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Preview

Molecular Engineering of Peptides

Deeper understanding of the role of short peptides in lipid layers has revealed potential applications for rationally designed synthetic replacements. A recent report illustrates the succesful design of a peptoid mimic of SP-C, a protein linked to respiratory distress syndrome.

Traditionally, rational drug design has primarily relied on biomolecular physics and chemistry. However, improved technologies have illustrated the importance of understanding the transport properties of compounds, forging collaborations between teams that include both chemists and engineers. An excellent example of the progress that can be made through such cross-disciplinary research is provided Wu and colleagues in a paper published in this issue of *Chemistry & Biology* [1] which describes the efforts of the groups of Annelise Barron of the Chemical Engineering Department at Northwestern University and of Ka Yee Lee of the University of Chicago Chemistry Department. This team endeavors to use rational design to develop replacements for the natural lung surfactant proteins that are essential for proper function of the human lung and have focused in particular on the family of "peptoids," poly-N-substituted glycines [2], as sequence-specific oligomers whose properties can be made to mimic natural peptides.

Lipids, such as dipalmitoylphosphatidylcholine, are the major component of lung surfactant, which lowers the interfacial tension of the aveolar lining of the lung to permit breathing. However, lung surfactant also contains four different proteins, SP-A, SP-B, SP-C, and SP-D [3]. Two of these, SP-B and SP-C, appear to modulate the fluidity of the membrane, particularly during the breathing cycle, and the lack of one of these proteins, SP-B, results in respiratory distress syndrome in severely premature infants [4], leading to death unless a functional replacement is supplied. This condition is echoed in transgenic mice that do not express SP-B protein because the corresponding gene has been ablated [5], and replacement therapy with surfactants containing either SP-B or SP-C has been shown to restore lung function to surfactant-deficient animals [6, 7]. While lung surfactant from animals can successfully substitute for the missing protein, risks of contamination and immunogenic reaction motivate the search for synthetic replacements for the natural proteins.

In the work of Wu et al. [1], a Langmuir-Wilhelmy surface balance is exploited as a platform for examining the effects of synthetic lung surfactant components on lipid monolayer properties under conditions that to some extent mimic breathing. In contrast to other systems containing vesicles and multilayer stacks that contain many lipid layers, providing an abundant experimental signal, the Langmuir trough contains only one lipid monolayer, which gives insufficient signal for some important experimental methods such as NMR but, more importantly, allows the area of a lipid film overlying water to be controlled dynamically and studied by various methods including fluorescence microscopy, X-ray diffraction, and pressure-area isotherm measurements. The use of the Langmuir trough to measure interactions between lipids and lung surfactant peptides, specifically a 25-amino-acid, truncated portion of the 78-residue SP-B protein, was pioneered by the groups of Zasadzinski and Waring [8].

The Barron and Lee collaboration focused on SP-C, a 35-residue peptide that evidently adopts a transmembrane orientation [3,9]. While the membrane-spanning helix of SP-C (residues 9–34) appears to be important in maintaining peptide function, the exact residues do not appear to be critical [9], suggesting that nonpeptide mimics might be viable replacements for SP-C. To this end, the authors chose to synthesize and study in vitro peptoid mimics of SP-C. As Wu et al. explain, peptoids are stable against protease degradation [10], less prone to immune recognition than proteins, and relatively easy to synthesize [2]. While their backbone is achiral, chirality and helix formation can be induced through attachment of chiral side groups, which can also be chosen to mimic the side chains of natural proteins, including their charge, hydrophobicity, and hydrophilicity [11]. Peptoid versions of short arginine-rich peptides have already been synthesized that successfully transport proteins across membranes, as their peptide counterparts do [12]. This discovery, combined with the observation that at least the helical portion of SP-C is not sensitive to amino-acid sequence (as long as helicity is preserved), suggests that suitably designed peptoids should be good candidates for mimicing SP-C function.

Indeed, the researchers found strong similarities between a slightly modified SP-C peptide and a peptoid mimic in their effects on the physical properties of membranes, including surface-tension versus time in a pulsating bubble surfactometer and pressure-area isotherms and fluorescence microscopy in a Langmuir-Wilhelmy surface balance. The group now plans to carry out animal studies with the peptoid mimic of SP-C and to synthesize and study a peptoid mimic of the more complex SP-B lung surfactant peptide.

In general, the most promising protein candidates for mimicry are short ones, whose function does not require precisely designed active sites. Thus, short membrane peptides are among the most obvious choices. In addition to membrane transporters and lung surfactant peptides (which meet these criteria), antimicrobial peptides come to mind. These are usually 15–45-residue peptides, with predominantly α-helical, β sheet, or mixed secondary structure, frequently both cationic and amphipathic [13, 14]. They function by thinning the membrane or perforating it, causing leakage of electrolytes. Why these peptides work selectively on bacterial membranes and not on host cell membranes is still rather

mysterious, although charge is evidently a factor [14]. Antimicrobial peptide activity does not usually depend on interactions with membrane proteins and is equivalent in D- and L-enantiomers [15], making them good candidates for mimicry. Indeed, mimics consisting of single chain arginine surfactants [16] and cyclic D,L-α peptides [17] have been shown to have antibacterial activity. Peptoids have evidently not yet been investigated for this purpose, but the need for new, easily synthesized antibiotics provides an obvious motivation to pursue this possibility.

Finally, short membrane-associated peptides and their mimics offer an excellent opportunity not only to study basic molecular physical chemistry through Langmuir trough and related experiments, but also to test and sharpen molecular simulation tools, particularly molecular dynamics (MD) methods. Such methods are best suited to relatively small biological molecules, such as lipids and short peptides, whose function is not regulated by specific interactions that would be difficult to capture accurately with empirical force fields used in most MD methods. Thus, MD methods are now being increasingly applied to membranes with small peptides, including antimicrobial peptides [18, 19], and preliminary work on lung surfactant peptides [20]. Presumably, efforts to simulate interactions of peptoids with lipid layers and to compare these simulations with those of their peptide analogs will soon be forthcoming.

It seems clear that small peptide-lipid systems offer teams involving experimental, theoretical scientists and engineers a treasure trove of opportunities, with the potential for eventual big payoffs in medicinal applications.

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Selected Reading

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