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Review

Pulmonary surfactant: functions and molecular composition

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Abstract

This review briefly notes recent findings important for understanding the surface mechanical functions of pulmonary surfactant. Currently known surfactant-specific proteins and lipids are discussed, with an eye to their possible functions. Competing models of the alveolar subphase life cycle of surfactant are also presented. It is concluded that, in spite of much effort, we still do not understand the basic molecular mechanisms underlying surfactant's rapid adsorption to the air-water interface.

Keywords: Dipalmitoylphosphatidylcholine; Surfactant protein; Surface tension; Surface balance

Contents

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; SP-A, -B, -C, -D, surfactant proteins A, B, C, and D

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1. Introduction

It is well over 40 years since Pattle revived interest in pulmonary surfactant [1] and perhaps a good time to see where we find current investigators working. This chapter on surfactant composition and function has been limited to introducing some issues related to its surface activity, leaving subsequent authors to fill in many of the details. Surfactant functions other than its surface activity have similarly been sidestepped in this chapter but are covered in depth later in this special issue of *Biochimica et Biophysica Acta*. An excellent recent review of all surfactant functions is available [2].

2. Surfactant distribution

2.1. Surfactant source

The source for most studies of surfactant composition has been bronchoalveolar lavage [2]. The same lipid and protein components appear in analyses of human and other mammalian species [3,4] with proportions of different lipid classes varying somewhat, perhaps due to methodological differences. Many of the principal lipids found in mammals are also found in the lungs of air-breathing fishes [5], so 'pulmonary' surfactant may have a long history.

2.2. In airways and alveolar monolayer

Some surfactant components [6], or their effects [7], have been seen in the tracheobronchial tree, but the mass of material retrieved by lavage probably originates in the alveoli. The alveolar air-water surface film is the site of surfactant's major action on lung mechanics, but its visualization there has proven difficult. Nevertheless, an innovative technique using fluorocarbon fluid to deliver the fixative osmium tetroxide could discern regions of up to seven stacked, presumably single lipid layers at what was once the fluorocarbon-water interface [8]. Whether continuous multilayers always line the air-water interface is still a matter of conjecture, however.

2.3. In aqueous alveolar subphase

In a recent scanning electron micrographic study of rat lungs, the aqueous alveolar subphase lying beneath the interface film had an average thickness of 0.14 μm over flat alveolar walls, and 0.89 μm in alveolar corners, for an area-weighted average thickness of 0.2 μm [9]. It appeared continuous, without evident breaks. Fig. 1 is a transmission electron micrograph of adult rat alveolar subphase, showing lamellar bodies (LB) apparently recently released from type II cells, tubular myelin (TM) in the process of being stitched together by a 'quilting bee' of several lamellar bodies, and many particles of presumed 'used' surfactant. The relations of these surfactant compartments are discussed below.

3. Surfactant composition

3.1. Fractionation of bronchoalveolar lavage

Bronchoalveolar lavage has commonly been separated into subfractions by differential centrifugation, with the most dense fractions being enriched in tubular myelin [10]. In associated tracer experiments these tubular myelin-rich fractions also appeared to be labeled earlier than the remaining liposomal fractions. In more recent studies, Gross and associates centrifuged mouse bronchoalveolar lavage to equilibrium on continuous sucrose gradients, producing three rather distinct fractions: an 'ultraheavy' fraction (9 weight %) consisting of large aggregates of tubular myelin with what appeared to be lamellar bodies, a 'heavy' fraction (48%) with large empty vesicles and some tubular myelin, and a 'light' fraction (43%) consisting of small unilamellar vesicles [11]. They further demonstrated that cyclic adsorption/desorption at the air-water interface somehow converted the ultraheavy into the light fraction in a

