Self-Assembling Amphiphiles for Construction of Protein Molecular Architecture

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Abstract: Peptide-amphiphiles with collagen-model head groups and dialkyl chain tails have been synthesized and shown to self-assemble into highly ordered polyPro II like triple-helical structures when dissolved in aqueous subphases. Evidence for this self-assembly process has been obtained from (a) compression of stable peptide-amphiphile monolayers to molecular areas comparable with triple-helical areas, (b) circular dichroism spectra and melting curves characteristic of triple-helices, and (c) two-dimensional NMR spectra indicative of stable triple-helical structure at low temperatures and melted triple-helices at high temperatures. The thermal stability of the collagen-like structure in the peptide-amphiphile is substantially higher ($\Delta T_m = 15-20$ °C) than that of peptides without lipidation. The assembly process driven by the hydrophobic tail may provide a general method for creating well-defined protein molecular architecture using a minimalist peptide-based approach.

Introduction

Biomolecules are remarkable in their capability to self-assemble into well-defined and intricate structures. The most intriguing self-assembly process is the folding of peptide chains into native protein structures. The fundamental building blocks in proteins are not simple canonical secondary structures (such as α-helices and β-sheets) but characteristic assemblies of secondary structural elements.[1] Among the protein assemblies are the simple β/α/β motif, the hairpin, and the α-helical-coiled coil, as well as the more complicated four α-helical bundle, the doubly wound β-sheet, the Jelly roll, and the Greek key.[1] Many researchers have attempted to create protein-like assemblies for the purpose of studying protein folding.[2] In addition, functional synthetic proteins have been created from designed assemblies. The most common assembly used for protein design is the four α-helical bundle, which has been developed as a synthetic enzyme,[3] for redox catalysis,[4] for antibody production,[5] as ion channels in lipid bilayers,[6] and as surface mimetics of human class I MHC.[7] The collagen-model triple-helix has also been used for functional protein design. Synthetic triple-helical proteins have incorporated type IV collagen sequences that promote adhesion and spreading of tumor cells,[8,9] type I collagen sequences that promote adhesion of fibroblasts,[10] type III or IV collagen sequences that induce the aggregation of platelets,[11] and macrophage scavenger receptor sequences that bind acetylated low-density lipoproteins.[12]

The triple-helix is a supersecondary structure characteristic of collagen.[13] Collagen-like triple-helices are also found in macrophage scavenger receptors types I and II[14] and bacteria-binding receptor MARCO,[15] complement component C1q,[16] pulmonary surfactant apoprotein,[17] acetylcholinesterase,[18] and mannose binding protein.[19] The triple-helix consists of three polypeptide chains, each in an extended, left-handed polyPro II like helix, which are staggered by one residue and then supercoiled along a common axis in a right-handed manner.[13] Geometric constraints of the triple-helical structure require that

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every third amino acid is Gly, resulting in a Gly-X-Y repeating sequence. The stability of the triple-helix depends upon the imino acid content.[20] Furthermore, hydroxyproline (Hyp) stabilizes the triple-helical structure by facilitating the formation of a hydrogen-bonding network with surrounding water molecules.[21] For simple collagen-model peptides, $(Gly\text{-}Pro\text{-}Hyp)_n$ forms the most thermally stable triple-helices, with a melting temperature (T_m) of 58−60 °C when $n = 10$.[22]

Several strategies have been employed in order to induce triple-helical structure formation in isolated collagen sequences that promote cellular and enzymatic activities.[23] Simply adding a number of Gly-Pro-Hyp repeats to both ends of a collagenous sequence can, under certain circumstances, induce triple-helical conformation. However, even with more than 50% of the peptide sequence consisting of Gly-Pro-Hyp repeats, the resulting triple-helices still may not have sufficient thermal stability (T_m < 37 °C) to survive physiological conditions. If synthetic proteins are to be utilized for biological and biomaterial applications, physiological stability is an important design criteria. Substantial stabilization of the triple-helical structure can be achieved with the introduction of covalent links between the *C*-terminal regions of the three peptide chains[8,10,24,25] or by use of a Kemp triacid (KTA) template linked to the *N*-terminus of three peptide chains.[26] However, the large size (90−125 amino acid residues) of some of the resulting triple-helical peptide compounds makes them difficult to synthesize and/or purify. Ideally, one would like to create a system by which synthetic linear peptide chains self-assemble into desirable secondary and tertiary structures. In the present paper, we report a new, noncovalent, self-assembly approach to building a collagen-like structural motif which uses the alignment of amphiphilic compounds at the lipid−solvent interface to facilitate peptide alignment and structure initiation and propagation.

