Chemistry & Biology, Vol. 10, 1057–1063, November, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.chembiol.2003.10.008

Helical Peptoid Mimics of Lung Surfactant Protein C

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Summary

Among the families of peptidomimetic foldamers under development as novel biomaterials and therapeutics, poly-*N*-substituted glycines (peptoids) with α-chiral side chains are of particular interest for their ability to adopt stable, helical secondary structure in organic and aqueous solution. Here, we show that a peptoid 22-mer with a biomimetic sequence of side chains and an amphipathic, helical secondary structure acts as an excellent mimic of surfactant protein C (SP-C), a small protein that plays an important role in surfactant replacement therapy for the treatment of neonatal respiratory distress syndrome. When integrated into a lipid film, the helical peptoid SP mimic captures the essential surface-active behaviors of the natural protein. This work provides an example of how an abiological oligomer that closely mimics both the hydrophobic/polar sequence patterning and the fold of a natural protein can also mimic its biophysical function.

Introduction

Recent research has been directed toward the creation of nonnatural, sequence-specific oligomers with bioinspired structures that capture both the amino acid sequence patterning and three-dimensional folds of natural proteins [1–3]. Such biomimetic oligomers may eventually serve as useful peptide replacements with better in vivo stability than the natural molecules. The hypothesis is that compounds with bioinspired structures may also offer good functional mimicry. Several different families of abiological oligomers have been synthesized [2, 3] and proposed as novel mimics of natural molecules such as magainin, a helical, amphipathic antimicrobial peptide [4–6]. One such family of molecules is the poly-*N*-substituted glycines or "peptoids," which have close structural similarity to peptides but are essentially invulnerable to protease degradation [7] and hence are biostable and less prone to immune system recognition [8]. Despite the achirality of the *N*-substituted glycine backbone and its absence of hydrogen bond donors, oligopeptoids are able to adopt stable, chiral helices when substituted with α-chiral, sterically bulky side chains [9, 10]. Their ability to form stable helices makes peptoids an excellent candidate for mimicry of bioactive molecules that rely on helical structure for proper function.

There is a clinical need for good mimics of the hydrophobic human lung surfactant proteins (SP), which perform critical functions in lung surfactant (LS) replacements used to treat respiratory distress syndrome (RDS) in premature infants. LS is a complex mixture of lipids and surfactant proteins that dramatically reduces alveolar surface tension and the work of breathing [11]. Animal-derived surfactants are widely used and show good efficacy in rescuing premature infants who suffer from respiratory distress due to a lack of surfactant at birth [11]; however, this approach raises some safety concerns [12]. In recent years, there has been increasing interest in the development of a synthetic, biomimetic LS replacement that functions as well as the animal-derived material [13]. The success of this endeavor requires an understanding of the roles of the lipid and surfactant protein components of natural LS [11, 13] and an ability to closely mimic their surface-active properties. Natural LS is composed primarily of dipalmitoylphosphatidylcholine (DPPC), unsaturated phosphatidylcholines (PC), unsaturated phosphatidylglycerol (PG), palmitic acid (PA), and surfactant proteins (SP-A, B, C, and D), along with other minor lipid components and cholesterol [11]. Although SP comprise $<10\%$ of LS by weight [11], surfactant replacements composed of lipids alone and lacking SP have been shown to be ineffective in capturing the requisite properties of LS for therapeutic purposes [14]. Researchers have shown that in particular, the hydrophobic, amphipathic proteins SP-B and SP-C are critical for the proper biophysical functioning of LS, due to their ability to enhance the adsorption and respreading of the lipid components at the air-liquid interface [15]. With this realization, several groups have developed synthetic peptide or recombinant protein versions of SP-B and SP-C and investigated their ability to enhance the performance of surfactant formulations composed of synthetic lipids [16–20]. These mimics are currently being used to investigate the mechanisms of action of SP-B and SP-C [21–24]; however, it is still not conclusively understood whether these two proteins work synergistically or individually to affect and enhance the biophysical surface activity of the natural lipid mixture.

SP-C is the smaller and simpler of the two amphipathic LS proteins, at just 35 monomers in length. The three-dimensional structure of human SP-C has been solved by 2D NMR in methanol solution and is predominantly helical [25]. In its correctly folded form, this amphipathic protein contains an α helix ~37 Å long comprising residues 9–34. Within this region is a 26 Å long, valyl-rich stretch of hydrophobic amino acids. FTIR studies have shown that the SP-C α helix orients in a transbilayer

