Modulation Of Membrane Properties By Hydrophobic Surfactant Proteins Sp-B And Sp-C As Assessed In Giant Vesicles

Elisa Parra, Lara H. Moleiro, Iván López-Montero, Antonio Cruz, Francisco Monroy, Jesús Pérez-Gil

Dept. Biochemistry, Fac. Biology and Dept. Physical Chemistry, Fac. Chemistry, UCM, Madrid.

Pulmonary surfactant is a complex mixture of lipids and proteins whose main function is to reduce surface tension at the alveolar air–liquid interface of lungs in order to avoid alveolar collapse at the end of expiration and to facilitate the work of breathing. It is composed by around 90% lipids and 8-10% specific surfactant proteins, including the hydrophobic proteins SP-B and SP-C. In this study, we have analyzed the permeability of two fluorescent water-soluble probes, FM®1-43 and calcein, in model phospholipid membranes supplemented with SP-B and/or SP-C. The membrane-sensitive probe FM®1-43, which is non-fluorescent in water, only labels the external leaflet of membranes due to its amphiphilic character, and calcein emits green fluorescence in aqueous media. The behaviour of these probes has been observed by fluorescence microscopy, studying the effect of SP-B and SP-C on the arrangement and accessibility of membranes in giant POPC vesicles.

In the presence of physiological amounts of both hydrophobic proteins SP-B and SP-C, giant oligolamellar vesicles incorporated almost instantaneously the probe FM®1-43 in every single membrane once added to the external medium. In contrast, oligolamellar vesicles made of pure POPC were only labelled in the outermost membrane layer. Lipid vesicles were impermeable for calcein, while this probe could permeate through membranes supplemented with SP-B, SP-C or mixtures of both proteins. Some differences were noticed between the effect of SP-B and SP-C on giant vesicles: suspensions containing only SP-B were stable but those containing only SP-C were quite dynamic, undergoing frequent fluctuations, reorganizations and ruptures as observed under the microscope. Structural differences caused by hydrophobic surfactant proteins in POPC vesicles were confirmed by electron microscopy studies.

These results suggest that SP-B and SP-C have different contributions to inter- and intra-membrane lipid dynamics, and that the combined action of both proteins could provide a unique synergic effect to modulate structure and dynamics of pulmonary surfactant membranes and films.