues at positions 11 and 12 interact with the polar head groups of the phospholipids in the bilayer while the palmitoyl groups may be inserted into the same or a juxtaposed bilayer. Synthetic SP-C without palmitoyl groups had a lower helical content than native SP-C and showed a rather low surface activity [7]. Replacement of the poly-valyl α -helical part of SP-C with the amino acid sequence of the second transmembrane helix of bacteriorhodopsin gives a hybrid (SP-C/BR) which in a lipid bilayer has

a transmembrane α -helix oriented parallel to the acyl chains [8]. Addition of this peptide to the lipid mixture accelerates the spreading at the air-liquid interface and decreases the equilibrium surface ten-

184

Biol Neonate 1996;69:165-212

International Symposium on Recent Advances in Neonatal Medicine

sion of the lipid film [7]. The studies with synthetic analogues reveal that the α -helical conformation as such and not the amino acid sequence seems to be essential for the spreading kinetics of the surface-active phospholipids at an air-liquid interface. However, the physiological activities of surfactant preparations based on these analogues are inferior to those of natural surfactants. This may reflect the complex and still to a large extent unknown biophysical interactions between various components of the surfactant system.

The other hydrophobic protein in natural surfactant preparations, SP-B, also increases the spreading of phospholipids at an air-liquid interface but seems to be more effective in decreasing both minimum and maximum surface tension during cyclic film compression in a pulsating bubble system [2]. This protein is a homodimer with two 79-residue polypeptide chains linked together by a disulfide bridge [3, 4]. The results indicate that the polypeptide chain is composed of four amphipathic α -helices [9]. These α -helices probably interact preferentially with superficial parts of the lipid bilayer while the basic residues of SP-B are thought to interact with anionic phospholipids. Since SP-B is a dimer it is possible that each polypeptide chain interacts with a phospholipid bilayer, thus cross-linking two bilayers.

The proposed cross-linking of juxtaposed phospholipid bilayers by the hydrophobic proteins may indicate that the lining layer at an air-liquid interface in reality is a multilayer with an excess of surfactant which can be used during expansion. This is in agreement with the results obtained by Schürch et al. [10] using the captive bubble technique.

SP-B and SP-C are different both in many structural aspects and in the way of interaction with phospholipid bilayers indicating that they fulfill different functional roles. By replacement of SP-B and SP-C in artificial surfactant preparations with synthetic analogues which have the ability to cross-link bilayers and to form transmembrane helices, respectively, the structural features essential for the functional properties of surfactant may be clarified.

References

Collaborative European Multicenter Study Group: Surfactant replacement therapy for severe neonatal respiratory distress syndrome: An international randomized clinical trial. *Pediatrics* 1988;82:683-691. Curstedt T, Jörnvall H, Robertson B, Bergman T, Berggren P: Two hydrophobic low-molecular-mass protein fractions of pulmonary surfactant. *Eur J Biochem* 1987; 168:255-262.

Curstedt T, Johansson J, Persson P, Eklund A, Robertson B, Löwenadler B, Jörnvall H: Hydrophobic surfactant-associated polypeptides: SP-C is a lipopeptide with two palmitoylated cysteine residues, whereas SP-B lacks covalently linked fatty acyl groups. *Proc Natl Acad Sci USA* 1990;87:2985-2989.

Johansson J, Curstedt T, Robertson B: The proteins of the surfactant system. *Eur Respir J* 1994;7:372-391. Vandenbussche G, Clercx A, Curstedt T, Johansson J, Jörnvall H, Ruyschaert JM: Structure and orientation of the surfactant-associated protein C in a lipid bilayer. *Eur J Biochem* 1992;203:201-209. Johansson J, Szyperski T, Curstedt T, Wüthrich K: The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a

valyl-rich- α -helix. *Biochemistry* 1994;33:6015-6023. Johansson J, Nilsson G, Strömberg R, Robertson B, Jörnvall H, Curstedt T: Secondary structure and biophysical activity of synthetic analogues of the pulmonary surfactant polypeptide SP-C. *Biochem J* 1995; 307:535-541.

10

Clercx A, Vandenbussche G, Curstedt T, Johansson J, Jörnvall H, Ruyschaert JM: Structural and functional importance of the C-terminal part of the pulmonary surfactant polypeptide SP-C. *Eur J Biochem* 1995;229:465-472. 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. Schürch S, Qanbar R, Bachofen H, Possmayer F: The surface-associated surfactant reservoir in the alveolar lining. *Biol Neonate* 1995;67: (suppl 1):61-76.

Extended Abstracts

Biol Neonate 1996;69:165-212

18?