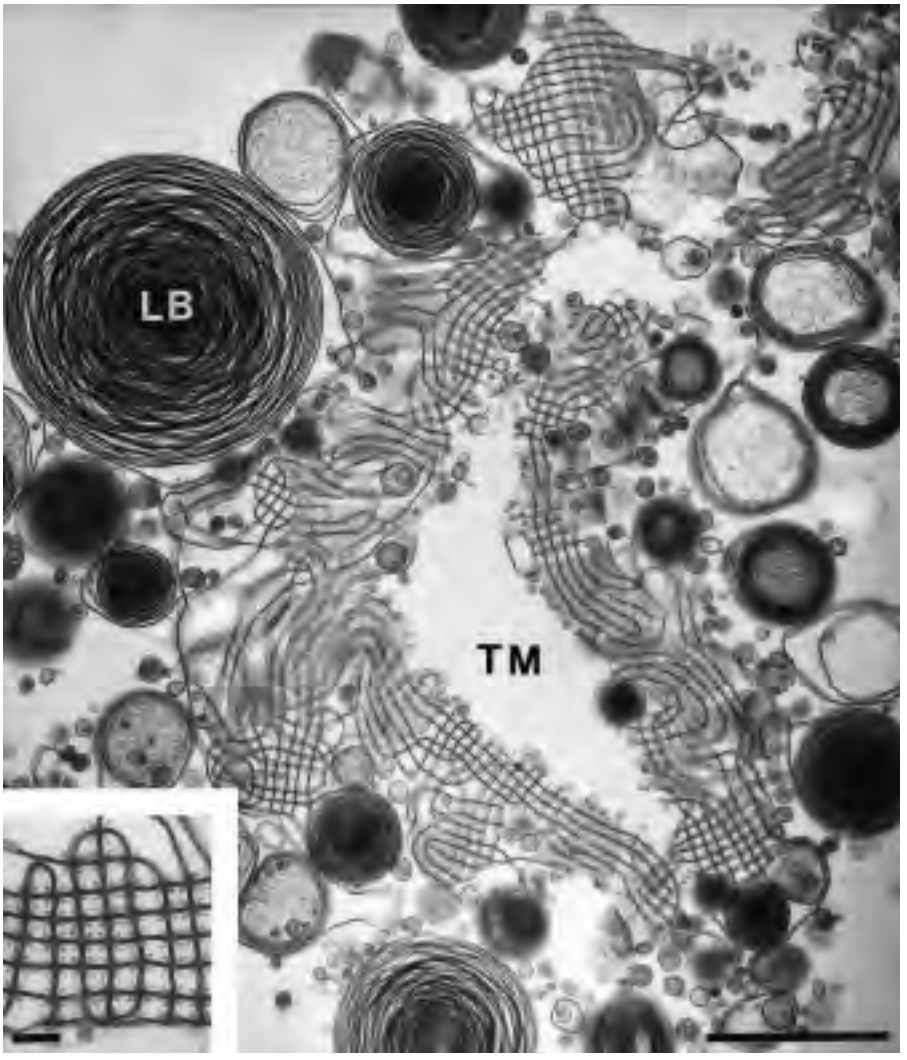

Fig. 1. Particles in the alveolar subphase. In this electron micrograph section of a rat lung, lamellar bodies (LB) are seen forming tubular myelin (TM) (bar at lower right = 1.0 μm). The remaining vesicular structures may represent both used and rejected surfactant materials. Inset: detail of tubular myelin at lower left, showing small projections in the corners, thought to represent SP-A (bar = 0.1 μm). This adult rat was exposed to NO_2 for 48 h, but no differences were seen from controls.

process that may possibly involve esterase activity. Surfactant is now more commonly separated by differential centrifugation into only two fractions, deemed heavy and light subtypes.

3.2. Lipid composition

Lipids, particularly phospholipids, make up the bulk of materials retrieved by bronchoalveolar lavage [2]. Although phosphatidylcholine predominates among the phospholipids, phosphatidylglycerol is present in amounts up to 10 mol%, and smaller amounts of phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and the plasmalogen analog of phosphatidylcholine are also present. Lipid analysis most recently seems to involve high-performance liquid chromatography, with [12] or without [6,13,14] derivatization, although thin-layer followed by gas chromatography is still used [15]. Investigators using these last techniques have found that alveolar surfactant subfractions may differ somewhat in their lipid composition.

Among the phosphatidylcholines, dipalmitoylphosphatidylcholine (DPPC) predominates, making

up 41–70% of phosphatidylcholine [2,16]. Fatty acid saturation has been assayed by a variety of methods, perhaps explaining the very large deviation in reported saturation indices between and even within animal species. A method using osmium tetroxide to oxidize or complex unsaturated phospholipids, leaving behind the fully saturated species, has been popular because of its simplicity [17,18], but high-performance liquid chromatography is also frequently employed [12,13,16]. Recently Holm et al. have questioned the osmium method in a study comparing it with more traditional gas chromatographic techniques [19]. Perhaps uncertainties with the method have contributed to the same group finding that the pool sizes for saturated phosphatidylcholine is 1.9 μmol/kg in humans [3], 8 μmol/kg in mice [20], and 11.3 μmol/kg in adult sheep [4]. Values obtained using the osmium method for rabbit surfactant, 5.2 μmol/kg [10], have also fallen within this range. Based on his own experience comparing several variants of the osmium method with gas chromatography of derived fatty acid methyl esters, the author is inclined to choose the latter, somewhat more laborious method for determination of phospholipid saturation. While the values noted above for different species may be accurate, a reality check is provided by the fact that a close packed DPPC film has a molecular area of about 38 $Å^2$. Assuming an adult human's alveolar area of 1.0 m^2/kg body weight, one would predict that a fully compressed film at functional residual capacity would contain approx. 5 μmol saturated phosphatidylcholine per kg. This value lies between the human and sheep values cited above, and of course would not account for 'multilayers' of surfactant at the interface or any alveolar subphase particles.

Cholesterol is also present in amounts up to 8 weight % (15 mol%), in addition to trace amounts of triglycerides and free fatty acids.

3.3. Protein composition

Surfactant-specific proteins make up about 10 weight % of material retrieved from bronchoalveolar lavage. An excellent recent review of their properties is available [21].

Surfactant proteins A and D (SP-A and SP-D) represent the larger of the two subgroups. They are members of the Ca^{2+}-dependent carbohydrate-binding collectin family, and will be discussed in great depth in later chapters of this issue. The unique electron microscopic morphology of both SP-A and -D has yet to be linked to their surface activity [22]. SP-D mRNA and protein have been detected in mouse alveolar type II and non-ciliated bronchiolar epithelial cells, as well as in cells of the tracheal epithelium and submucosal glands [23].

Surfactant proteins B and C (SP-B and SP-C) are extremely hydrophobic in nature and were found in heavy but not light surfactant subfractions separated by differential centrifugation [24]. SP-B is usually present as a dimeric structure, and SP-C is found as thio-esters of one or more fatty acids [25]. SP-C has also been found in association with isolated alveolar type II cells [26].

4. Surfactant surface activity

The author has limited himself to discussing pulmonary surfactant's surface tension lowering function, leaving the growing list of its other roles to later chapters in this issue.