Experimental Section

Synthesis of Peptides and Peptide-Amphiphiles. The three dialkyl ester tail precursors used in this study, 1′,3′-dihexadecyl *N*-[*O*-(4-nitrophenyl)succinyl]-L-glutamate [designated $(C_{16})_2$-Glu-C_2-pNp], 1′,3′-ditetradecyl *N*-[*O*-(4-nitrophenyl)succinyl]-L-glutamate [designated $(C_{14})_2$-Glu-C_2-pNp], and 1′,3′-didodecyl *N*-[*O*-(4-nitrophenyl)succinyl]-L-glutamate [designated $(C_{12})_2$-Glu-C_2-pNp], were synthesized as described previously for $(C_{16})_2$-Glu-C_2-pNp.[27] The C_{14} and C_{12} tails were prepared using 1-tetradecyl alcohol and 1-dodecyl alcohol, respectively. $(C_{16})_2$-Glu-C_2-Gly was prepared as described previously.[27] All standard peptide synthesis chemicals and solvents were analytical reagent grade or better and purchased from Applied Biosystems, Inc. (Foster City, CA), or Fisher Scientific (Pittsburgh, PA). ((Fluorenylmethoxy)carbonyl) [Fmoc]-4-((2′,4′-dimethoxyphenyl)aminomethyl)phenoxy resin (substitution level = 0.46 mmol/g) was purchased from Novabiochem (La Jolla, CA). All Fmoc-amino acid derivatives were from Novabiochem and are of L-configuration. 1-Hydroxybenzotriazole (HOBt) was purchased from Novabiochem, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) from Richelieu Biotechnologies (St. Hyacinthe, Quebec), and *N*,*N*-diisopropylethylamine (DIEA) from Fisher Scientific.

Peptide-resin assembly was performed by Fmoc solid phase methodology on an ABI 431A peptide synthesizer.[28,29] Peptide-resins were characterized by Edman degradation sequence analysis as described previously for "embedded" (noncovalent) sequencing.[29] Peptide-resins were then either (a) cleaved or (b) lipidated[27] with the appropriate $(C_n)_2$-Glu-C_2 tail and then cleaved. Cleavage and side chain deprotection of peptide-resins and peptide-amphiphile-resins proceeded for 1 h using either ethanedithiol−thioanisole−phenol−H_2O−trifluoroacetic acid (TFA) (2.5:5:5:5:82.5) or H_2O−TFA (1:19) as described.[30] Peptide-amphiphile cleavage solutions were not extracted with methyl *tert*-butyl ether prior to purification.

Peptide Purification and Analysis. Preparative RP(reverse phase)-HPLC purification was performed on a Rainin AutoPrep system. Peptides were purified with a Vydac 218TP152022 C_{18} column (15−20 μm particle size, 300 Å pore size, 250 × 25 mm) at a flow rate of 5.0 mL/min. The elution gradient was either 0−60% B or 0−100% B in 60 min where A was 0.1% TFA in H_2O and B was 0.1% TFA in acetonitrile. Detection was at 229 nm. Peptide-amphiphile purification was achieved with a Vydac 214TP152022 C_4 column (15−20 μm particle size, 300 Å pore size, 250 × 22 mm) at a flow rate of 10 mL/min. The elution gradient was 55−90% B in 20 min where A was 0.05% TFA in H_2O and B was 0.05% TFA in acetonitrile. Detection was at 229 nm. Analytical RP-HPLC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a Hypersil C_{18} column (5 μm particle size, 120 Å pore size, 200 × 2.1 mm) a flow rate of 0.3 mL/min. The elution gradient was 0−60% B in 45 min where A and B were the same as those used for peptide purification. Diode array detection was at 220, 254, and 280 nm.

Edman degradation sequence analysis was performed on an Applied Biosystems 477A protein sequencer/120A analyzer. Laser desorption mass spectroscopy (LDMS) was performed on a Hewlett-Packard G2025A LD-TOF (time-of-flight) mass spectrometer. We previously found LDMS to be effective for peptide-amphiphile characterization.[27] FABMS was performed on a VG 7070E-HF with a glycerol matrix.

