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Review

Structure of pulmonary surfactant membranes and films: The role of proteins and lipid–protein interactions

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ABSTRACT

The pulmonary surfactant system constitutes an excellent example of how dynamic membrane polymorphism governs some biological functions through specific lipid–lipid, lipid–protein and protein–protein interactions assembled in highly differentiated cells. Lipid–protein surfactant complexes are assembled in alveolar pneumocytes in the form of tightly packed membranes, which are stored in specialized organelles called lamellar bodies (LB). Upon secretion of LBs, surfactant develops a membrane-based network that covers rapidly and efficiently the whole respiratory surface. This membrane-based surface layer is organized in a way that permits efficient gas exchange while optimizing the encounter of many different molecules and cells at the epithelial surface, in a cross-talk essential to keep the whole organism safe from potential pathogenic invaders. The present review summarizes what is known about the structure of the different forms of surfactant, with special emphasis on current models of the molecular organization of surfactant membrane components. The architecture and the behaviour shown by surfactant structures in vivo are interpreted, to some extent, from the interactions and the properties exhibited by different surfactant models as they have been studied in vitro, particularly addressing the possible role played by surfactant proteins. However, the limitations in structural complexity and biophysical performance of surfactant preparations reconstituted in vitro will be highlighted in particular, to allow for a proper evaluation of the significance of the experimental model systems used so far to study structure–function relationships in surfactant, and to define future challenges in the design and production of more efficient clinical surfactants.

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1. Introduction

The lungs establish the largest surface contact that most air-breathing vertebrates have with their environment. Exposure of a sufficiently large surface to the air is required to facilitate appropriated

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levels of gas exchange to support metabolic functions [1,2]. A complex macromolecular system has evolved in the pulmonated organisms as part of their environmental interface, to provide optimal properties in terms of structural stability and accessibility to the air phase while raising an efficient barrier against environmental insults, including the entrance of pathogens. Pulmonary surfactant, a membrane-based lipid–protein complex, which is assembled and secreted onto the respiratory surface by specialized cells of the alveolar epithelium, contains molecular components simultaneously responsible for biophysical stabilizing activities [3] and innate defence mechanisms [4,5]. Which of these activities evolved first is a matter of debate, but it is becoming clear that the two functions, biophysics and defence, are now inseparably coordinated in the surfactant system.

Lack of an operative surfactant system is associated with severe respiratory dysfunctions [6–8]. The pulmonary surfactant system matures during the last few weeks of gestation, and babies delivered prematurely before a threshold amount of surfactant has been produced are at risk of developing Infant Respiratory Distress Syndrome (IRDS), a major cause of mortality and morbidity in neonates, particularly before supplementation with exogenous surfactant preparations was established as a routine therapeutical practice [9,10]. On the other hand, patients suffering from acute lung injury (ALI), arising from a number of different potential causes, often develop Acute Respiratory Distress Syndrome (ARDS) ending in severe respiratory failure due, at least in part, to inactivation of the surfactant system by inflammatory by-products and blood components leaked into the airways through a deteriorated alveolar-capillary barrier [11–13]. Much research in this field, carried out in the last decades, has been devoted to elucidating the role of the different lipid and protein components of surfactant, with the primary purpose of understanding the molecular mechanisms associated with surfactant function. An additional objective has been defining the minimal compositional requirements for an effective therapeutic surfactant [14–17]. Extensive knowledge is available today about structural and physicochemical properties of most surfactant components in simplified model systems, although we still do not fully understand how the whole surfactant complex is assembled and developed in vivo or the molecular mechanisms by which surfactant proteins and membranes modulate respiratory physiology. The clinical surfactant preparations available today are effective in preventing and treating IRDS in preterm babies, but have not proven effective in reverting or ameliorating ARDS, suggesting that clinical formulations are still suboptimal, but also that we need to get further insight into the molecular events defining surfactant action in the complex context of the alveolar spaces, both in normal and injured lungs.