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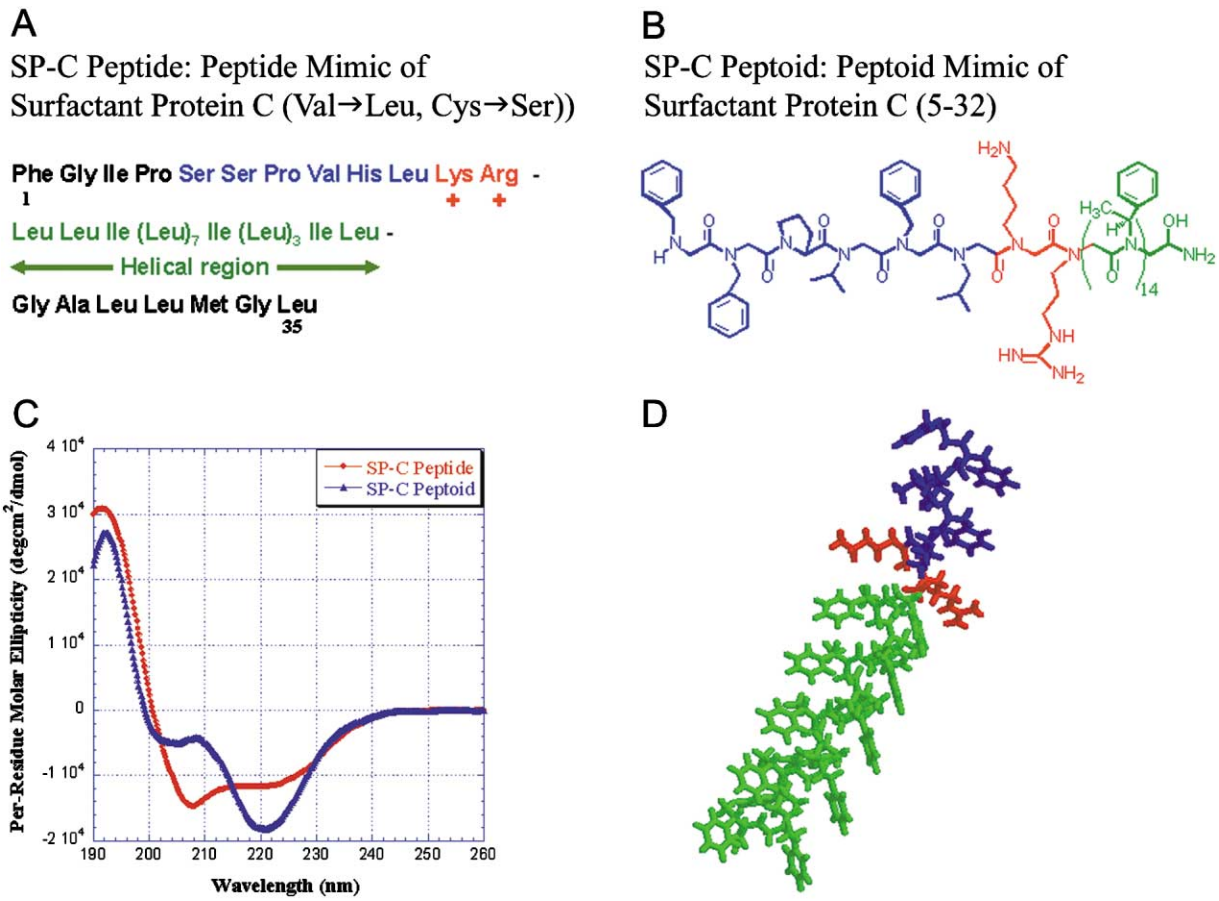

Figure 1. SP-C Peptide and Peptoid Sequence and Structure

(A) A modified SP-C peptide sequence.

(B) The chemical structure of a peptoid 22-mer created as a mimic of (A).

(C) CD spectra of the peptoid-based SP-C mimic in comparison to that of the SP-C peptide shown in (A).

(D) A molecular model of the SP-C peptoid structure. The helical, hydrophobic region of the peptoid is displayed in green, the charged residues (*N*Lys and *N*Arg) are shown in red, and the flexible, achiral amino-terminal region is shown in blue.

orientation in a fluid lipid film, where the helical region interacts hydrophobically with lipid acyl chains [26]. Two adjacent, positively charged residues at positions 11 and 12 interact with anionic phospholipid head groups and promote SP binding to the monolayer or bilayer by ionic interactions [27]. Cysteine residues 5 and 6 are posttranslationally modified with palmitoyl groups, the function and importance of which are debated in the literature [28–32]. In an LS film, SP-C promotes phospholipid insertion into the air-liquid interface [24] and thereby enhances the rate of lipid adsorption [30, 31] as well as the respreading of the alveolar film upon inhalation [33].

Here, we show that a nonnatural peptoid with a specific, 22-monomer sequence and an amphipathic, helical structure serves as an excellent mimic of lung surfactant protein C. When integrated into a lipid film, the peptoid analog of SP-C captures, to a significant extent, the unique surface-active behaviors of the natural protein. These results show that a nonnatural oligomer that mimics both the hydrophobic/polar sequence patterning and the folded structure of a natural protein can also mimic that protein's biophysical functioning. The novel LS replacement we have created offers an intriguing and potentially advantageous alternative to animal-derived and peptide-based surfactants.

Results and Discussion

Various peptide mimics of SP-C have been created and have shown promise as spreading agents for surfactant lipids [18–20, 34, 35]. Due to its extreme hydrophobicity and strong tendency to misfold and aggregate in the absence of phospholipids [36], SP-C peptide with the natural human sequence is very challenging to synthesize and purify in good yield. Therefore, researchers have identified alternative peptide sequences, closely related to that of human SP-C, comprising amino acid substitutions that reduce the peptide's tendency to aggregate in solution without appearing to compromise its activity [36, 37]. After finding a protein with the natural human sequence very difficult to work with, we chose to use a modified polypeptide for comparison to our peptide mimic, following the previous work cited above. The primary structure of the modified polypeptide [SP-C

