Pulmonary Surfactant: The Key to the Evolution of Air Breathing

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Pulmonary surfactant controls the surface tension at the air-liquid interface within the lung. This system had a single evolutionary origin that predates the evolution of the vertebrates and lungs. The lipid composition of surfactant has been subjected to evolutionary selection pressures, particularly temperature, throughout the evolution of the vertebrates.

Lungs have evolved independently on several occasions over the past 300 million years in association with the radiation and diversification of the vertebrates, such that all major vertebrate groups have members with lungs. However, lungs differ considerably in structure, embryological origin, and function between vertebrate groups. The bronchoalveolar lung of mammals is a branching “tree” of tubes leading to millions of tiny respiratory exchange units, termed alveoli. In humans there are ~25 branches and 300 million alveoli. This structure allows for the generation of an enormous respiratory surface area (up to 70 m² in adult humans). Generally, in nonmammals, lungs are baglike with either smooth walls or large, bellows-shaped respiratory units (termed faveoli) extending from the outer wall of the lung into a central air space. Birds have the most strikingly different lung structure, with a pair of small parabronchial lungs connected to a series of air sacs. Air is propelled via the airsacs, which act like bellows, in a unidirectional manner through the lung. The lungs consist of a series of tubes (parabronchi) from which emanate the very-small-diameter, rigid air capillaries, which lie in close apposition with blood capillaries and represent the site of gas exchange. Some reptiles and amphibia have a complex and dense arrangement of septate compartments. However, in contrast to mammals, the lungs of fish, amphibians, and reptiles always lack a bronchial tree, a diaphragm, and a separate pleuroperitoneal chamber, and they have respiratory units up to 100 times larger than alveoli of similar-sized mammals.

But all lungs have one common characteristic. They are internal, fluid-lined, gas-holding structures that inflate and deflate cyclically. As a result, all lungs face potential problems related to the surface tension of the fluid. Pulmonary surfactant is produced in the lung to decrease surface tension of this fluid lining (hypophase). Von Neergaard (quoted in Ref. 6) first demonstrated that the surfactant forces at the gas-liquid interface of the lung contribute substantially to the retractive pressure, and hence static compliance, of the lung. However, surfactant can also vary surface tension with the radius of curvature of each alveolus (or more accurately, regions within an alveolus) so that the pressures within all alveoli are maintained at similar values, permitting alveoli of different sizes to coexist. Surfactant also helps the narrowest airways to remain open, thereby reducing the resistance to air flow and controlling fluid balance in the lung. However, the alveoli, being interdependent units, do not necessarily stretch upon inflation but unpleat or unfold in a complex manner. Moreover, the many fluid-filled corners and crevices in the alveoli open and close as the lung inflates and deflates.

Surfactant in nonmammals exhibits an antiadhesive function, lining the interface between apposed epithelial surfaces within regions of a collapsed lung. As the two apposing surfaces peel apart, the lipids rise to the surface of the hypophase fluid at the expanding gas-liquid interface and lower the surface tension of this fluid, thereby decreasing the work required to separate the two surfaces. However, for surfactant to act as an antiadhesive, the respiratory tissues must “fold” in on themselves, possibly during exhalation, or when the ventilatory period is punctuated by protracted nonventilatory periods with low lung volume. These conditions occur frequently in the ventilatory pattern of nonmammals.

The pulmonary surfactant system

Pulmonary surfactant is a complex mixture of phospholipids (PL), neutral lipids (particularly cholesterol [Chol]), and surfactant proteins. The PL are assembled in the endoplasmic reticulum and the Golgi apparatus of alveolar type II cells and are stored in lamellar bodies until exocytosis (Fig. 1). The assembly of surfactant proteins into lamellar bodies is less clear and may proceed via the endoplasmic reticulum and Golgi apparatus to multivesicular bodies before combining with the lamellar bodies (10). The source(s) of alveolar Chol remains unknown (16). The lamellar bodies consist of a dense proteinaceous core with lipid bilayers arranged in concentric, stacked lamellae surrounded by a limiting membrane. After the lamellar bodies have been released into the alveolar space, they swell and unravel into a characteristic cross-hatched structure termed tubular myelin (Fig. 1). It is this structure that supplies the lipids for the surface film, which regulates the surface tension of the liquid lining the lung (10).