4.1. Models of the life cycle of alveolar surfactant

4.1.1. Physicochemical and physiological constraints

Surface films are obliged to follow certain physicochemical constraints, and adequate pulmonary surfactant films in particular must produce appropriate physiological behavior [27]. For example, a given material in the alveolar subphase will not adsorb into the air-water interface unless film surface tension is above the equilibrium value for that material. When conditions are appropriate for adsorption to occur, a good lung surfactant usually adsorbs very rapidly, with a time constant measured in milliseconds. This process most often requires a deep breath (e.g., a sigh) and will generally not occur during quiet tidal breathing. Only when a surface film is compressed below the equilibrium surface tension of a particular component, will there be a net desorption of that component from the surface film. In good, physiologically functioning lung surfactant films, this process is usually very slow, with a time constant measured in hours.

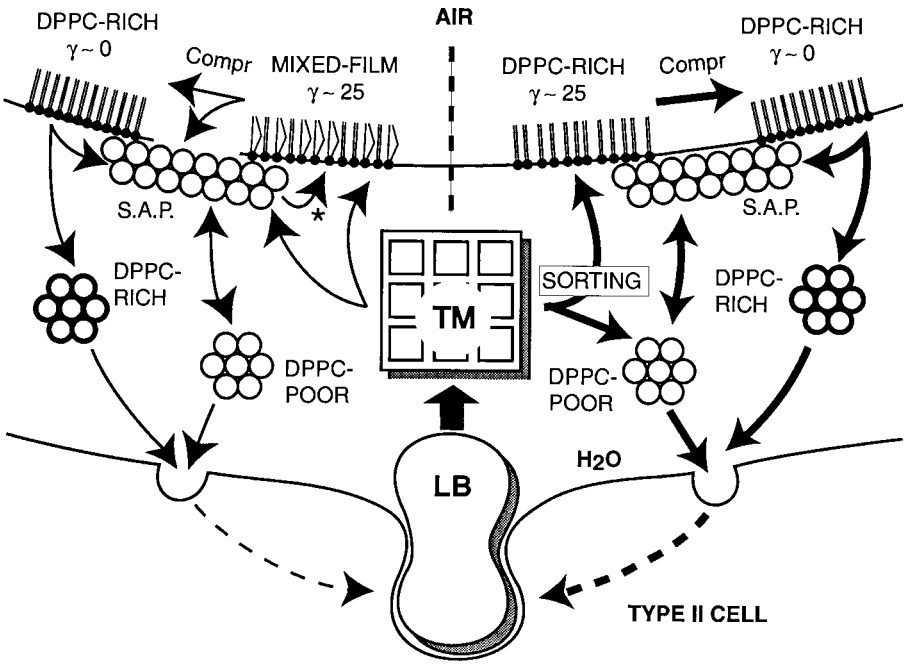

Fig. 2. Two models of the life cycle of pulmonary surfactant in the alveolar subphase. The left-hand half of the diagram (thin arrows) represents the most widely accepted model of the cycle, the right-hand half (thick arrows) represents the author's current biased views. An alveolar type II cell sits at the bottom of the figure, releasing lamellar bodies (LB), which are transformed into tubular myelin (TM). During deep inspiration, materials adsorb extremely rapidly from TM to the film in the air-water interface at the top, and during expiration the film is compressed to the low surface tensions that promote alveolar stability. Losses from the surface into the subphase at various stages are recycled by type II cells into new lamellar bodies (dashed arrows). Both models produce a DPPC-rich film at near zero surface tension (γ, upper right and left of figure), and both produce a surface-associated phase (S.A.P.) and DPPC-rich and -poor particles for eventual type II cell retrieval. The right-hand model differs from the left in that it sorts DPPC-rich lipids directly into the film during adsorption, whereas the left-hand model must compress (Compr) the film several times in order to convert an initially mixed-lipid film into one that is DPPC-enriched. The sequential compressions squeeze non-DPPC components out of the film, perhaps into the surface associated phase. Possible reentry of material into the film is also indicated (*). Some likely inter-particle and particle-film transfers are indicated by the arrows, but other possible arrows have been eliminated in the interests of clarity.

4.1.2. Commonly accepted parts of the life cycle

The following commonly accepted steps followed by alveolar components in the subphase can be seen along with some more controversial paths in Fig. 2.

1. Secretion of LB into alveolar subphase from type II cells.
2. Formation of TM, usually from multiple LB.
3. Rapid adsorption of TM into the interface, forming a film.
4. Compression of film, lowering surface tension.
5. Eventual slow collapse of low tension film into subphase.
6. Retrieval of used subphase surfactant by type II cells.
7. Similar retrieval by macrophages (not shown).
8. Repackaging of retrieved materials into new LB.

4.1.3. The author's preferred changes to the life cycle

The following modifications seem likely to the author, and are based on what the author believes to be important recent observations by others. They are diagrammed in Fig. 2, to the right of the dashed line under 'AIR'.

(3a) Sorting of surfactant during adsorption of TM forms a DPPC-rich film [28,29] and DPPC-poor lipid-protein particles in the subphase.

(5a) Slow collapse of the DPPC-rich film contributes to DPPC-rich particulate forms in the subphase and possibly to a surface-associated phase (SAP) which remains in close proximity to the active film [30].

4.1.4. The more widely accepted version of the model

These modified steps are portrayed on the left-

hand side of Fig. 2, and represent the current, traditional view of the surfactant cycle [2].

(3b) All TM components adsorb into the initial film.

