Comment to the Editor

Properly Interpreting Lipid-Protein Specificities in Pulmonary Surfactant

Recent studies have provided new information about the lateral distribution of lipids and proteins in pulmonary surfactant interfacial films, with a particular focus on the potential interaction of surfactant protein SP-B with the anionic surfactant phospholipid phosphatidylglycerol (PG) (1,2). SP-B is an essential protein for pulmonary surfactant to form surface active films at the air-liquid interface of the alveoli and to maintain very low surface tensions along the successive compression-expansion cycles during respiratory dynamics (3). In a study using electron spin resonance (ESR) spectroscopy, it was reported that the cationic character of SP-B makes it interact preferentially with anionic over zwitterionic phospholipids, and that SP-B/phospholipid interactions are particularly selective for PG under physiological conditions of pH and ionic strength (4). As a matter of fact, SP-B exhibits, in PG-containing membranes, a particular rotational dynamic, which is clearly different from that shown by the protein in zwitterionic bilayers, reflecting conformational effects of SP-B/PG electrostatic interactions on the disposition/organization of SP-B in lipid-protein complexes (5). This is relevant, because although the main phospholipid in surfactant is the zwitterionic phosphatidylcholine (PC), the anionic PG fraction, unusual in other animal cell membranes, is considered essential for surfactant action.

Two studies published recently by Dr. Galla's group (1,2) have approached an analysis of the lateral distribution of SP-B in PC/PG interfacial films, by applying fluorescence microscopy and time-of-flight secondary ion mass spectrometry to lipid/protein films transferred onto solid supports. These studies provide evidences that in a wide range of environmental conditions, binary mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylgycerol segregate under compression into different regions at the interface, and that SP-B colocalizes with PC rather than with PG-containing domains. These results have been interpreted as proof against selective SP-B/PG interactions, giving readers the impression that SP-B/PG interaction is a controversial matter. However, it is important to note that the significance of the information provided by spectroscopic techniques is essentially different than that obtained by techniques with only microscopic resolution such as the techniques applied by the Galla group. Data obtained by fluorescence resonance energy transfer or ESR, for instance, refer to specific molecular properties and therefore provide information about particular interactions. Our ESR measurements showed a direct effect of SP-B to decrease the mobility of a PG spin probe, which was not shown toward any other phospholipid species of those tested (4). This is not incompatible by any means with a preferential distribution of SP-B/lipid molecular complexes into PC-enriched lipid phases, as detected by Galla and co-workers in interfacial films (1,2). The data by Galla et al. do not discard the likely possibility that SP-B would be accompanied, when distributed in the more disordered PC-enriched phase, by one or few PG molecules bound in defined protein sites. These specific PG molecules would be the ones detected as selectively perturbed by SP-B in the ESR experiments. Detection of the particular spectral properties of these few SP-B-perturbed PG molecules requires saturation of lipid/protein interaction, and that is the reason why ESR experiments have to be carried out at particularly high protein/lipid contents. Data obtained at such high protein/lipid ratios have been criticized by some authors as potentially related to nonnatural effects. In our opinion, the molecular interactions unraveled by ESR at high protein/lipid ratios probably also exist at lower ratios, close to the physiological range of protein proportions.

Some differences between spectroscopic and microscopic experimental models cannot be discarded as additional potential sources of variation in detecting apparent lipid specificities. Choice of saturated or unsaturated phospholipid species, incorporation of spurious fluorescent probes, or transfer of interfacial films onto solid supports as required for atomic force microscopy imaging, could all cause differences in the distribution of lipids and protein. SP-B has been proposed to associate in between adjacent lipid layers (3). Multilayer-like structures, formed in certain regions of the interfacial films upon compressing under high pressures, could favor a lateral redistribution of SP-B independent of lipid composition. Furthermore, microscopic images may only show a picture coming from lipid-protein layers subjected to relatively long periods of equilibration, whereas spectroscopic measurements have the potential to provide information on processes occurring at very different timescales.

Still, without a doubt, the information provided by microscopic and spectroscopic techniques should be considered complementary, as these techniques can inform about the structure of surfactant films at very different space and time resolution. We need enough information in the full range from the molecular up to the microscopic scales to understand how pulmonary surfactant performs its exigent physiological function.

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