The ability to lower and vary surface tension with changing surface area is attributed to the interactions between the saturated PLs (DSPs), particularly dipalmitoylphosphatidylcholine (DPPC), and the other lipids, such as the unsaturated PLs (USPs) and Chol. Upon expiration, dynamic compression of the mixed surfactant film results in the “squeezing out” of USP and Chol, such that the surface film is enriched in DPPC.
The DPPC molecules can be compressed tightly together by virtue of their two fully saturated fatty acid chains. In so doing, they exclude water molecules from the air-liquid interface, thereby eliminating surface tension (19). Lipids can exist in either a fluid liquid-crystalline state or in a solid gel state. The transition between these two phases occurs at the phase transition temperature \( T_m \) of that lipid. For the surfactant lipids to spread over the alveolar surface upon inspiration, the surfactant film must exist in the liquid-crystalline state. Because DPPC has a \( T_m \) of 41°C, a pure DPPC film will exist in the gel form at mammalian body temperatures and hence adsorb extremely slowly to the air-liquid interface (19). The addition of other lipids, e.g., Chol or USP, into the surface film upon inspiration lowers the \( T_m \) of the lipid mixture, enabling it to exist in the fluid state at the same body temperature. In this state, the lipids are able to disperse to coat the surface of the expanding fluid layer.

Temperature therefore has a profound influence on the structure and function of surfactant lipids. Given that the majority of animals have much lower body temperatures than homeothermic mammals, how can animals regulate the fluidity of their surfactant film at low and/or fluctuating body temperatures? Furthermore, the low metabolic rates of nonmammals also have profound implications for the rates of synthesis of new components. Hence, for example, the need for additional quantities of USP in surfactant requires PL synthesis.
which may take some time. Hence USPs do not appear to be appropriate for controlling fluidity of surfactant in the face of the very rapid or step changes in body temperature, which commonly occur in most nonmammals and in torpid or hibernating mammals.

Chol is able to affect the fluidity of PLs directly. Chol is thought to increase the separation between PL molecules, thus disrupting the intermolecular interactions between their head groups and allowing greater rotational movement. At 10% by weight, or 20 mol%, Chol is the second most abundant lipid component of pulmonary surfactant. Although the source of surfactant Chol is not known, the ready availability of Chol in the alveolar compartment and in the circulating plasma makes this molecule a good candidate for the rapid regulation of surfactant fluidity (16). Hence, titrating the Chol/DSP ratio in the face of rapid body temperature changes is an excellent method for maintaining fluidity and a functional surfactant at low body temperatures.

The protein component of pulmonary surfactant represents ~10% by weight, and four surfactant proteins have been described. These are SP-A, SP-B, SP-C, and SP-D, which are synthesized in alveolar type II cells and are all associated with purified surfactant (11). Both the secretion and the reuptake of surfactant PLs into type II cells appear to be regulated by SP-A (11). Both the secretion and the reuptake of surfactant PLs into type II cells appear to be regulated by SP-A. Both SP-A and SP-B are essential for the formation and structural integrity of surfactant components (Fig. 1A). The hydrophobic surfactant proteins, SP-B and SP-C, strongly interact with the lipids and promote the formation and adsorption of the surfactant film to the air-liquid interface. However, the hydrophilic surfactant proteins, SP-A and SP-D, are predominantly involved in the innate host-defense system of the lung (11).