(3.5b) The surface-associated phase can readsorb to the air-water interface (arrow with asterisk).

(4b) Compression/expansion cycles purify the film during tidal breathing by squeezing out most non-DPPC components into the surface associated phase and aqueous particulate phases [31].

The author believes that these last steps may indeed occur in special circumstances, but that they are not found during normal physiologic breathing. They may be regarded as 'back-up' mechanisms during situations of surfactant shortage. He feels that during normal ventilation, tubular myelin usually places DPPC into the interface too fast for competing readsorption of used surfactant to be quantitatively significant. If the author's belief is indeed true, then it is likely that most surface balance studies of pulmonary surfactant components involving solvent spreading of mixed monolayers are actually studying this 'traditional' model to the exclusion of the sorting model. Since a normal newborn's lungs do not collapse during their first expiration, the initial adsorbed air-water interface must somehow have become enriched in DPPC during the prior inspiration. If purification by several cycles of respiration were required, some lung collapse would have to occur before a usable working film would be achieved.

4.2. *The effects of surfactant function on pulmonary physiology*

4.2.1. *Pulmonary mechanics*

Beyond the importance of low alveolar surface tension in promoting lung stability [27], one should also consider the effects of surfactant adsorption and desorption kinetics on surfactant function. More detailed kinetic studies and some computer model-building will be required [32,33]. Clinically evident repetitive collapse and re-expansion of lungs may be a result of kinetic dysfunctions [34], and a better understanding of lung damage from overinflation should follow a better picture of how surfactant function is linked to mechanics [35,36].

4.2.2. *Surfactant in airways*

Surfactant also has roles to play in small airways. Thus it can act to maintain patency [37], to prevent resistance increase in allergen-challenged animals [38], and to enhance mucociliary clearance of unwanted contaminants [7,39].

4.2.3. *Pulmonary vessels and blood flow*

Not surprisingly, surfactant effects on lung mechanics have also been found to produce concomitant lung circulatory changes [40].

4.3. *Means of assessing alveolar-stabilizing function of surfactant*

Adequately measuring surfactant function in vitro and in vivo has proven to be very difficult. Different techniques are required to answer different questions, one size does not fit all.

4.3.1. *Wilhelmy balance*

The modified Langmuir-Wilhelmy surface balance, usually consisting of a Teflon trough with either tightly fitting or ribbon barriers, has been used for a longer time than most other lung surfactant methods, and is still being suitably employed. For example, recent work on the respreading of used surfactant has made appropriate use of this technique [41].

4.3.2. *Captive bubble surfactometer*

Because films with very low tensions could not be adequately contained in Langmuir-Wilhelmy balances at physiological temperatures, Schürch and colleagues developed several models of a captive bubble surfactometer [42–44]. The use of such a balance was critical in demonstrating the importance of low film compressibility as an indicator of good surfactant function [29]. Reaching near-zero surface tension with a mere 15–20% film area compression is probably the most difficult goal to achieve, and represents the hallmark of excellent surfactant function. It is becoming apparent from these in vitro studies that surfactant concentrations of at least 1 mg phospholipid/ml must be present to obtain optimal results.

4.3.3. *Pulsating bubble surfactometer*

The pulsating bubble surfactometer, developed by Enhorning [45], is a predecessor of the captive bubble

device, and is still in more common use in spite of some surface leakage difficulties. It offers a much smaller sample size and far greater ease of use than its derivative machine, but falls short when asked to provide critical information from the first film compression. A comparison of both bubble devices with a recommendation for addressing surface leakage problems with the pulsating bubble surfactometer has been published [46]. Most studies using this latter device ignore first compression isotherm data, and report the lowest surface tensions found in 50–100 cyclic bubble compressions [47].

4.3.4. Administration of surfactants to preterm animals, rabbits and lambs

Preterm rabbits and preterm lambs have increasingly been used to study the in vivo effects of surfactant preparations. They provide excellent, 'real-world' tests of function, and good comparisons between artificial and natural products [48], free of the many qualifications inherent to the above-described in vitro methods.

4.3.5. Ethanol resistive microbubble test

Another microbubble method for assaying surfactant [49] is quite closely related to the bubble behavior originally described by Pattle [1], and offers a good opportunity to study film stability.

4.4. Function of surfactant fractions and individual components

4.4.1. General concerns

We know something about what a few of the components of surfactant can do in vitro, but almost nothing about how they do it, even under such controlled conditions. Our further linking of in vitro to in vivo performance still relies largely on faith. Even genetic knockouts have left us with more questions than answers, given most animals' superb means for compensation of losses. A 'rescued' animal need not be as normal as he appears, always bringing into question the true physiologic function of the deleted gene.

4.4.2. Functions of surfactant fractions

Heavier, tubular myelin-enriched preparations studied in a surface balance, adsorb and spread more rapidly and compress with smaller area changes to low surface tensions than do 'lighter' small vesicle preparations [10]. Lipid adsorption seems to be necessary for conversion of heavy to light subfractions [50]. Whereas Putman et al. [24] found that their original bronchoalveolar lavage adsorbed rapidly, neither their resuspended heavy nor light fractions did so. They concluded that subtleties in the handling surfactant materials can greatly affect their properties. We have found repeatedly that careful, thorough dispersion of heavy fractions destroys their ability to adsorb very rapidly (unpublished observations), perhaps explaining the results of Putman et al.