The following $[M + H]^+$ values for peptides and peptide-amphiphiles were obtained: [IV-H1], 1436.8 Da (theoretical 1436.6 Da); $(Gly\text{-}Pro\text{-}Hyp)_4$-[IV-H1], 2502.5 Da (theoretical 2502.7 Da); [IV-H1]-$(Gly\text{-}Pro\text{-}Hyp)_4$, 2502.6 Da (theoretical 2502.7 Da); $(Gly\text{-}Pro\text{-}Hyp)_4$-[IV-H1]-$(Gly\text{-}Pro\text{-}Hyp)_4$, 3574.2 Da (theoretical 3574.9 Da); $(C_{16})_2$-Glu-C_2-[IV-H1]-Tyr, 2277.2 Da (theoretical 2278.4 Da); $(C_{16})_2$-Glu-C_2-$(Gly\text{-}Pro\text{-}Hyp)_4$-[IV-H1], 3184.6 Da (theoretical 3183.8 Da); $(C_{14})_2$-Glu-C_2-[IV-H1]-$(Gly\text{-}Pro\text{-}Hyp)_4$, 3130.8 Da (theoretical 3127.8 Da); $(C_{12})_2$-Glu-C_2-[IV-H1], 2003.6 Da (theoretical 2002.6 Da); $(C_{12})_2$-Glu-C_2-$(Gly\text{-}Pro\text{-}Hyp)_4$-[IV-H1], 3075.8 Da (theoretical 3071.8 Da); $(C_{12})_2$-Glu-C_2-[IV-H1]-$(Gly\text{-}Pro\text{-}Hyp)_4$, 3076.9 Da (theoretical 3071.8 Da). For the $(C_{12})_2$-Glu-C_2-$(Gly\text{-}Pro\text{-}Hyp)_4$-[IV-H1]-$(Gly\text{-}Pro\text{-}Hyp)_4$ peptide-amphiphile, $[M + Na]^+$ = 4166.8 Da (theoretical 4162.9 Da).

Pressure−Area Isotherms. All isotherms were obtained at 22 °C after spreading a peptide-amphiphile solution in hexane−$CHCl_3$−methanol (5:4:1) over a pure water subphase. After 15 min, the monolayer was compressed laterally with constant speed of 10 mm/min on a computerized KSV LB5000 Langmuir−Blodgett instrument

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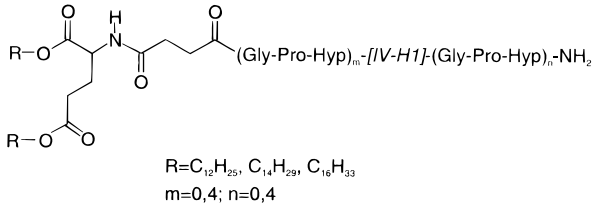

Figure 1. General structure of the peptide-amphiphiles used in this study. The sequence of [IV-H1] is Gly-Val-Lys-Gly-Asp-Lys-Gly-Asn-Pro-Gly-Trp-Pro-Gly-Ala-Pro.

and the surface pressure was detected using a film balance with a platinum Wilhemy plate.

Circular Dichroism Spectroscopy. Spectra were recorded on a JASCO J-710 spectropolarimeter using a thermostated 0.1 mm quartz cell. Thermal transition curves were obtained by recording the molar ellipticity ([θ]) in the range of 10−80 °C at $\lambda = 225$ nm. The peptide and peptide-amphiphile concentrations were 0.5 mM in H_2O at 25 °C.

NMR Spectroscopy. Freeze-dried samples for NMR spectroscopy were dissolved in D_2O or D_2O-H_2O (1:9) at peptide and peptide-amphiphile concentrations of 3−5 mM. NMR spectra were acquired on a 500 MHz Bruker AMX-500 spectrometer at 10, 25, 50, and 80 °C. Two-dimensional total correlation spectroscopy (TOCSY) and nuclear Overhauser effect spectroscopy (NOESY) were performed with 256 T_1 increment and 1024 complex data points in the T_2 dimension. TOCSY spectra were obtained at mixing times of 40−150 ms. NOESY spectra were obtained at mixing times of 60−250 ms. The spectral widths were 6024 Hz in both dimensions.

