Answer

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Our 1996 study [1] was undertaken to characterize the secondary structure and the orientation of the KL_4 peptide in a lipid environment, and to relate these data to the known structural properties of pulmonary surfactant proteins B and C (SP-B and SP-C).

Using polarized Fourier transform infrared (FTIR) spectroscopy and CD spectroscopy we unequivocally demonstrated a helical structure and a transmembrane orientation of KL_4 in a DPPC/PG bilayer. The monolayer model of KL_4 proposed by Cochrane and Revak [2] is partly based on fluorescence data [2] and Raman spectroscopy data [3] of the peptide in lipid bilayers. Due to the differences between lipid bilayers and monolayers, it is difficult to predict how a transmembrane stretch of 21 residues with charged residues distributed around the helical circumference can be accommodated in a lipid monolayer. No attempt was made in our study to relate the orientation of KL_4 in a lipid bilayer to the orientation in a lipid monolayer [1]. Further studies are needed in order to establish the orientation of KL_4 in a lipid monolayer. For example, it has been demonstrated that native SP-C adopts a transmembrane orientation in a lipid bilayer [4,5] but the SP-C helix tilt angle to the interface normal changes from $\sim 24°$ in lipid bilayers to $\sim 70°$ in DPPC monolayer films [6]. We noticed that the orientation of KL_4 in a lipid bilayer closely resembled that of native SP-C [4–7] but contrasted to the proposed interaction of SP-B with lipid bilayers [8–10], and therefore suggested that the mechanism of action of KL_4 may be similar to that of SP-C rather than to that of SP-B. We strongly believe that interactions between SP-B, SP-C, and synthetic analogues on the one hand and lipid bilayers on the other are relevant, since these proteins are hydrophobic and need to be transported in a lipid bilayer environment to the interfacial monolayer. It should be remembered that the main location and site of action (bilayer, monolayer, or both) of SP-B and SP-C in lung surfactant remain to be established.

We also pointed out that the KL_4 helix exhibits a mixed nonpolar/polar surface which is different from the amphipathic SP-B helices [8,11] and nonpolar SP-C helix [12]. The spreading of KL_4/DPPC/PG/PA vesicles at an air/water interface is rapid, but slower than the spreading of native SP-C/DPPC/PG/PA vesicles or vesicles composed of an SP-C/bacteriorhodopsin hybrid mixed with the same lipids [1]. We suggested that this may be caused by the fact that SP-C exhibits a 'mobility gradient' in a phospholipid bilayer, i.e. only the N-terminal end of the helix contains basic residues that can interact with the phospholipid head groups [7], while KL_4 is expected to interact similarly with the phospholipid head groups at both ends of the helix [1].

Studies of the relationships between structure, orientation and activity of SP-B and SP-C, and their analogues, are necessary in order to understand their roles in the adsorption of phospholipids to the alveolar air-liquid interface. Such knowledge is also required for rational design of synthetic analogues of the surfactant proteins.

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