4.4.3. Functions of individual components

4.4.3.1. Lipids. The active alveolar film seems to be very highly enriched in DPPC compared to the composition of bronchoalveolar lavage, although pure DPPC suspensions have usually been found to adsorb and spread extremely slowly at the air-water interface. By preparing DPPC suspensions of large particles in buffer at temperatures near 5°C, however, one can create a form that adsorbs rapidly in systems at 37°C (unpublished observations), hence the sometimes unknown physical state of any component may greatly influence its surface properties. The particular roles of the other lipid components remain uncertain in spite of intensive study. For example, surface balance experiments involving solvent-spread lipid and lipid/protein mixtures give suggestive evidence for the possible in vivo behavior of non-DPPC surfactant components [51,52], but generally such experimental conditions do not closely replicate those in the lung.

4.4.3.2. Proteins. Hydrophobic apoproteins SP-B and -C usually promote very rapid adsorption of lipids to the air-water interface, and SP-A speeds this process even further under certain circumstances. These three surfactant proteins have also been found to improve pulmonary function in a premature rabbit model [53].

SP-A and -D, the hydrophilic surfactant proteins, both demonstrate unusual morphologies (Fig. 1), and seem to aid the other proteins in promoting adsorption [22]. Although binding studies indicate strong interactions between surfactant proteins and surfactant lipids, these findings have not led to any

detailed understanding of surfactant proteins' surface tension-lowering function, although they do help us understand better the uptake and release of surfactant lipids by type II cells [54]. Some recent data provide suggestions about mechanisms: phospholipid membrane fusion is commonly taken as an indicator of surfactant adsorption potential, and has been found to be induced by both SP-A and -B [55], addition of SP-A to Curosurf, a commercial surfactant replacement, can overcome added surfactant inhibitors [56], and surface tension-area isotherms with compression to near zero surface tension were consistent with the interpretation that SP-D incorporated by adsorption was apparently not squeezed out of the film [57].

SP-B and -C, the hydrophobic surfactant proteins, accelerate adsorption of mixed surfactant lipids [58]. There is evidence that both proteins are required for rapid adsorption of surfactant heavy fraction, but that this activity can be lost during handling of samples [24] if large aggregates are disrupted. Both proteins can form stable air-water monolayers by themselves or as mixtures with DPPC, and both (SP-B > SP-C) promote readsorption of materials from collapsed, DPPC-containing monolayers [59,60]. Although some protein content was evidently squeezed out of compressed monolayers, the retained proteins did not prevent the reaching of near zero surface tensions, and appeared to promote respreading of lipid-protein on film expansion. The film association of these proteins was later confirmed using fluorescent labels [61], although the labels could not distinguish material in the adjacent subphase from that retained within the monolayer film itself. Further external reflection absorption infrared spectroscopic data for SP-B and -C films show that SP-B is more easily squeezed out than SP-C [62].

SP-B's effects have also been studied alone. For example, antibody against SP-B produced deleterious physiological effects in near-term newborn rabbits [63], and the positive charge on SP-B has been shown to promote segregation of negatively charged lipids within mixed-lipid monolayers [64]. This last action may be a clue to the mechanism underlying the apparent sorting behavior of tubular myelin during adsorption.

SP-C-oriented studies show it to exist in palmitoylated and non-palmitoylated forms [65]. In investigations of the activity of the major acylated form versus non-acylated SP-C, palmitoylation was found to enhance respreading and stability of mixed DPPC/phosphatidylglycerol films in a captive bubble surfactometer [66]. This same paper showed that low film tensions could be achieved with the same lipid mixture at very low pH without any proteins at all. Others have shown that SP-C's positively charged residues are important for binding phospholipid vesicles to the monolayer, preceding their insertion [67], and that the hydrophobic C-terminal helix is crucial for its rapid adsorption [68].

4.5. Effects of genetic manipulations and diseases

Selected modifications of surfactant genes and some human diseases often affect surfactant in ways that may help us understand more about the function of its components.

4.5.1. Genetic manipulations of surfactant proteins

Much current research in surfactant uses newly available, powerful genetic tools. In the first published SP-A gene knockout, Korfhagen et al. found that the animals survived, appeared grossly normal and had normal lung function, although tubular myelin figures were decreased in number [69]. Minimum surface tensions of compressed films from centrifuged bronchoalveolar lavage were the same for wild type and knockout at higher concentrations, but somewhat higher for knockouts at low concentrations. A follow-up paper found that tissue and alveolar pools of saturated phosphatidylcholines were actually larger in the knockout mice.

SP-B knockouts have produced more dramatic results. Homozygous null mice did not survive after delivery unless rescued with SP-B proprotein or continually managed with liquid fluorocarbon ventilation [70]. Heterozygous mice demonstrated decreased lung compliance and air trapping secondary to airway collapse [71]. In both sets of mice, SP-A, -C and -D were present in normal amounts. Surfactant pools of saturated phosphatidylcholine were also normal in these mice [72].

To date no SP-C knockouts have been reported. Conservation of its gene has been strong throughout evolution [73], so SP-C is likely to prove valuable for function and survival.

4.5.2. Association with diseases

The respiratory distress syndrome of the newborn has long been associated with prematurity, but there appears to be at least one genetic predisposition as well. Floros et al. have found a significant increase in the incidence of SP-B variants among respiratory distress infants [74], perhaps indicating an increased susceptibility to the disease due to SP-B inadequacy. Surface balance data for these variants have not been published as yet. To date the acute (often called 'adult') form of the respiratory distress syndrome has not been linked to genetic defects, but bronchoalveolar lavage of these patients has been found to contain lowered heavy/light fraction ratios, with decreased SP-A content in the heavy fractions [75].