Results

We have recently described the synthesis of novel peptide-amphiphiles which incorporate a long-chain dialkyl ester lipid "tail" onto a peptide head group.[27] For the present study, the α1(IV)1263−1277 collagen sequence Gly-Val-Lys-Gly-Asp-Lys-Gly-Asn-Pro-Gly-Trp-Pro-Gly-Ala-Pro ([IV-H1]), which is known to promote melanoma cell adhesion and spreading,[8,31,32] has been combined with the lipid to create collagen-like peptide-amphiphiles (Figure 1). Initially, the surface activity of peptides and peptide-amphiphiles was investigated. While the [IV-H1] peptide and variants without lipid tails were not surface active, formation of monolayers at the air−water interface was observed for all investigated collagen-like peptide-amphiphiles. For $(C_{16})_2$-Glu-C_2- and $(C_{14})_2$-Glu-C_2-derived peptide-amphiphiles, surface pressure (which can be interpreted as a measure of resistance of amphiphile molecules against lateral compression) could be detected at surface areas of 2−3 nm^2/molecule (Figure 2). The surface pressure increased gradually as the monolayer was compressed for peptide-amphiphiles containing both [IV-H1] and Gly-Pro-Hyp repeats. At a surface area of 0.6 nm^2/molecule, no further compression was possible and the monolayer reached the maximum surface pressure and collapsed. The common value of 0.6 nm^2/molecule for the limiting surface area of the $(C_{16})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1] and $(C_{14})_2$-Glu-C_2-[IV-H1]-(Gly-Pro-Hyp)$_4$ peptide-amphiphiles can only be explained assuming a fully stretched, elongated peptide head group.[27] Prior X-ray crystallographic analyses of a triple-helical peptide revealed hexagonal-packed trimers with axis-to-axis distances of 1.4 nm.[21] The calculated surface area for this triple-helical peptide would be 1.7 nm^2/trimer, very close to the surface area of 1.8 nm^2/trimer for the peptide-amphiphiles studied here.

We did not observe a dependency of the π-A isotherm on the length of the dialkyl tail for the investigated peptide head groups for alkyl chains larger than C_{14} (data not shown). The π-A isotherms for C_{12} amphiphiles repeat the trend that was observed for amphiphiles with longer alkyl chains, although monolayers of the former are not as stable at room temperature. However, good solubility in the aqueous subphase makes C_{12} compounds well suited for spectroscopic investigations. We report spectral observations only for the C_{12} amphiphiles (see below), but the main spectral features have been observed independent of the amphiphile tail length.

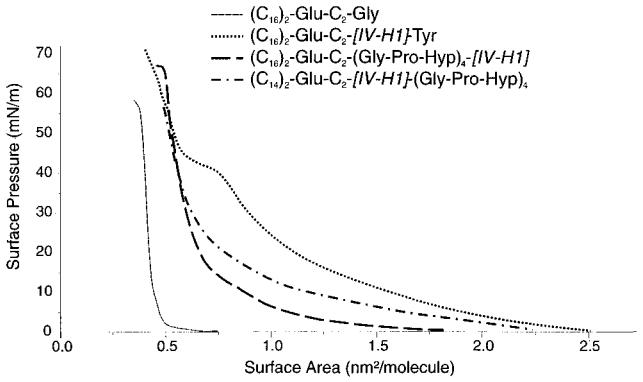

Figure 2. Surface pressure−area isotherms of collagen-model peptide-amphiphiles. All peptide-amphiphile isotherms converge at surface pressure values of above 50 mN/m at a surface area of 0.6 nm^2/molecule. This surface area is different from the exclusion volume (0.4 nm^2/molecule) for an dialkyl chain amphiphile molecule [e.g., $(C_{16})_2$-Glu-C_2-Gly].

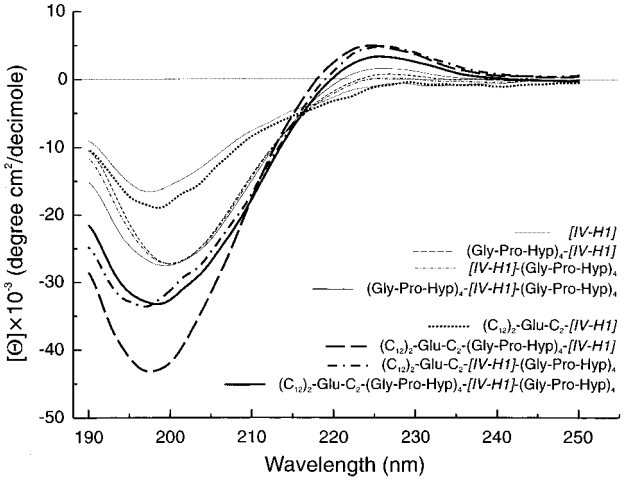

Figure 3. Circular dichroism spectra of collagen-model peptides and peptide-amphiphiles. Positive values of ellipticity in the λ range of 215−245 nm are attributed to an ordered, polyPro II like structure.[33] Among the investigated peptides, only (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ shows this structure distinctly. However, except for $(C_{12})_2$-Glu-C_2-[IV-H1], all peptide-amphiphiles display a positive signal with the residual ellipticity corresponding to the maximum values reported for triple-helical structures. Solutions of $(C_{12})_2$-Glu-C_2-Gly (the lipid tail without a collagenous head group) show little positive or negative ellipticity over the λ range 190−250 nm (data not shown).