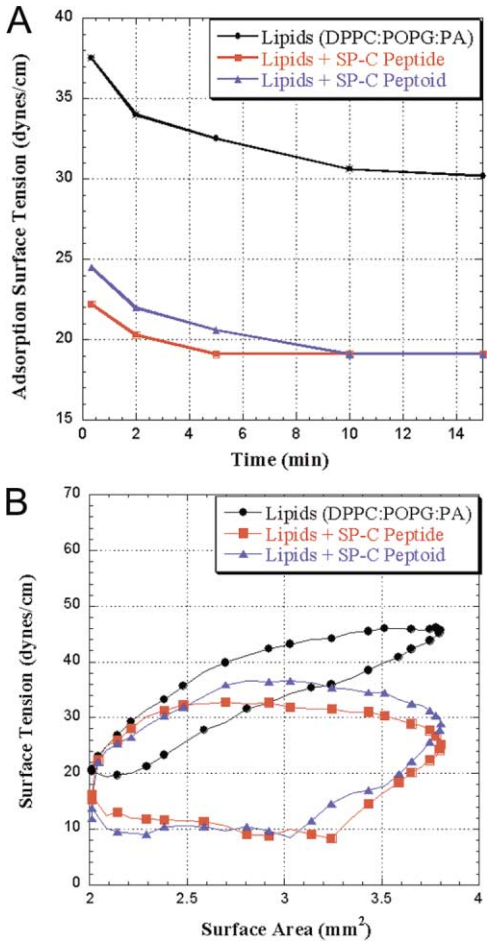

Figure 2. Pulsating Bubble Surfactometry Results

Static and dynamic characterization of surfactant film properties performed on a pulsating bubble surfactometer (PBS).

(A) Adsorption surface tension γ versus time t for lipids alone and with addition of either SP-C peptide or SP-C peptoid. The presence of the SP-C mimics significantly improves adsorption kinetics.

(B) Surface tension versus area (compression and expansion loops obtained by cycling at a frequency of 20 cycles/min) of a pure lipid mixture, a lipid mixture with SP-C peptide, and a lipid mixture with SP-C peptoid. The addition of either the SP-C peptide or the SP-C peptoid alters the loop shape so that less area compression is required to obtain low surface tension, relative to the observation for the pure lipid mixture. Additionally, the maximum surface tension is substantially reduced upon addition of either mimic.

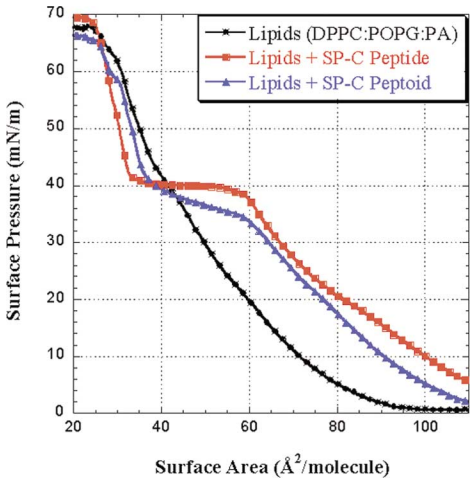

Figure 3. Langmuir-Wilhelmy Surface Balance Isotherms

Surface pressure (π)-area isotherms obtained for a lipid mixture alone and with 10% (w/w) of either SP-C peptide or SP-C peptoid added. Results indicate that the addition of the SP-C mimics engenders biomimetic surface activity, as indicated by liftoff at a higher molecular area and the introduction of a biomimetic plateau.

(Val→Leu, Cys→Ser)] used in these studies is shown in Figure 1A.

A peptoid oligomer was designed to capture both the amphipathic and helical characteristics of the SP-C protein. The chemical structure of the peptoid mimic we created, a 22-mer ("SP-C peptoid"), is shown in Figure 1B. The SP-C peptoid mimics the patterning of hydrophobic and polar side chain moieties in a truncated version of SP-C, including residues 5–32 of the natural 35-mer, which has been shown to have comparable activity to native SP-C [18]. Oligopeptoids comprised of *N*-(S)-1-phenylethyl (*N*spe) monomers, with their bulky, aromatic side chain moieties, adopt particularly stable polyproline type I-like helices with *cis*-amide bonds, 3 residues/turn, and a pitch of ~6 Å [9, 10, 38]. Taking into account the differences in helical pitch between a peptide α helix and a peptoid helix, we estimated that 14 *N*spe residues are sufficient to mimic the length of the hydrophobic helical stretch in SP-C. The 8-residue amino-terminal stretch of the SP-C peptoid is composed of achiral residues, most of which have side chain structures similar to the amino acids found in human SP-C (5–12) and conserve the basic patterning of charged and polar residues in the natural peptide. We substituted *N*-phenylmethyl (*N*pm) monomers for the palmitoylated cysteines found at positions 5 and 6 in the human sequence (similar to a natural, phenylalanine substitution found at position 6 in canine SP-C [39]) and the histidine found at position 9 in natural, human SP-C.

The peptoid oligomer shown in Figure 1B exhibits circular dichroism (CD) spectral features in organic solution that are similar to that of SP-C peptide (Val→Leu, Cys→Ser) and are characteristic of a stable helical structure, with an intense maximum at 192 nm and double minima at λ ~205 nm and 220 nm (Figure 1C) [40]. These are the characteristic spectral signatures of a helical structure with highly ordered backbone amide bonds [9]. Hence, the SP-C peptoid satisfies one of the major structural criteria—helicity—believed to be important for mimicry of the natural protein. An idealized molecular model of the SP-C peptoid we designed is shown in Figure 1D, where the hydrophobic, helical region is shown in green, the two positively charged residues in red, and the remainder of the amino-terminal region in blue.