The bronchoalveolar lavage of patients with alveolar proteinosis, a rare condition characterized by voluminous amounts of lipid- and protein-rich material in the airways, has been found to contain both normal and multimerized SP-A [76]. Further investigation has established that the abnormal SP-A aggregates are associated with significant amounts of immunoglobulin G [77], suggesting an immunological basis for the condition. Silicosis in humans and animals produces somewhat similar increases in bronchoalveolar lipid and protein. Cell culture studies of rat type II alveolar cells [78] showed that silica itself did not produce similar changes, but that bronchoalveolar wash from silica-exposed animals produced an increase in lipid synthesis, a decrease in secretion of surfactant materials, an increase in DPPC reuptake by type II cells, and a decreased uptake by alveolar macrophages.

In cystic fibrosis, a disease of variously modified ion transporter genes, bronchiolar lavage of infants with associated pulmonary inflammation or infection showed the saturated phosphatidylcholine/total phosphatidylcholine ratio to be lower and the SP-A concentration to be higher than in either normals or non-inflamed cystic fibrosis patients [18]. A direct surfactant involvement is thus found lacking at present.

Smoking has also been studied for its possible effects on the surfactant system. Rats chronically exposed to cigarette smoke showed significant decreases in total lavage SP-B, but SP-A and SP-B mRNAs were not different from sham-exposed animals [79].

Other diseases will undoubtedly be linked to surfactant effects in the future, but the alveolar proteinosis/silicosis links seem most promising at present for leads to the function of surfactant components.

5. Unresolved issues and future perspectives

Although pulmonary surfactant has been subjected to very sophisticated analytical and experimental techniques for over 40 years, we are still in the dark as to how some of its basic actions take place at a molecular level. In particular, the tools of surface chemistry and other physicochemical subfields, and of molecular biology have not helped us discover the detailed mechanisms by which selected surfactant components can adsorb so very rapidly to the air-water interface.

There are hints of these mechanisms in what we already know, however. Genetic knockout experiments and less leaky surface balances are teaching us more about what the various surfactant components can do, or more correctly, fail to do if they are absent. There is even a new hint in the oft used analogy between surface adsorption and liposomal fusion. Very recently Weber et al. have successfully tested a liposome model of protein-mediated membrane fusion in neuronal exocytosis [80] in which 'SNAREs, SNAPS, and SNAREpins' all participate. Is it too great a stretch of the imagination to envision the present surfactant lipids and proteins or even a protein yet to be discovered (perhaps SP-V = VELCRIN) playing similar roles at the air-water surface? Time will tell.

Acknowledgements

This special issue of *Biochimica et Biophysica Acta* is dedicated to John A. Clements, who pioneered the use of surface balances in studying pulmonary surfactant, and who has probably been the most influential leader in the field since its rebirth in the 1950s. Many of the authors of the chapters in this special issue have worked directly with him, and all are indebted to his vision. This author in particular wishes to thank John Clements for his many years of scientific guidance and good fellowship. It has been a

privilege each day to watch the master working with equal ease developing new theoretical ideas, operating on animals, mixing chemicals, soldering together new circuits, and even, outside the laboratory, tickling the ivories: truly a renaissance man. Ms. Lennell Allen's excellent preparation of the electron micrograph is gratefully acknowledged. The author is supported by NIH Grant HL 24075.