Collagens in triple-helical conformation exhibit a circular dichroism (CD) spectrum similar to a polyPro II helix, with positive ellipticity at $\lambda = 215-240$ nm.[33] At 25 °C, (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ was found to exhibit this characteristic CD spectrum (Figure 3). For (Gly-Pro-Hyp)$_4$-[IV-H1] and [IV-H1]-(Gly-Pro-Hyp)$_4$ a small magnitude of positive ellipticity at $\lambda = 225$ nm was observed, while the [IV-H1] peptide did not show any positive ellipticity at this wavelength. Of the peptide-amphiphiles, $(C_{12})_2$-Glu-C_2-[IV-H1] displayed

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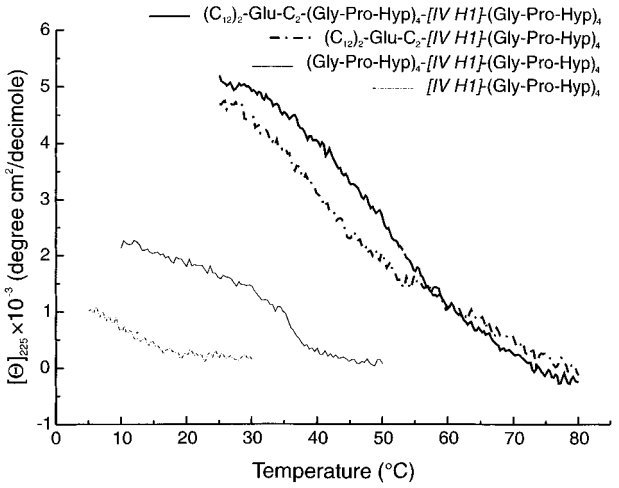

Figure 4. Temperature dependence of molar ellipticity per amino acid residue for collagen-model peptides and peptide-amphiphiles. Among the peptides, only (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ displays a thermal denaturation curve typical for collagen-like triple-helices, with a $T_m \approx 36$ °C. All peptide-amphiphiles, except $(C_{12})_2$-Glu-C_2-[IV-H1], show a more gradual transition starting at 30−40 °C and finishing at about 80 °C.

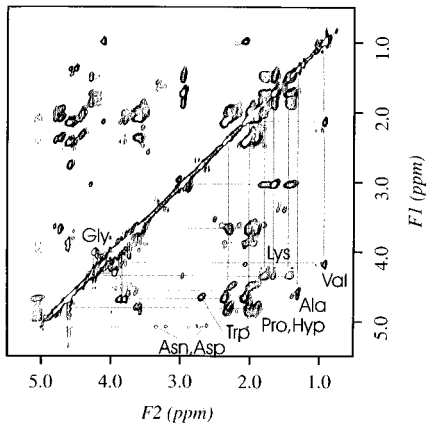

Figure 5. Total correlation spectroscopy (TOCSY) spectrum of (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ in D_2O at 10 °C. Peptide concentration was 5 mM. For the preliminary assignment of amino acid residues present in the peptide, data from additional TOSCY experiments (D_2O−H_2O, 1:9) and nuclear Overhauser effect spectroscopy (NOESY) experiments were used. The Pro/Hyp region was identified from the lack of an amide ^{1}H signal and the shown connectivity pattern in TOCSY.

a CD spectrum similar to that of [IV-H1] (no positive ellipticity at $\lambda = 225$ nm), while the other three amphiphiles showed a large magnitude of positive ellipticity at $\lambda > 220$ nm. Most remarkably, the ellipticity per residue for the amphiphilic compounds $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1], $(C_{12})_2$-Glu-C_2-[IV-H1]-(Gly-Pro-Hyp)$_4$, and $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ was about 5 times larger than that of (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ (Figure 3) and approximately equal to that of the triple-helical peptide (Gly-Pro-Hyp)$_{10}$.[34] These ellipticity per residue values indicate maximal ordered structures for $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1], $(C_{12})_2$-Glu-C_2-[IV-H1]-(Gly-Pro-Hyp)$_4$, and $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$. It appears that all residues in these three peptide-amphiphiles are in triple-helical conformation.