The biophysical film characteristics that are important to capture with an LS replacement include the abilities of natural LS to adsorb rapidly to an air-water interface, to reduce and control surface tension as a function of surface area, and to respread quickly upon surface expansion [11]. Based on a previous report [41], we chose an optimized lipid formulation comprised of DPPC:POPG:PA (68:22:9, by weight) that has been shown to closely

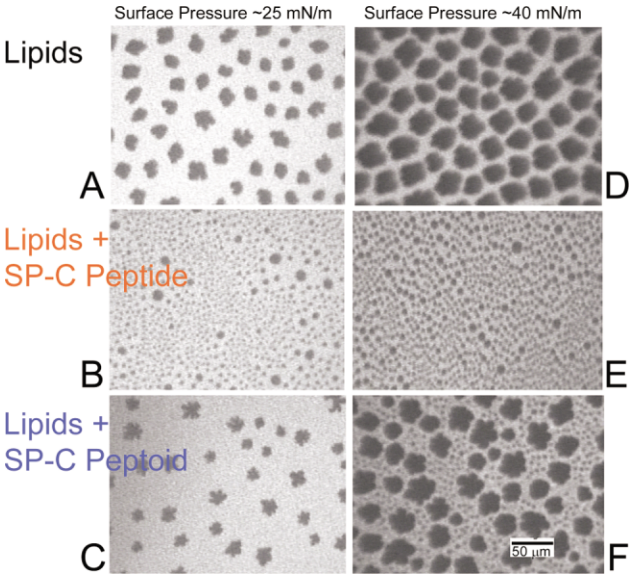

Figure 4. Fluorescence Microscopy Images

Fluorescence microscope images of surfactant film morphology on the LWSB. Images correspond to surface pressures of 25 mN/m (left) and 40 mN/m (right) for a pure lipid mixture (A and D), a lipid/SP-C peptide mixture (B and E), and a lipid/SP-C peptoid mixture (C and F).

mimic the behaviors of the lipid portion of LS [11]. The performance of this lipid mixture alone and with the addition of 10% (w/w) SP-C peptide or peptoid was characterized using a pulsating bubble surfactometer (PBS) at 37°C. Figure 2A compares the adsorption surface tension (γ_{ads}) as a function of time for the three surfactant formulations, obtained by running the PBS in static mode, i.e., without cycling bubble area. In the absence of SP mimics, the lipid mixture fails to reach γ_{ads} lower than 30 dynes/cm even after 15 min (natural, animal-derived LS reaches $\gamma_{ads} \sim$20–25 dynes/cm within a few seconds [42]). We find that the addition of the SP-C peptide significantly accelerates the kinetics of lipid adsorption to the interface, allowing the film to reach $\gamma_{ads} <$25 dynes/cm within 20 s. With the addition of the helical SP-C peptoid, we observe similarly accelerated kinetics of adsorption.

It is also important for an LS replacement to reduce and control surface tension, γ, as surface area is cyclically expanded and compressed. Figure 2B shows a plot, obtained on the PBS in a dynamic mode, showing γ as a function of bubble surface area for a lipid mixture, a lipid/SP-C peptide mixture, and a lipid/SP-C peptoid mixture. The compression-expansion loop for the pure lipid mixture shows that a significant degree of film compression is required to achieve a minimum γ of 20 dynes/cm, whereas a relatively high maximum γ of 46 dynes/cm is reached upon film expansion. The addition of the SP-C peptide substantially reduces the degree of film compression required to achieve a much lower minimum γ of 10 dynes/cm and also allows the attainment of a much lower maximum γ of $\sim$30 dynes/cm. These surface-active behaviors are unique to lung surfactant mixtures and are due to the interaction of surfactant proteins with phospholipids in the film [42]. Interestingly, a lipid film spiked with the SP-C peptoid displays a compression-expansion loop that is highly similar to that of the SP-C peptide. Hence, it appears that this completely nonnatural peptoid oligomer can capture the essential biophysical behaviors of SP-C peptide in a lipid film.

As a complement to the PBS experiments, a home-built Langmuir-Wilhelmy surface balance (LWSB) [43] was used to evaluate the surface activity of these biomimetic surfactant formulations. Previous studies have shown that LS replacements that exhibit good biophysical activity on a LWSB also generally perform well in in vivo tests using animal or animal lung models of RDS [44, 45]. Surface pressure-area (π-A) isotherms were obtained at 37°C for the pure lipid mixture, the lipid/SP-C peptide mixture, and the lipid/SP-C peptoid mixture (Figure 3). We find that the addition of either the SP-C peptide or the SP-C peptoid results in the liftoff of the isotherm at a higher molecular area, demonstrating that both the surface-active peptide and the peptoid rapidly take physical positions at the interface. We also observe the occurrence of a pronounced plateau in the isotherm for both the peptide and the peptoid SP-C mimics. This plateau is observed for natural LS and has been postulated to coincide with the removal of lipids and proteins from the monolayer to a surface-associated surfactant reservoir near the interface, resulting in a multilayered film [46, 47]. The presence of this reservoir offers an explanation for how natural SPs interact with lipids to provide low surface tension in the surfactant film upon compression (exhalation) and yet respread rapidly on film expansion (inhalation), an activity that is also important for stabilizing the alveolar network of the lung [46, 48, 49]. The π-A isotherms obtained with the SP-C peptoid are similar to those obtained with the SP-C peptide, but show a somewhat later liftoff and a less pronounced plateau. However, the substantial similarity observed in the isotherms suggests that the SP-C peptoid captures many of the critical surface-active behaviors of SP-C peptide.