References

[1] R.E. Pattle, Nature 175 (1955) 1125–1127.
[2] L.A. Creuwels, L.M. van Golde, H.P. Haagsman, Lung 175 (1997) 1–39.
[3] C.M. Rebello, A.H. Jobe, J.W. Eisele, M. Ikegami, Am. J. Respir. Crit. Care Med. 154 (1996) 625–628.
[4] M. Ikegami, C.M. Rebello, A.H. Jobe, J. Appl. Physiol. 81 (1996) 2517–2522.
[5] A.W. Smits, S. Orgeig, C.B. Daniels, Am. J. Physiol. 266 (1994) R1309–R1313.
[6] W. Bernhard, H.P. Haagsman, T. Tschernig, C.F. Poets, A.D. Postle, M.E. van Eijk, H. von der Hardt, Am. J. Respir. Cell Mol. Biol. 17 (1997) 41–50.
[7] V. Im Hof, P. Gehr, V. Gerber, M.M. Lee, S. Schürch, Respir. Physiol. 109 (1997) 81–93.
[8] M.M. Lee, F.H.Y. Green, S. Schürch, Am. J. Respir. Crit. Care Med. 157 (1998) 445A.
[9] J. Bastacky, C.Y. Lee, J. Goerke, H. Koushafar, D. Yager, L. Kenaga, T.P. Speed, Y. Chen, J.A. Clements, J. Appl. Physiol. 79 (1995) 1615–1628.
[10] M.W. Magoon, J.R. Wright, A. Baritussio, M.C. Williams, J. Goerke, B.J. Benson, R.L. Hamilton, J.A. Clements, Biochim. Biophys. Acta 750 (1983) 18–31.
[11] N.J. Gross, K.R. Narine, J. Appl. Physiol. 66 (1989) 342–349.
[12] T. Akino, in: Robertson, van Golde, Batenburg (Eds.), Pulmonary Surfactant: from Molecular Biology to Clinical Practice, Elsevier, Amsterdam, 1992, pp. 19–31.
[13] A.D. Postle, J. Chromatogr. 415 (1987) 241–251.
[14] H. Bunger, U. Pison, J. Chromatogr. B Biomed. Appl. 672 (1995) 25–31.
[15] F. Guthmann, R. Haupt, M. Schlame, P.A. Stevens, B. Rustow, Int. J. Biochem. Cell Biol. 27 (1995) 1021–1026.
[16] M.C. Kahn, G.J. Anderson, W.R. Anyan, S.B. Hall, Am. J. Physiol. 269 (1995) L567–L573.
[17] R.J. Mason, J. Nellenbogen, J.A. Clements, J. Lipid Res. 17 (1976) 281–284.
[18] J. Hull, M. South, P. Phelan, K. Grimwood, Am. J. Respir. Crit. Care Med. 156 (1997) 161–165.
[19] B.A. Holm, Z. Wang, E.A. Egan, R.H. Notter, Pediatr. Res. 39 (1996) 805–811.
[20] M. Ikegami, T.R. Korfhagen, M.D. Bruno, J.A. Whitsett, A.H. Jobe, Am. J. Physiol. 272 (1997) L479–L485.
[21] J. Johansson, T. Curstedt, Eur. J. Biochem. 244 (1997) 675–693.
[22] F.R. Poulain, R. Chang, L. Allen, J. Goerke, S. Hawgood, J. Invest. Med. 175 (1996) 132A.
[23] C.J. Wong, J. Akiyama, L. Allen, S. Hawgood, Pediatr. Res. 39 (1996) 930–937.
[24] E. Putman, L.A. Creuwels, L.M. van Golde, H.P. Haagsman, Biochem. J. 320 (1996) 599–605.
[25] M. Gustafsson, T. Curstedt, H. Jornvall, J. Johansson, Biochem. J. 326 (1997) 799–806.
[26] R.A. Pinto, S. Hawgood, J.A. Clements, B.J. Benson, A. Naidu, R.L. Hamilton, J.R. Wright, Biochim. Biophys. Acta 1255 (1995) 16–22.
[27] J. Goerke, J.A. Clements, in: Macklem, Mead (Eds.), Handbook of Physiology, American Physiological Society, Washington, DC, 1986, pp. 247–261.
[28] S. Schürch, F. Possmayer, S. Cheng, A.M. Cockshutt, Am. J. Physiol. 263 (1992) L210–L218.
[29] S. Schürch, D. Schürch, T. Curstedt, B. Robertson, J. Appl. Physiol. 77 (1994) 974–986.
[30] S. Schürch, R. Qanbar, H. Bachofen, F. Possmayer, Biol. Neonate 67, (Suppl. 1) (1995) 61–76.
[31] B.M. Discher, W.R. Schief Jr., V. Vogel, K.M. Maloney, D.W. Grainger, S.B. Hall, Am. J. Respir. Crit. Care Med. 157 (1998) 446A.
[32] J. Goerke, in: Robertson, van Golde, Batenburg (Eds.), Pulmonary Surfactant: from Molecular Biology to Clinical Practice, Elsevier, Amsterdam, 1992, pp. 165–192.
[33] J. Goerke, in: Robertson, Taeusch (Eds.), Surfactant Therapy for Lung Disease, Marcel Dekker, New York, 1995, pp. 349–369.
[34] V. Taskar, J. John, E. Evander, B. Robertson, B. Jonson, Am. J. Respir. Crit. Care Med. 155 (1997) 313–320.
[35] L.J. Bjorklund, J. Ingimarsson, T. Curstedt, J. John, B. Robertson, O. Werner, C.T. Vilstrup, Pediatr. Res. 42 (1997) 348–355.
[36] K. Wada, A.H. Jobe, M. Ikegami, J. Appl. Physiol. 83 (1997) 1054–1061.
[37] G. Enhorning, J. Appl. Physiol. 42 (1977) 976–979.
[38] M. Liu, L. Wang, E. Li, G. Enhorning, Clin. Exp. Allergy 26 (1996) 270–275.
[39] G.T. De Sanctis, R.P. Tomkiewicz, B.K. Rubin, S. Schürch, M. King, Eur. Respir. J. 7 (1994) 1616–1621.
[40] M.H. Wagner, H. Segerer, H. Koch, A. Scheid, M. Obladen, Exp. Lung Res. 22 (1996) 667–676.
[41] Z. Wang, S.B. Hall, R.H. Notter, J. Lipid Res. 36 (1995) 1283–1293.
[42] G. Putz, J. Goerke, S. Schürch, J.A. Clements, J. Appl. Physiol. 76 (1994) 1417–1424.
[43] G. Putz, J. Goerke, J.A. Clements, J. Appl. Physiol. 77 (1994) 597–605.
[44] W.M. Schoel, S. Schürch, J. Goerke, Biochim. Biophys. Acta 1200 (1994) 281–290.
[45] G. Enhorning, J. Appl. Physiol. 43 (1977) 198–203.
[46] G. Putz, J. Goerke, H.W. Taeusch, J.A. Clements, J. Appl. Physiol. 76 (1994) 1425–1431.