A triple-helical assembly can be distinguished from a simple, non-intercoiled polyPro II structure by its thermal denaturation behavior. A triple-helix is relatively sensitive to temperature, since it is stabilized by a hydrogen bonded intra- and interstrand water network.[21] Triple-helical melts are highly cooperative.[33] The thermal stability of peptides and peptide-amphiphiles were studied by monitoring ellipticity at $\lambda = 225$ nm as a function of increasing temperature. Among the peptides, only (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ gave a typical sigmoidal transition associated with the transformation of triple-helical to single-stranded structure ($T_m = 36$ °C) (Figure 4). [IV-H1]-(Gly-Pro-Hyp)$_4$ showed a small magnitude of positive ellipticity which decreased nearly linearly from 5 to 20 °C and then flattened out (Figure 4), as did (Gly-Pro-Hyp)$_4$-[IV-H1] (data not shown). The molar ellipticities of the peptide-amphiphiles decreased very gradually between 10 and 30 °C and then more markedly starting at around 30−40 °C, with some traces of positive CD detectable up to 80 °C (Figure 4). The midpoint of the transitions (T_m) was found to be at 50 ± 5 °C, and the melting curve was fully reversible upon cooling. Although the change in ellipticity was large, thermal transitions for the peptide-amphiphiles were broad. These observations suggest that the (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ and $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ structures consist of packed polyPro II like helices, possibly triple-helical, and that the lipid tail remarkably enhanced the stability of this assembly.

The structures of the collagen-model peptides and peptide-amphiphiles were further investigated by 2D ^{1}H NMR spectroscopy (Figure 5). The Pro and Hyp spin systems in TOCSY were identified by the lack of amide protons and reference to the chemical shifts of the side chain protons from other collagen-like peptides.[34] The chemical shift of the Pro and Hyp side chain protons is sensitive to their conformation.[34] For our peptides and peptide-amphiphiles, there are Pro residues that occur both surrounding and within the [IV-H1] sequence while Hyp residues surround the [IV-H1] sequence. At 10 °C, the relatively few crosspeaks found in the Pro/Hyp region of the ^{1}H NMR spectra (Figure 6) indicate that the Pro and Hyp residues of (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ are in a limited number of conformations, as expected for a compound with an ordered structure. The crosspeaks at 4.6 ppm are comparable to those observed for the triple-helical, template-bound peptide KTA-[Gly-(Gly-Pro-Hyp)$_3$-NH_2]$_3$.[26] The spectra of (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 50 °C shows additional crosspeaks at 4.85 ppm, indicating less-ordered conformation at higher temperature. Some of these additional crosspeaks are consistent with the multiple states that exist for the Pro residues within the [IV-H1] sequence when in a non-triple-helical conformation.[32] After the (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ peptide is lipidated with a C_{12} tail, similar NMR spectra are obtained (Figure 6). For example, $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 25 °C shows a few well-defined crosspeaks, indicating ordered conformation of the peptide-amphiphile. Consistent with our CD observations, the NMR spectra of $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 80 °C indicates more disorder than at 25 °C. Additional crosspeaks are seen at 4.85 ppm, in similar fashion to (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 50 °C. Overall, the CD and NMR spectra of the (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ peptide and the $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ peptide-amphiphile suggest that both can spontaneously form a well-ordered polyPro II like triple-helical structure. Similar NMR spectra were obtained for the $(C_{12})_2$-Glu-C_2-[IV-H1]-(Gly-Pro-Hyp)$_4$ and $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1] peptide-amphiphiles (data not shown).