To further compare the surface-active properties of the lipid mixture with and without the peptide and peptoid SP-C mimics, fluorescence microscopy (FM) was used in conjunction with the LWSB to study the surface morphology of lipid, lipid/peptide, and lipid/peptoid films. Figure 4 displays FM images of surfactant film

morphology at surface pressures of 25 mN/m (left) and 40 mN/m (right) for the lipid mixture (Figures 4A and 4D), lipid/peptide mixture (Figures 4B and 4E), and a lipid/peptoid mixture (Figures 4C and 4F). The FM image for the lipid mixture reveals dark, flower-shaped domains that correspond to a liquid-condensed (LC) phase (Figure 4A). Upon surface compression, these domains greatly increase in size and area fraction, while the extent of the lighter liquid-expanded (LE) region is substantially reduced (Figure 4D). A "dark" film like that shown in Figure 4D is enriched in DPPC and does not respread well upon subsequent surface expansion [50]. In comparison, the images taken with added SP-C peptide show that the peptide interacts with the lipids to retain the fluidity of the film upon compression, as evidenced by the larger extent of light LE regions and the dramatic decrease in LC domain size in Figure 4E. This increase in fluidity allows better respreading of surfactant upon subsequent surface compressions. This important behavior is partially mimicked by the addition of SP-C peptoid, as illustrated in Figure 4F, which displays a film morphology intermediate between that of the pure lipid mixture (Figure 4D) and of the film spiked with SP-C peptide (Figure 4E). The change in morphology provided by the SP-C peptoid, relative to that of the lipid mixture, signals an increased fluidity of the film. This similarity in the action of the SP-C peptoid on the surfactant film to the peptide's behavior provides evidence that the peptoid has a substantial biomimetic interaction with the phospholipids. Taken together, the CD, PBS, LWSB, and FM results all suggest that the peptoid analog captures the essential structural and surface-active properties of lung surfactant protein C.

Significance

We have designed a peptoid-based SP-C mimic that, when integrated into a lipid film, holds promise as a biomimetic lung surfactant replacement for the treatment of RDS in premature infants and potentially adults as well. Oligopeptoids with α-chiral side chains offer several advantages for mimicry of SP-C, including their unusual stability in a helical secondary structure and their strong resistance to aggregation [9, 10, 51]. In addition, peptoid oligomers have been shown to be protease resistant and are easily and inexpensively produced by solid-phase synthesis [7, 52], traits that are critical for the successful development of a peptidomimetic therapeutic agent.

The structured, amphipathic peptoid analog that we have created for biological mimicry of surfactant protein C, comprised of 22 monomers of seven different types, is one of the longest and most complex nonnatural peptidomimetic oligomers yet evaluated as a protein replacement. It combines three important features: a specific, biomimetic sequence of monomers, precise chain length, and mimicry of a biological (helical) secondary structure. The results presented here indicate that peptoids and other nonnatural oligomers have excellent potential to be developed for therapeutic applications, particularly where the goal is to mimic bioactive protein domains that interact with lipid bilayers. Optimization of this formulation for an even closer mimicry of calf lung surfactant is ongoing. For example, this biomimetic LS formulation lacks a mimic of SP-B, which has also been shown to be important for LS functioning [15]; peptoid-based SP-B mimics are currently under development in our lab. Further investigations, including animal studies, will allow us to evaluate the efficacy and safety of peptoid-containing surfactants for in vivo application.

Experimental Procedures

Materials

Peptide and peptoid synthesis reagents were purchased from Applied Biosystems (Foster City, CA) or Sigma-Aldrich (Milwaukee, WI). Fmoc-protected amino acids, resins, and t-Boc were purchased from NovaBiochem (San Diego, CA), PMC was purchased from Omega Chemical (Quebec, Canada), and primary amines were purchased from Sigma-Aldrich. HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA). DPPC, POPG, and NBD-PC were purchased from Avanti Polar Lipids (Alabaster, AL), and PA was purchased from Sigma-Aldrich. All chemicals were used without further purification.

Peptide and Peptoid Synthesis

An SP-C peptide with a sequence of amino acids modified from the natural sequence for improved stability of α-helical secondary structure [37] was made by solid-phase synthesis on an ABI 433A automated peptide synthesizer using standard Fmoc chemistry. The peptoid 22-mer was also synthesized on the 433A on solid support (Rink amide resin) via the submonomer method [52] with Boc protection of *N*Lys [53] and PMC protection of *N*Arg [54] groups during the synthesis. When the synthesis was complete, peptoid oligomers were cleaved from the resin with 95% TFA/water along with necessary protecting group scavengers and HPLC purified using a linear gradient of 25%–100% solvent B in solvent A over 50 min (solvent A, 0.1% TFA in water [v/v]; solvent B, 0.1% TFA in isopropanol [v/v]). The final purity of both compounds was confirmed by analytical reversed-phased HPLC to be $>$97%. MALDI-TOF mass spectrometry confirmed the molar masses of the purified compounds to be correct (SP-C peptide, 3803 Da; SP-C peptoid, 3309 Da).

Circular Dichroism

Peptide and peptoid samples were prepared in propanol:1% acetic acid (4:1, v/v) solution at a 60 μM concentration. CD was carried out at room temperature on a Jasco J-715 instrument using a cylindrical quartz cuvette (Hellma, Plainview, NY). Spectra shown represent the average of 40 data accumulations.

Molecular Modeling

A model of the helical SP-C peptoid was created using Insight II (Accelrys, San Diego, CA) by inputting the central backbone dihedral angles of a closely related, helical peptoid pentamer structure comprised of *para*-substituted *N*spe monomers previously solved in methanol solution by 2D NMR [38].

Pulsating Bubble Surfactometry

Static and dynamic characterization of surfactant film properties was performed on a pulsating bubble surfactometer (PBS) (General Transco, Largo, FL). The lipid mixture (DPPC:POPG:PA, 68:22:9 [by weight]) was dissolved in chloroform [41] to a total phospholipid concentration of 1 mg/ml. The lipid mixture was then spiked with 10% (w/w) SP-C mimic. The sample was prepared in an Eppendorf tube, dried under vacuum, and resuspended in an aqueous solution of 0.15 M NaCl and 5 mM $CaCl_2$. Measurements on the PBS were made at 37°C and with a bulk surfactant concentration of 1 mg/ml. When a constant, equilibrium surface tension had been obtained after initial surfactant adsorption to the interface, surface tension versus interfacial area data were gathered, with compression and expansion loops obtained by cycling the bubble size at a frequency of 20 cycles/min.