[47] N.J. Gross, R. Veldhuizen, F. Possmayer, R. Dhand, Am. J. Physiol. 273 (1997) L907–L912.
[48] M.D. Henry, M. Ikegami, A.H. Jobe, Biol. Neonate 71 (1997) 181–189.
[49] K. Cho, S. Chida, M. Sasaki, T. Fujiwara, Acta Paediatr. Jpn. 38 (1996) 322–327.
[50] R.A. Veldhuizen, S.A. Hearn, J.F. Lewis, F. Possmayer, Biochem. J. 300 (1994) 519–524.
[51] S.H. Yu, F. Possmayer, Biochim. Biophys. Acta 1211 (1994) 350–358.
[52] S. Taneva, K.M. Keough, Biochemistry 36 (1997) 912–922.
[53] K. Yukitake, C.L. Brown, M.A. Schlueter, J.A. Clements, S. Hawgood, Pediatr. Res. 37 (1995) 21–25.
[54] F.X. McCormack, J. Stewart, D.R. Voelker, M. Damodarasamy, Biochemistry 36 (1997) 13963–13971.
[55] F.R. Poulain, S. Nir, S. Hawgood, Biochim. Biophys. Acta 1278 (1996) 169–175.
[56] B. Sun, T. Curstedt, G. Lindgren, B. Franzen, A.A. Alaiya, A. Calkovska, B. Robertson, Eur. Respir. J. 10 (1997) 1967–1974.
[57] S. Taneva, D.R. Voelker, K.M. Keough, Biochemistry 36 (1997) 8173–8179.
[58] Z. Wang, S.B. Hall, R.H. Notter, J. Lipid Res. 37 (1996) 790–798.
[59] S.G. Taneva, K.M. Keough, Biochemistry 33 (1994) 14660–14670.
[60] M.A. Oosterlaken-Dijksterhuis, H.P. Haagsman, L.M. van Golde, R.A. Demel, Biochemistry 30 (1991) 10965–10971.
[61] K. Nag, S.G. Taneva, J. Perez-Gil, A. Cruz, K.M. Keough, Biophys. J. 72 (1997) 2638–2650.
[62] B. Pastrana-Rios, S. Taneva, K.M. Keough, A.J. Mautone, R. Mendelsohn, Biophys. J. 69 (1995) 2531–2540.
[63] G. Grossmann, Y. Suzuki, B. Robertson, T. Kobayashi, P. Berggren, W.Z. Li, G.W. Song, B. Sun, J. Appl. Physiol. 82 (1997) 2003–2010.
[64] L.A. Creuwels, L.M. van Golde, H.P. Haagsman, Biochim. Biophys. Acta 1285 (1996) 1–8.
[65] L.A. Creuwels, R.A. Demel, L.M. van Golde, H.P. Haagsman, Biochim. Biophys. Acta 1254 (1995) 326–332.
[66] R. Qanbar, S. Cheng, F. Possmayer, S. Schürch, Am. J. Physiol. 271 (1996) L572–L580.
[67] L.A. Creuwels, E.H. Boer, R.A. Demel, L.M. van Golde, H.P. Haagsman, J. Biol. Chem. 270 (1995) 16225–16229.
[68] A. Clercx, G. Vandenbussche, T. Curstedt, J. Johansson, H. Jornvall, J.M. Ruysschaert, Eur. J. Biochem. 229 (1995) 465–472.
[69] T.R. Korfhagen, M.D. Bruno, G.F. Ross, K.M. Huelsman, M. Ikegami, A.H. Jobe, S.E. Wert, B.R. Stripp, R.E. Morris, S.W. Glasser, C.J. Bachurski, H.S. Iwamoto, J.A. Whitsett, Proc. Natl. Acad. Sci. USA 93 (1996) 9594–9599.
[70] H.T. Akinbi, J.S. Breslin, M. Ikegami, H.S. Iwamoto, J.C. Clark, J.A. Whitsett, A.H. Jobe, T.E. Weaver, J. Biol. Chem. 272 (1997) 9640–9647.
[71] J.C. Clark, T.E. Weaver, H.S. Iwamoto, M. Ikegami, A.H. Jobe, W.M. Hull, J.A. Whitsett, Am. J. Respir. Cell Mol. Biol. 16 (1997) 46–52.
[72] K. Tokieda, J.A. Whitsett, J.C. Clark, T.E. Weaver, K. Ikeda, K.B. McConnell, A.H. Jobe, M. Ikegami, H.S. Iwamoto, Am. J. Physiol. 273 (1997) L875–L882.
[73] D. Hatzis, G. Deiter, D.E. deMello, J. Floros, Exp. Lung Res. 20 (1994) 57–72.
[74] J. Floros, S.V. Veletza, P. Kotikalapudi, L. Krizkova, A.M. Karinch, C. Friedman, S. Buchter, K. Marks, Biochem. J. 305 (1995) 583–590.
[75] R.A. Veldhuizen, L.A. McCaig, T. Akino, J.F. Lewis, Am. J. Respir. Crit. Care Med. 152 (1995) 1867–1871.
[76] A. Hattori, Y. Kuroki, T. Katoh, H. Takahashi, H.Q. Shen, Y. Suzuki, T. Akino, Am. J. Respir. Cell Mol. Biol. 14 (1996) 608–619.
[77] A. Hattori, Y. Kuroki, H. Takahashi, H. Sohma, T. Akino, Am. J. Respir. Crit. Care Med. 155 (1997) 1785–1788.
[78] O. Lesur, T. Bouhadiba, B. Melloni, A. Cantin, J.A. Whitsett, R. Begin, Int. J. Exp. Pathol. 76 (1995) 287–298.
[79] S. Subramaniam, J.A. Whitsett, W. Hull, C.G. Gairola, Toxicol. Appl. Pharmacol. 140 (1996) 274–280.
[80] T. Weber, B.V. Zemelman, J.A. McNew, B. Westermann, M. Gmachl, F. Parlati, T.H. Sollner, J.E. Rothman, Cell 92 (1998) 759–772.