Materials

Amino acids, amino acid derivatives, and resins were from Novabiochem. Gel filtration chromatography was performed with Sephadex G-50 resin from Sigma.

Instrumentation

Analytical HPLC was performed with an Aligent C8 column or a Varian Dynamax C18 column with linear gradients. Mass spectrometry was performed with an Applied Biosystems Voyager DE-Pro MALDI–TOF mass spectrometer with sinapinic acid (Fluka) as the matrix at the University of Wisconsin Biophysics Instrumentation Facility (www.biochem.wisc.edu/bif). Ultraviolet/visible spectra were recorded with either a Cary 50 Bio or a Cary 3 spectrometer.

Strand and Fragment Synthesis

Synthesis of (Pro-Pro-Gly)$_8$-Cys(StBu)-Gly, Pro-α1(StBu). Fluorenylmethoxycarbonyl (Fmoc)-ProProGly-OH was synthesized as described (1). Pro-α1-*tert*-butylthio (StBu) was then synthesized on Fmoc-Gly-polyethyleneglycol-polystyrene copolymer (PEG-PS) resin (Applied Biosystems): 1 × Fmoc-Cys(StBu)-OH, 8 × Fmoc-ProProGly-OH, cleaved from the resin with 95:2.5:2.5 trifluoroacetic acid (TFA)/H_2O/isopropyl $(iPr)_3SiH$ (8 ml, 2 h) and precipitated from *tert*-butylmethyl ether: 58% yield; MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{105}H_{155}N_{26}O_{27}S_2$ 2277.6, found 2277.6; HPLC t_R 17.79 min [Aligent C8 column, 85:15–15:85 H_2O/CH_3CN containing TFA (0.1% vol/vol) over 50 min].

Synthesis of (Pro-Hyp-Gly)$_8$-Cys(StBu)-Gly, Hyp-α1(StBu). FmocProHypGly was synthesized as described (1), but with Hyp in the Yaa position (no *tert*-butyl protection was necessary). Hyp-α1(StBu) was then synthesized on Fmoc-Gly-PEG-PS resin: 1 × Fmoc-Cys(StBu)-OH, 8 × Fmoc-ProHypGly-OH, cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH (8 ml, 2 h) and precipitated from *tert*-butylmethyl ether: 64% yield; MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{105}H_{155}N_{26}O_{35}S_2$ 2405.6, found 2406.2; HPLC t_R 14.89 min [Aligent C8 column, 85:15–15:85 H_2O/CH_3CN containing TFA (0.1% vol/vol) over 50

min]. The sample was heated at 80°C for 5 min prior to injection onto the column; otherwise a peak for folded trimers (t_R ~22 min) was observed.

Attachment of Fmoc-Gly-OH to 4-Hydroxymethylphenoxyacetyl-Polyethyleneglycol-Polyacrylamide Copolymer (HMPA–PEGA) Resin, Fmoc-Gly-PEGA. HMPA–PEGA resin was obtained from Novabiochem already preswollen in MeOH with an approximate wet loading of 0.072 mmol/g. Preswollen HMPA–PEGA resin (1.545 g, 0.111 mmol) was washed with CH_2Cl_2 (3 × 5 ml) and dried under high vacuum for 1 h to remove all MeOH. The resin was then swollen in *N*,*N*-dimethylformamide (DMF) for Fmoc-Gly-OH attachment.

In a flame-dried flask under Ar(g) atmosphere, Fmoc-Gly-OH (331 mg, 1.11 mmol) was dissolved in CH_2Cl_2 (10 ml) and DMF (0.5 ml). The clear solution was cooled to 0°C, diisopropylcarbodiimide (87 ml, 0.56 mmol) was added, and the mixture was stirred at 0°C for 30 min to generate the symmetrical anhydride. The solution was concentrated to remove CH_2Cl_2, DMF (3 ml) was added, and the anhydride solution was filtered through cotton (to remove the insoluble urea) into the swelled resin suspension. A solution of dimethylaminopyridine (1.4 mg, 0.11 mmol) in DMF (0.1 ml) was added, the vessel was purged with Ar(g), and the suspension was agitated gently for 1.5 h. The resin was then drained, washed with DMF (7 × 10 ml) and CH_2Cl_2 (7 × 10 ml), and dried under high vacuum to give Fmoc-Gly-PEGA. Resin-loading was measured by ultraviolet spectroscopy to be 0.25 mmol/g.