Measurement of Adsorption Isotherms on a Langmuir-Wilhelmy Surface Balance

Surface pressure (π)-area (A) isotherms were obtained using a Langmuir-Wilhelmy surface balance (LWSB) for a lipid mixture (DPPC:POPG:PA, 68:22:9 [by weight]) alone and with 10% (w/w) of either SP-C peptide or SP-C peptoid added. The measurements were performed on a home-built LWSB instrument [55] filled with distilled water (Milli-Q, Millipore) as the subphase and heated to 37°C, whereupon the sample of interest was spread in chloroform solution and the solvent allowed to evaporate for 5 min. Barriers were compressed at a rate of 0.10 mm/sec to obtain data for the compression-expansion cycles.

Fluorescence Microscopy Imaging of Surfactant Film Morphology

FM images of surfactant film morphology on the LWSB were obtained as previously described [55] after spiking the lipid mixture with 1 mol% of a fluorescently labeled lipid, NBD-PC.

Acknowledgments

We thank Mark Johnson, Ronald N. Zuckermann, Edward P. Ingenito, Elias I. Frances, Ajaykumar Gopal, Canay Ege, and Nathan J. Brown for their assistance. We acknowledge use of the Keck Biophysics Facility at Northwestern University for CD measurements. We acknowledge support for this work from the National Science Foundation (Grant Nos. BES-9870386 and BES-0101195) and the National Institute of Health (Grant No. 1R01HL67984-01). C.W.W. was supported by an NIH Molecular Biophysics Training Grant (Grant No. 5 T32 GM08382-10); K.Y.C.L. is grateful for the support from March of Dimes (6-FY03-429). The building of the LWSB apparatus was made possible by an NSF CRIF/Junior Faculty Grant (CHE-9816513).

Received: August 5, 2003
Revised: August 28, 2003
Accepted: August 28, 2003
Published online: October 20, 2003