Using surfactant protein analysis, we determined that surfactant had a single evolutionary origin that predated the evolution of the vertebrates. We demonstrated that an SP-A-like protein is present in surfactant from all vertebrate classes, including in goldfish swimbladders (20) (Fig. 2A). Furthermore, the ultrastructure of the surfactant system is highly conserved. Lamellar bodies and tubular myelin-like structures have been observed in the lungs of reptiles (Fig. 1), birds, and amphibians. Lamellar bodies have been observed in the three extant species of lungfish, in the lungs of primitive air-breathing fish, and in the swimbladder of the rainbow trout. Thus surfactant from nonmammalian vertebrates appears to be produced, stored, and released in a similar manner to mammalian surfactant. Moreover, the system predates the evolution of lungs (reviewed in Ref. 4).

Lungs developed as out-pouchings of the gut, and the primary selection pressure for the evolution of lungs was probably aquatic hypoxia (18). The ancestral bony vertebrate was most likely lunged, inhabiting warm stagnant pools and gulping air to gain sufficient oxygen. The cells that produce surfactant, and contain SP-A, have been located in the gut of mammals (9). In the gut, surfactant may be important in controlling fluid-fluid interactions between liquids of different viscosities (e.g., the mucus and serous fluid layers). Alternatively, or perhaps additionally, gut surfactant may be involved in innate immunity (11). Hence it makes sense to propose that the surfactant-secreting cells migrated with the air-filled out-pouchings of the gut before the lung surfactant took on its current surface tension-controlling functions. Hence the surfactant system predated the evolution of lungs and was crucial for the evolution of air breathing.

**Surfactant and the evolution of air breathing**

Here we examine the evolution of the surfactant system in association with three of the major evolutionary steps for the vertebrates: 1) the separation of the Actinopterygian (bony) fish from the Sarcopterygia (lungfish) and the Tetrapods (land-dwelling vertebrates); 2) the “Land-Water Transition”; and 3) changes in body temperature, particularly the general pattern of increasing from cold ectotherms to warm heliotherms and endotherms. An evolutionary analysis of surfactant composition of the vertebrates ranging from air-breathing fish, teleosts, and lungfish to amphibians, reptiles, birds, and mammals has revealed that fish lungs and the lungs of the primitive Australian lungfish, Neoceratodus forsteri, have surfactant with approximately threefold greater amounts of Chol relative to DSP than any of the other vertebrate groups (5, 15) (Fig. 2B). However, DSP as a percentage of PL demonstrates the opposite trend, with only the mammals and some reptiles having high levels of 40–50% (5, 6) (Fig. 2C). Teleost swimbladders and the lungs of the air-breathing fish and N. forsteri contain PLs that are two- to fourfold less saturated than those of the derived dipnoans, the African and South American lungfish, and the majority of the amphibians and fivefold less than those of reptiles and mammals (5, 6). These opposite trends in Chol/PL and DSP/PL ratios result in a very dramatic pattern for the Chol/DSP ratio (5, 16). The fish and N. forsteri with their relatively simple baglike lungs have a Chol/DSP ratio up to an order of magnitude greater than the reptiles and mammals. The amphibians and the derived Dipnoans have intermediate levels of Chol relative to DSP, i.e., the ratio is approximately double that of the reptiles and mammals (6, 16).

Differences among terrestrial groups in the composition of surfactant probably reflect the temperature-dependent fluidity of surfactant PL. Because DPPC undergoes a phase transition from a gel to a liquid-crystalline state at 41°C, and realistically only homeotherms and the most heliothermic of the reptiles are likely to have body temperatures that approach this value, it follows that only these groups are likely to be capable of tolerating high DSP/PL ratios (5) (Fig. 2C). In contrast, advanced Dipnoans and amphibians generally have much lower body temperatures and have incorporated less DSP in their surfactant, with DSP/PL ratios of only 15–30% (5). Chol is not as effective as DSP at reducing surface tension but is able to lower the normal T_m of DSP, thereby maintaining the mixture...
in a fluid, easily adsorbable state over a much broader range of temperatures (reviewed in Ref. 16). Similarly, USP have a much lower Tm than their saturated counterparts. The surfactant of fish and amphibians, having greater Chol/DSP ratios (20–130%) compared with those of the warmer reptiles and mammals (10–15%), appear to benefit from this increased fluidity (5, 6). Bats have the lowest amount of surfactant Chol ever recorded. The two species we examined had 6 and 15 times less surfactant Chol than all other mammals studied to date (1) (Fig. 2B). The extraordinarily complex structure of bat lungs, with the tiny alveoli and high ventilatory capacity, correlates with the most sophisticated surfactant so far recorded.