Synthesis of (Pro-Pro-Gly)$_3$-Gly-Cys(Acm)-Cys(StBu)-Gly-(Pro-Pro-Gly)$_5$-(HMPA–PEGA), Pro-α2(Acm,StBu)-PEGA. Pro-α2(Acm,StBu)-PEGA (Acm = acetamidomethyl) was synthesized by starting with Fmoc-Gly-(HMPA–PEGA) resin (0.33 g, 80 μmol): 2 × Fmoc-Pro-OH, 4 × Fmoc-ProProGly-OH, 1 × Fmoc-Gly-OH, 1 × Fmoc-Cys(StBu)-OH, 1 × Fmoc-Cys(Acm)-OH, 1 × Fmoc-Gly-OH, 3 × Fmoc-ProProGly-OH. A small amount of peptide was cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH for analysis: MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{113}H_{168}N_{29}O_{30}S_3$ 2507.2, found 2508.0.

Synthesis of (Pro-Hyp-Gly)$_3$-Gly-Cys(Acm)-Cys(StBu)-Gly-(Pro-Hyp-Gly)$_5$-(HMPA–PEGA), Hyp-α2(Acm,StBu)-PEGA. Hyp-α2(Acm,StBu)-PEGA was synthesized by starting with Fmoc-Gly-(HMPA–PEGA) resin (0.25 g, 61 μmol): 1 × Fmoc-Hyp(tBu)-OH, 1 × Fmoc-

Pro-OH, 4 × Fmoc-ProHypGly-OH, 1 × Fmoc-Gly-OH, 1 × Fmoc-Cys(StBu)-OH, 1 × Fmoc-Cys(Acm)-OH, 1 × Fmoc-Gly-OH, 3 × Fmoc-ProHypGly-OH. A small amount of peptide was cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH for analysis: MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{113}H_{168}N_{29}O_{38}S_3$ 2635.1, found 2635.6.

Disulfide Bond Formation

Pro-α2(*p*-Npys,Acm)-PEGA. Pro-α2(Acm,StBu)-PEGA (7.7 μmol) was swollen in 95:5 TFE/H_2O (1 ml, 5 min), Bu_3P (38 μl, 150 μmol) was added, and the suspension was agitated gently for 2 h. The resin was drained, washed with trifluoroethanol (3 × 1 ml), DMF (8 × 1 ml) and CH_2Cl_2 (8 × 1 ml), and dried under high vacuum for 30 min. The resin was then swollen in CH_2Cl_2 (0.6 ml, 5 min). A solution of *p*-$Npys_2$ (2,2′-dithiobis(5-nitropyridine), 24 mg, 77 μmol) in CH_2Cl_2 (0.4 ml) was added to the swelled resin, the vessel was purged with Ar(g) and the suspension was agitated gently for 2 h. The resin was drained, washed with CH_2Cl_2 (3 × 1 ml), DMF (20 × 1 ml), and CH_2Cl_2 (8 × 1 ml), and dried under high vacuum. A small amount of peptide was cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH for analysis: MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{114}H_{162}N_{31}O_{32}S_3$ 2573.1, found 2573.6.

Pro-α1. A solution of Pro-α1(StBu) (28 mg, 12 μmol) and Bu_3P (61 μl, 250 μmol) in 95:5 TFE/H_2O (1 ml) was agitated gently for 2 h. The peptide was precipitated from *tert*-butylmethyl ether (15 ml) to give Pro-α1: MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{101}H_{147}N_{26}O_{27}S$ 2188.1, found 2188.8.