References

1. Kirshenbaum, K., Zuckermann, R.N., and Dill, K.A. (1999). Designing polymers that mimic biomolecules. Curr. Opin. Struct. Biol. *9*, 530–535.
2. Gellman, S.H. (1998). Foldamers: a manifesto. Acc. Chem. Res. *31*, 173–180.
3. Barron, A.E., and Zuckermann, R.N. (1999). Bioinspired polymeric materials: in-between plastics and proteins. Curr. Opin. Chem. Biol. *3*, 681–687.
4. Wender, P.A., Mitchell, D.J., Pattabiraman, K., Pelkey, E.T., Steinman, L., and Rothbard, J.B. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. Proc. Natl. Acad. Sci. USA *97*, 13003–13008.
5. Porter, E.A., Wang, X., Lee, H.-S., Weisblum, B., and Gellman, S.H. (2000). Non-haemolytic β-amino-acid oligomers. Nature *404*, 565.
6. Hamuro, Y., Schneider, J.P., and DeGrado, W.F. (1999). De novo design of antibacterial β-peptides. J. Am. Chem. Soc. *121*, 12200–12201.
7. Miller, S.M., Simon, R.J., Ng, S., Zuckermann, R.N., Kerr, J.M., and Moos, W.H. (1995). Comparison of the proteolytic susceptibilities of homologous L-amino acid, D-amino acid, and N-substituted glycine peptide and peptoid oligomers. Drug Dev. Res. *35*, 20–32.
8. Gibbons, J.A., Hancock, A.A., Vitt, C.R., Knepper, S., Buckner, S.A., Brune, M.E., Milicic, I., Kerwin, J.F., Jr., Richter, L.S., Taylor, E.W., et al. (1996). Pharmacologic characterization of CHIR 2279, an N-substituted glycine peptoid with high-affinity binding for alpha 1-adrenoceptors. J. Pharmacol. Exp. Ther. *277*, 885–899.
9. Wu, C.W., Sanborn, T.J., Zuckermann, R.N., and Barron, A.E. (2001). Peptoid oligomers with α-chiral, aromatic sidechains: Effects of chain length on secondary structure. J. Am. Chem. Soc. *123*, 2958–2963.
10. Kirshenbaum, K., Barron, A.E., Armand, P., Goldsmith, R., Bradley, E., Cohen, F.E., Dill, K.A., and Zuckermann, R.N. (1998). Sequence-specific polypeptoids: A diverse family of heteropolymers with stable secondary structure. Proc. Natl. Acad. Sci. USA *95*, 4303–4308.
11. Notter, R.H. (2000). Lung Surfactants: Basic Science and Clinical Applications (New York: Marcel Dekker Inc.).
12. Robertson, B., Johansson, J., and Curstedt, T. (2000). Synthetic surfactants to treat neonatal lung disease. Mol. Med. Today *6*, 119–124.
13. Wu, C.W., and Barron, A.E. (2002). Biomimetic lung surfactant replacements. In Biomimetic Materials and Design: Interactive Biointerfacial Strategies, Tissue Engineering, and Targeted Drug Delivery, A.K. Dillow and A. Lowman, eds. (New York: Marcel Dekker), pp. 565–633.
14. Cockshutt, A., Absolom, D., and Possmayer, F. (1991). The role of palmitic acid in pulmonary surfactant: Enhancement of surface activity and prevention of inhibition by blook proteins. Biochim. Biophys. Acta *1085*, 248–256.
15. King, R.J., and Clements, J.A. (1972). Surface active materials from dog lung. II. Composition and physiological correlations. Am. J. Physiol. *223*, 715–726.
16. Veldhuizen, E.J.A., Batenburg, J.J., Vandenbussche, G., Putz, G., van Golde, L.M.G., and Haagsman, H.P. (1999). Production of surfactant protein C in the baculovirus expression: the information required for correct folding and palmitoylation. Biochim. Biophys. Acta *1416*, 295–308.
17. Waring, A., Taeusch, W., Bruni, R., Amirkhanian, J., Fan, B., Stevens, R., and Young, J. (1989). Synthetic amphipathic sequences of surfactant protein-B mimic several physiochemical and in vivo properties of native pulmonary surfactant proteins. Pept. Res. *2*, 308–313.
18. Takei, T., Hashimoto, Y., Aiba, T., Sakai, K., and Fujiwara, T. (1996). The surface properties of chemically synthesized peptides analogous to human pulmonary surfactant protein SP-C. Biol. Pharm. Bull. *19*, 1247–1253.
19. Johansson, J., Nilsson, G., Stromberg, R., Robertson, B., Jornvall, H., and Curstedt, T. (1995). Secondary structure and biophysical activity of synthetic analogs of the pulmonary surfactant polypeptide SP-C. Biochem. J. *307*, 535–541.
20. Cochrane, C.G., Revak, S.D., Merritt, T.A., Heldt, G.P., Hallman, M., Cunningham, M.D., Easa, D., Pramanik, A., Edwards, D.K., and Alberts, M.S. (1996). The efficacy and safety of KL4 surfactant in preterm infants with respiratory distress syndrome. Am. J. Respir. Crit. Care Med. *153*, 404–410.
21. von Nahmen, A., Schenk, M., Sieber, M., and Amrein, M. (1997). The structure of a model pulmonary surfactant as revealed by scanning force microscopy. Biophys. J. *72*, 463–469.
22. Ding, J., Takamoto, D.Y., von Nahmen, A., Lipp, M.M., Lee, K.Y.C., Waring, A., and Zasadzinski, J.A. (2001). Effects of lung surfactant proteins, SP-B and SP-C, and palmitic acid on monolayer stability. Biophys. J. *80*, 2262–2272.
23. Lipp, M.M., Lee, K.Y.C., Takamoto, D.Y., Zasadzinski, J.A., and Waring, A.J. (1998). Coexistence of buckled and flat monolayers. Phys. Rev. Lett. *81*, 1650–1653.
24. Oosterlaken-Dijksterhuis, M.A., Haagsman, H.P., van Golde, L.M.G., and Demel, R.A. (1991). Interaction of lipid vesicles with monomolecular layers containing lung surfactant proteins SP-B or SP-C. Biochemistry *30*, 8276–8281.
25. Johansson, J., Szyperki, T., Curstedt, T., and Wüthrich, K. (1994). The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α-helix. Biochemistry *33*, 6015–6023.
26. Gericke, A., Flach, C.R., and Mendelsohn, R. (1997). Structure and orientation of lung surfactant SP-C and L-α-dipalmitoylphophatidylcholine in aqueous monolayers. Biophys. J. *73*, 492–499.
27. Creuwels, L.A.J.M., Boer, E.H., Demel, R.A., van Golde, L.M.G., and Haagsman, H.P. (1995). Neutralization of the positive charges of surfactant protein C: Effects on structure and function. J. Biol. Chem. *270*, 16225–16229.
28. Gustafsson, M., Palmblad, M., Curstedt, T., Johansson, J., and