It also appears that the combination of extremely high surfactant Chol and very low DSP may be a universal function of simple, largely avascular lungs, which are either not used or only infrequently used for respiratory purposes (16). This is supported by the fact that the smooth baglike or saccular lung used for gas storage in the rattlesnake has a threefold greater Chol/DSP ratio than the septated faveolar lung containing the respiratory tissue (8). Furthermore, the goldfish swimbladder, which is an internal gas-holding structure that is used predominantly for buoyancy, had one of the highest Chol/DSP ratios of any surfactant system (5). It is not clear why lungs and swimbladders have such high relative proportions of Chol. Is it a primitive feature, or does it represent an adaptive requirement? Possibly it is related to an increased need for spreadability in organs where the surfactant secretory cells are relatively few and far between. Alternatively, Chol may represent...
a primitive antioxidant, protecting the inner lining from oxidative damage. However, because fish surfactant is so simple, poorly surface active, dominated by Chol, and located within lungs only occasionally used for respiration (i.e., these species are facultative air breathers), we have termed the surfactant of fish and the Australian lungfish the “protosurfactant” (16).

In addition to the large-scale evolutionary changes attributable to temperature as the primary selection pressure, temperature is also the major acute controller for the lipid composition of surfactant. The first documentation that alveolar surfactant Chol can change dynamically in response to a physiological change occurred in the lizard, *Ctenophorus nuchalis* (2). This central Australian lizard withstands variations in body temperature from 13 to 44°C, with a mean preferred temperature of 36.4°C (2). A step decrease in temperature from 37 to 19 or 14°C for 4 h increased the Chol/PL ratio from 8% at 37°C to 15% at 19°C and 18% at 14°C (2). The increase in the ratio was due solely to an increase in the amount of Chol. Changes in the ratio were evident after as little as 2 h and were maintained for up to 48 h. Hence it appears that at low body temperatures the animals respond by selectively increasing the level of Chol in their surfactant, presumably to maintain fluidity (2). Similar changes in surfactant Chol occur in response to torpor in the fat-tailed dunnart, *Sminthopsis crassicaudata*, a desert-dwelling marsupial capable of reducing its body temperature to as little as 13°C for an average of 8 h and a maximum of 19 h. The absolute amounts of Chol and PL increased by up to 50% after as little as 1 h of torpor, and the Chol/PL and Chol/DSP ratios increased after 8 h in torpor (13). Similarly, an increase in Chol relative to total PL and DSP occurred in the microchiropteran bat, *Chalinolobus gouldii*, in response to a torpor bout at a body temperature of 25°C. However, another microchiropteran bat, *Nyctophilus geoffroyi*, which has the lowest Chol level ever recorded in a mammal, did not demonstrate this classic pattern (1).

**What is the evolutionary significance in surfactant function?**

We have proposed that the main function of surfactant in nonmammals is to act as an antiadhesive that prevents adhesion of adjacent epithelial surfaces at low lung volumes (Fig. 3, A and B). If the fluid lining the tissue-tissue interface has a high surface tension, then the work of initially separating...
ing the tissues will be high. As the surfaces are further separated and the fluid retreats to the corners, surface tension should play a smaller and smaller role in the work of breathing. Without an agent to lower the surface tension of the fluid intervening between the contacting epithelial surfaces (Fig. 3C), inspiration after lung collapse might be impossible, or at least extremely costly. The antiadhesive property of surfactant is therefore a biological manifestation of the ability of the lipids to lower surface tension.