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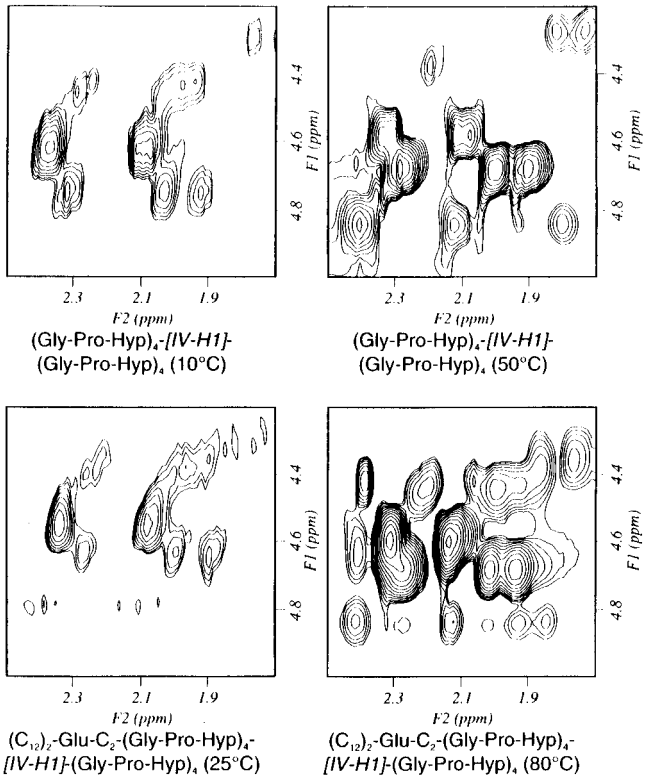

Figure 6. TOCSY spectra of the Pro/Hyp region for (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ (top panels) and $(C_{12})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ (bottom panels) in D_2O at peptide and peptide-amphiphile concentrations of 3−5 mM. The 25 °C spectra of the peptide-amphiphile indicates a highly ordered structure similar to (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 10 °C. At 80 °C, the additional crosspeaks at 4.85 ppm for the peptide-amphiphile are similar to those seen for (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ at 50 °C.

Discussion

The *de novo* design of proteins has been based on the knowledge of individual amino acid propensities to form distinct secondary structures, such as α-helices and *β*-sheets, and modeling of these secondary structures to associate by long-range interactions and create tertiary structures.[1,35] Mutter and colleagues developed the template-assembled synthetic protein (TASP) approach based on the concept that templates would promote secondary structure formation and minimize aggregation of larger complexes.[35b] When one considers the contributions to the free energy of folding, a template is predicted to decrease the chain entropy of the unfolded state (due to increased excluded volume effects), while favoring intramolecular interactions (formation of a hydrophobic core) instead of intermolecular interactions (aggregation).[35b] Lattice statistical mechanics predicts that surfaces would enhance secondary structure formation by minimizing unfolded states.[36] Surfaces have been shown to induce peptide secondary structures, either α-helical or *β*-sheet.[37] The template effect has since been documented for synthetic four α-helical bundles, since the α-helicity of the individual peptide chains was greatly enhanced upon incorporation onto a template containing several other α-helical peptide sequences.[38] Thus, one method for creating novel biomaterials with distinct protein-like structures is to synthesize the peptide onto a template that (a) induces secondary and tertiary structures and (b) either serves directly as a biomaterial or is compatible with biomaterial surfaces. We envisioned the construction of a novel "peptide-amphiphile" to serve this purpose, whereby a peptide "head group" has the propensity to form a distinct structural element (and contains a cell recognition site), while a hydrophobic "tail" serves to align the peptide strands and induce secondary and tertiary structure formation as well as providing a hydrophobic surface for self-association and/or interaction with other surfaces. As described by Kunitake,[39] design of synthetic lipids requires consideration of four building blocks: tail, linker/connector, spacer, and head group. Our design features C_{12}, C_{14}, and C_{16} dialkyl tails, a Glu linker, a -$(CH_2)_2$- spacer, and a collagen-model peptide head group (Figure 7).

The peptide-amphiphiles appear to self-assemble into highly ordered polyPro II like triple-helical structures when dissolved in aqueous subphases. Evidence for the self-assembly process has been obtained from monolayer observations and CD and NMR spectroscopies. Peptide-amphiphiles have surface areas, CD spectra, and Pro and Hyp side chain conformations characteristic of triple-helices and exhibit large structural transitions, as monitored by CD melting curves. The transitions were typically broader than those observed for associated triple-helices (Figure 4). However, broad transitions have been observed previously for triple-helices which are constrained at either the *N*- or *C*-termini.[10,12,24,26,40] Since these constraints prevent melting of the triple-helix from both ends simultaneously, broad transitions are most likely the result of a shifted equilibrium between unfolded and folded states compared with associated triple-helices. For our specific case, we are melting a mixture of amphiphile assemblies (monomers, micelles, vesicles, etc.) of different sizes and stabilities, which may additionally contribute to broad thermal transitions. Several of the prior studies of constrained triple-helices,[26,40] as well as the study presented here, have used 2D NMR spectroscopy to substantiate triple-helical structure.