Schürch, S. (2000). Palmitoylation of a pulmonary surfactant protein C analogue affects the surface associated lipid reservoir and film stability. Biochim. Biophys. Acta *1466*, 169–178.
29. Bi, X.H., Flach, C.R., Perez-Gil, J., Plasencia, I., Andreu, D., Oliveira, E., and Mendelsohn, R. (2002). Secondary structure and lipid interactions of the N-terminal segment of pulmonary surfactant SP-C in Langmuir films: IR reflection-absorption spectroscopy and surface pressure studies. Biochemistry *41*, 8385–8395.
30. Qanbar, R., Cheng, S., Possmayer, F., and Schürch, S. (1996). Role of palmitoylation of surfactant-associated protein C in surfactant film formation and stability. Am. J. Physiol. *271*, L572–L580.
31. Wang, Z., Gurel, O., Baatz, G.E., and Notter, R.H. (1996). Acylation of pulmonary surfactant protein-C is required for its optimal surface active interations with phospholipids. J. Biol. Chem. *271*, 19104–19109.
32. Flach, C.R., Gericke, A., Keough, K.M.W., and Mendelsohn, R. (1999). Palmitoylation of lung surfactant protein SP-C alters surface thermodynamics, but not protein secondary structure or orientation in 1,2-dipalmitoylphosphatidylcholine Langmuir films. Biochim. Biophys. Acta *1416*, 11–20.
33. Taneva, S., and Keogh, K.M.W. (1994). Pulmonary surfactant proteins SP-B and SP-C in spread monolayers at the air-water interface. III. Proteins SP-B plus SP-C with phospholipids in spread monolayers. Biophys. J. *66*, 1158–1166.
34. Takei, T., Hashimoto, Y., Ohtsubo, E., and Ohkawa, H. (1996). Characterization of poly-leucine substituted analogues of human surfactant protein SP-C. Biol. Pharm. Bull. *19*, 1550–1555.
35. McLean, L., Lewis, J., Krstenansky, J., Hagaman, K., Cope, A., Olsen, K., Matthews, E., Uhrhammer, D., Owen, T., and Payne, M. (1993). An amphipathic alpha-helical decapeptide in phosphatidylcholine is an effective synthetic lung surfactant. Am. Rev. Respir. Dis. *147*, 462–465.
36. Gustafsson, M., Thyberg, J., Naslund, J., Eliasson, E., and Johansson, J. (1999). Amyloid fibril formation by pulmonary surfactant protein C. FEBS Lett. *464*, 138–142.
37. Nilsson, G., Gustafsson, M., Vandenbusshe, G., Veldhuizen, E., Griffiths, W.J., Sjovall, J., Haagsman, H.P., Ruysschaert, J.M., Robertson, B., Curstedt, T., et al. (1998). Synthetic peptide-containing surfactants: evaluation of transmembrane versus amphipathic helices and SP-C polyvalyl to polyleucyl substitution. Eur. J. Biochem. *225*, 116–124.
38. Armand, P., Kirshenbaum, K., Goldsmith, R.A., Farr-Jones, S., Barron, A.E., Truong, K.T.V., Dill, K.A., Mierke, D.F., Cohen, F.E., Zuckermann, R.N., et al. (1998). NMR determination of the major solution conformation of a peptoid pentamer with chiral side chains. Proc. Natl. Acad. Sci. USA *95*, 4309–4314.
39. Hawgood, S., Benson, B.J., Schilling, J., Damm, D., Lements, J.A.C., and White, R.T. (1987). Nucleotide and amino acid sequences of pulmonary surfactant protein SP 18 and evidence for cooperation between SP 18 and SP 28–36 in surfactant lipid adsorption. Proc. Natl. Acad. Sci. USA *84*, 66–70.
40. Mayer-Fligge, P., Volz, J., Kruger, U., Sturm, E., Gernandt, W., Schafer, K., and Przyblski, M. (1998). Synthesis and structural characterization of human-identical lung surfactant SP-C protein. J. Pept. Sci. *4*, 355–363.
41. Tanaka, Y., Takei, T., Aiba, T., Masuda, K., Kiuchi, A., and Fujiwara, T. (1986). Development of synthetic lung surfactant. J. Lipid Res. *27*, 475–485.
42. Ingenito, E.P., Mark, L., Morris, J., Espinosa, F.F., Kamm, R.D., and Johnson, M. (1999). Biophysical characterization and modeling of lung surfactant components. J. Appl. Physiol. *86*, 1702–1714.
43. Lee, K.Y.C., Lipp, M.M., Takamoto, D.Y., Ter-Ovanesyan, E., Zasadzinski, J.A., and Waring, A.J. (1998). Apparatus for the continuous monitoring of surface morphology via fluorescence micrscopy during monolayer transfer to substrates. Langmuir *14*, 2567–2572.
44. Walther, F.J., Hernandez-Juviel, J., Bruni, R., and Waring, A. (1998). Protein composition of synthetic surfactant affects gas exchange in surfactant-deficient rats. Pediatr. Res. *43*, 666–673.
45. Walther, F.J., Hernandez-Juviel, J., Bruni, R., and Waring, A.J. (1997). Spiking Survanta with synthetic surfactant peptides improves oxygenation in surfactant-deficient rats. Am. J. Respir. Crit. Care Med. *156*, 855–861.
46. Takamoto, D.Y., Lipp, M.M., von Nahmen, A., Lee, K.Y.C., Waring, A.J., and Zasadzinski, J.A. (2001). Interaction of lung surfactant proteins with anionic phospholipids. Biophys. J. *81*, 153–169.
47. Schürch, S., Green, F.Y., and Bachofen, H. (1998). Formation and structure of surface films: captive bubble surfactometry. Biochim. Biophys. Acta *1408*, 180–202.
48. Pérez-Gil, J., and Keough, M.W. (1998). Interfacial properties of surfactant proteins. Biochim. Biophys. Acta *1408*, 203–217.
49. Galla, H.-J., Buordos, N., von Nahmen, A., Amrein, M., and Sieber, M. (1998). The role of pulmonary surfactant C during the breathing cycle. Thin Solid Films *327–329*, 632–635.
50. Discher, B.M., Schief, W.R., Vogel, V., and Hall, S.B. (1999). Phase separation in monolayers of pulmonary surfactant phospholipids at the air-water interface: composition and structure. Biophys. J. *77*, 2051–2061.
51. Sanborn, T.J., Wu, C.W., Zuckerman, R.N., and Barron, A.E. (2002). Extreme stability of helices formed by water-soluble poly-N-substituted glycines (polypeptoids) with alpha-chiral side chains. Biopolymers *63*, 12–20.
52. Zuckermann, R.N., Kerr, J.M., Kent, S.B.H., and Moos, W.H. (1992). Efficient method for the preparation of peptoids [oligo (N-substituted glycines] by submonomer solid-phase synthesis. J. Am. Chem. Soc. *114*, 10646–10647.
53. Krapcho, A.P., and Kuell, C.S. (1990). Mono-protected diamines—N-tert-butoxycarbonyl-alpha,omega-alkanediamines from alpha,omega-alkanediamines. Synthetic Communications *20*, 2559–2564.
54. Uno, T., Beausoleil, E., Goldsmith, R.A., Levine, B.H., and Zuckermann, R.N. (1999). New submonomers for poly N-substituted glycines (peptoids). Tetrahedron Lett. *40*, 1475–1478.
55. Gopal, A., and Lee, K.Y.C. (2001). Morphology and collapse transitions in binary phospholipid monolayers. J. Phys. Chem. B *105*, 10348–10354.