Apart from the clinical importance of understanding structure–function correlations in surfactant, the pulmonary surfactant system constitutes an excellent example of how dynamic membrane polymorphism governs some biological functions through specific lipid–lipid and lipid–protein interactions assembled in highly differentiated cells [18–20]. Lipid–protein surfactant complexes are assembled in pneumocytes in the form of tightly packed membranes, which are stored in specialized organelles called lamellar bodies (LB). Upon secretion of LBs, surfactant develops a membrane-based network that covers rapidly and efficiently the whole respiratory surface. This membrane-based surface phase is organized in such a way that it permits an efficient exchange of molecules between the gas and the liquid regions in alveoli, enabling gases to reach the blood stream flowing on the other side of the thin alveolar-capillary barrier. At the same time, the respiratory surfactant layer optimizes the encounter between many different molecules and cells at the epithelial surface, in a cross-talk essential to keep the whole organism safe from potential pathogenic invaders [5].

The present review summarizes what is known about the structure of the different forms of surfactant, with special emphasis on current model systems on the molecular organization of surfactant membrane components. The architecture and behaviour shown by surfactant structures in vivo will be interpreted to some extent from the interactions and properties exhibited by different surfactant models as they have been studied in vitro, particularly addressing the possible roles played by surfactant proteins. However, the limitations in structural complexity and biophysical performance of surfactant preparations reconstituted in vitro will be particularly highlighted, to allow a proper evaluation of the significance of the experimental models used so far to study structure–function relationships in surfactant, and to define future challenges in the design and production of more efficient clinical surfactants.

2. Pulmonary surfactant: the molecules and the basic interactions

The composition of pulmonary surfactant has been discussed in detail in other reviews, including its analysis as a reference either to interpret differences in performance of current clinical surfactant preparations of natural origin [14] or to rationalize the selection of protein and lipid components used to produce new artificial surfactants [15,21]. The present review will then sketch only the main structural features, the potential interactions and some of the self-organization properties of what are considered the key compositional elements in surfactant [22,23] (see cartoon in Fig. 1). It is important to consider that composition of native pulmonary surfactant is usually studied using material obtained from bronchoalveolar lavage of animal lungs. This lavage may collect structures that could coexist in the airspaces but were assembled separately and/or play different functions, or from different locations of the respiratory tract. The traditional approach has been to fractionate the lipid/protein material obtained from lavage into what has been called *large aggregates* (LA), large membrane-based structures with relatively high density and very good surface activity, and *small aggregates* (SA), of lighter density and much less surface active [24–26]. These two fractions are considered as two different stages of surfactant in its morphological transformation in the respiratory cycle. However, the possibility that both LA and the SA fractions are intrinsically heterogeneous and could contain more than one type of structure, cannot be discarded.

2.1. The lipids and the membrane phases

In general terms, pulmonary surfactant is composed of around 80% phospholipids, 5–10% neutral lipids –mainly cholesterol–, and 8–10% proteins, with 5–6% of total surfactant mass being constituted by specific surfactant proteins [22]. The phospholipid fraction of surfactant is mainly responsible for forming surface active films at the respiratory air–liquid interface [19,23], but it also provides the scaffold or matrix on which the different surfactant structures are assembled. In the bulk phase of aqueous environments, phospholipids usually self-organize in the form of bilayers, which is also the structural form in which surfactant is assembled and stored by the pneumocytes. At polar/non-polar interfaces such as the air–liquid interface, phospholipids form oriented monolayers, with the headgroups oriented towards the aqueous phase and the hydrophobic acyl chains pointing toward the air. The higher the concentration of phospholipid molecules at the interface, the fewer the number of water molecules exposed to air and the lower the surface tension, which also defines a lower energy required to enlarge the surface exposed while opening the alveoli during inspiration [22]. In most mammals, half of surfactant phospholipids by mass is composed of disaturated species, mainly dipalmitoylphosphatidylcholine (DPPC), a scarce phospholipid species in other tissues. Evolution has probably selected DPPC as the main phospholipid species in surfactant because at physiological temperature, the saturated chains of DPPC can be packed to a very high density at the air–water interface, providing the large reductions of surface tensions required to stabilize the lung at the end of expiration [17,27]. The kinked chains of unsaturated phospholipid species –constituting