Using scanning electron microscopy and computerized tomography scanning, a model was developed for the breathing dynamics of the lizard, Ctenophorus nuchalis (3). During lung deflation, the epithelial tissues, which are strung between the outer lung wall and the inner trabecular network, fold in on each other like a concertina (Fig. 3D). This causes large portions of epithelial tissue to come into contact, a situation in which the antiadhesive function of surfactant may be critical. Such a function can be demonstrated in nonmammalian vertebrates (reptiles, Actinopterygian fish, and salamanders) by demonstrating an increase in opening pressure following the removal of surfactant by rinsing with isotonic saline, a process termed lavage (5). Opening pressure is defined as the pressure required to inflate a completely collapsed isolated lung (Fig. 4, A and B). Surfactant also performed an antiadhesive function in the goldfish swimbladder (7). Furthermore, in almost all cases, filling pressure (i.e., the pressure required to continue to inflate the lung after initial lung opening) was extremely low (1–4 cmH2O) and remained unchanged before and after lavage (5). Therefore, the surfactant lipids appear to be important only during the initial phase of inflating a collapsed lung and not during further inflation.

The pattern and mode of breathing of nonmammalian vertebrates also indicate that an antiadhesive function might be essential for these animals, particularly when they are very cold. The aquatic amphibians Amphiuma and Siren collapse their lungs completely upon expiration, as do dipnoan lungfish and Polypterus. Complete collapse of the lung may also occur at end-expiration in some small frogs, and sea snakes cycle air by emptying and collapsing the saccular lung (reviewed in Ref. 5). Furthermore, at low body temperatures, the lizard C. nuchalis exhibits periods of apnea during which the lungs collapse and the epithelial surfaces may come into contact (5). The low metabolic rate of cold ectotherms decreases the need for frequent ventilations, and the lungs collapse for prolonged periods. Here surfactant is critical to decrease the work of separating the contacting epithelial surfaces when the occasional breath is required.

**Conclusion**

One of the great steps in vertebrate evolution has been the development of an internal lung. However, despite the morphological diversity of lungs, they are all essentially internal, fluid-lined structures that cycle air by changing volume. Moreover, lungs all face similar physical challenges. Organisms must maximize the surface for gas exchange while maximizing lung compliance to minimize the work of breathing. They must minimize the threat of infection, prevent the accumulation of irritants, and also prevent the lung from collapsing or filling with fluid. There are some constants in the evolution of aerial breathing, particularly the presence of a surfactant system. It appears that a surfactant system evolved before the evolution of lungs in vertebrates. It also appears that there are two different types of surfactant: one in the Actinopterygian fishes, which is high in Chol and USP, and one in the Sarcopterygian fishes and tetrapods, which is relatively low in Chol and USP and higher in DSP. The fish surfactant is more spreadable but not very surface active. The tetrapod surfactant is much more surface active and may have enabled the development of more complex lungs with smaller respiratory units and a greater total respiratory surface area, paving the way for the occupation of land. It is possible that fish surfactant is a "protosurfactant" that evolved into tetrapod surfactants but was retained as a protective lipid lining for the gas bladder of the modern fish and in gas-holding structures that are not used for respiration.

Within the tetrapods, the concentration of DSP increases with the evolution from cold, wet ectotherms (amphibians) to...
warm heliotherms and endotherms (some reptiles, birds, and mammals). It appears that the DSP/PL ratio is set by the “thermal programming” of the animal and does not represent a short-term method for maintaining fluidity. Chol declines in importance with the evolution of the vertebrates but retains an important function as a “rapid-response” method of maintaining fluidity of the surfactant lipids, particularly for ectotherms or heterothermic mammals faced with major diurnal fluctuations in body temperature.

The majority of nonmammalian lungs are essentially baglike, without a bronchial tree, and with only a few exceptions (e.g., tracheal lungs of snakes or parabronchial lungs of birds) regularly collapse completely. An important function of surfactant in these lung types is to prevent the epithelial surfaces from adhering to each other. But nonmammalian surfactant does not influence inflation compliance. Because there is little doubt that lungs of this type, and breathing patterns of this nature, represent the primitive form, we hypothesize that acting as an antiadhesive may be the original function of surfactant, but one which led naturally to the alveolar stability and compliance roles that dominate mammalian surfactant function.

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References