The lipid hydrophobic interactions of the peptide-amphiphiles exert a significant influence on collagen-model structure formation and stabilization. For example, although the [IV-H1]-(Gly-Pro-Hyp)$_4$ sequence has the potential of forming a triple-helix, it is realized only in the amphiphilic compound. The triple-helix is also exceptionally stable when formed in the presence of the lipid modification. The difference in the denaturation temperatures between the structured (Gly-Pro-Hyp)$_4$-[IV-H1]-(Gly-Pro-Hyp)$_4$ peptide and the corresponding C_{12} peptide-amphiphile is about 15−20 °C. The tight alignment of the *N*-terminal amino acids achieved through the association of the lipid part of the molecule in a monolayer could be a general tool for initiation of peptide folding. Model investigations with amphiphile monolayers mimic this general building principle and might therefore pave a new way to the design of artificial proteins and enzymes.

The peptide-amphiphiles described here provide a simple approach for building stable protein structural motifs using peptide head groups. One of the most intriguing features of this system is the possible formation of stable lipid films on

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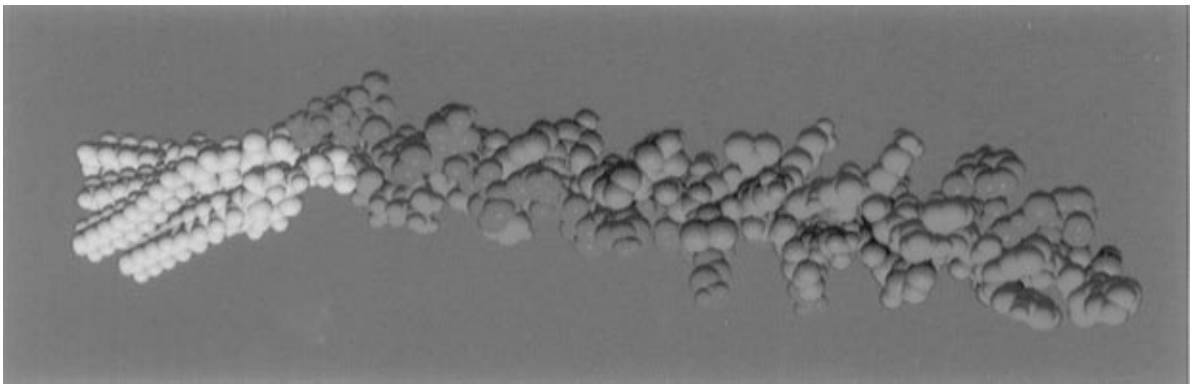

Figure 7. The computer-modeled structure of the peptide-amphiphile $(C_{16})_2$-Glu-C_2-(Gly-Pro-Hyp)$_4$-[IV-H1] following energy minimization with MacroModel.

solid substrates, or the use of the novel amphiphiles in bilayer membrane systems, where the lipid tail serves not only as peptide structure inducing agent but also as anchor of the functional head group to the lipid assembly.[41] In general, amphiphile systems may form a great variety of structures in solution including micelles and vesicles.[42] Israelachvili devised a dimensionless group, the surfactant number (N_S), to predict the geometry of the final amphiphile aggregate.[43] The surfactant number is defined as $N_S = v/a_0l$, where v is the volume of the hydrocarbon chain tail, a_0 is the optimal head group area, and l is the maximal extended length of the hydrocarbon chain tail. For $N_S < {}^1/_3$, spherical micelles are formed; for N_S between ${}^1/_3$ and ${}^1/_2$, cylindrical micelles are formed; for N_S between ${}^1/_2$ and 1, flexible bilayers and vesicles are formed.[43] Since our peptide-amphiphiles have a large head group (Figure 7) and thus a_0 is relatively large, it is anticipated that $N_S < {}^1/_3$ and micellization is probable. However, one can mix peptide-amphiphiles with vesicle-forming lipids (such as dilauryl phophatidylcholine) to form stable mixed vesicles with collagen-model, triple-helical peptide head groups. Vesicles featuring collagen coatings have already been shown to be advantageous for targeted drug delivery.[44] As a first application, we have undertaken the design of a drug-targeting system against melanoma cells using vesicles containing the [IV-H1] peptide-amphiphile.

The peptide-amphiphile system presented here offers extraordinary flexibility with regard to head group geometry and macromolecular structure. For building materials with molecular and cellular recognition capacity, it is essential to have a wide repertoire of tools to produce characteristic supersecondary structures at surfaces and interfaces.

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