Pro-α1α2(Acm)-PEGA. Pro-α2(Acm,*p*-Npys)-PEGA (7.7 μmol) was swollen in buffer (0.4 ml, 5 min). A solution of Pro-α1 (12 μmol) in buffer (1 ml) was added to the swelled resin, the vessel was purged with Ar(g), and the suspension was agitated gently for 12 h [buffer: 50 mM NH_4OAc, 2 mM EDTA, pH 5.4, degassed and sparged with Ar(g)]. The resin was drained, washed with H_2O (7 × 1 ml), DMF (7 × 1 ml), and CH_2Cl_2 (7 × 1 ml), and dried under high vacuum. A small amount of peptide was cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH for analysis: MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{210}H_{304}N_{55}O_{57}S_3$ 4607.2, found 4607.9.

Pro-α1α2(*o*-Npys)-PEGA. Pro-α1α2(Acm)-PEGA was swollen in DMF (0.4 ml, 5 min), a solution of *o*-Npys-Cl (3-nitro-2-pyridinesulfenyl chloride, 2.9 mg, 15 μmol) in DMF (0.6 ml) was added to the swelled resin, and the suspension was agitated gently for 2 h. The resin was drained, washed with DMF (8 × 1 ml) and CH_2Cl_2 (8 × 1 ml) and dried under high vacuum. A small amount of peptide was cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH for analysis: MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{212}H_{301}N_{56}O_{58}S_4$ 4690.2, found 4688.9.

Fragment 1 (Pro-α1α2α1′). Pro-α1α2(*o*-Npys)-PEGA (7.7 μmol) was swollen in buffer (0.4 ml, 5 min). A solution of Pro-α1 (12 μmol) in buffer (1 ml) was added to the swelled resin, the vessel was purged with Ar(g), and the suspension was agitated gently for 12 h [buffer: 50 mM NH_4OAc, 2 mM EDTA, pH 5.4, degassed and sparged with Ar(g)]. The resin was drained, washed with H_2O (7 × 1 ml), DMF (7 × 1 ml), and CH_2Cl_2 (7 × 1 ml), and dried under high vacuum. The fragment was then cleaved from the resin with 95:2.5:2.5 TFA/H_2O/iPr_3SiH (8 ml, 2 h) and precipitated from *tert*-butylmethyl. Crude **1** was dissolved in 20 mM NH_4CO_3 (pH 6.0, 1 ml), introduced onto a Sephadex G-50 column (21 cm × 2.5 cm) and eluted with the same 20 mM NH_4CO_3 buffer to give **1** (6.4 mg, 12% overall): MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{308}H_{443}N_{80}O_{83}S_4$ 6722.6, found 6723.3; HPLC t_R 18.75 min [Aligent C8 column, 90:10–10:90 H_2O/CH_3CN containing TFA (0.1% vol/vol) over 60 min].

Hyp-α2(*p*-Npys,Acm)-PEGA. Hyp-α2(*p*-Npys,Acm)-PEGA was prepared as described for Pro-α2(*p*-Npys,Acm)-PEGA starting with Hyp-α2(Acm,StBu)-PEGA (8.3 μmol): MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{114}H_{162}N_{31}O_{40}S_3$ 2701.1, found 2701.1.

Hyp-α1. Hyp-α1 was prepared as described for Pro-α1 starting with Hyp-α1(StBu) (32 mg, 13 μmol): MS (MALDI–TOF) $[M+H]^+$ calculated for $C_{101}H_{147}N_{26}O_{35}S$ 2316.0, found 2316.3.

Hyp-α1α2(Acm)-PEGA. Hyp-α1α2(Acm)-PEGA was prepared as described for Pro-α1α2(Acm)-PEGA starting with Hyp-α2(*p*-Npys, Acm)-PEGA (8.3 μmol) and Hyp-α1

(13 μmol): MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{210}H_{304}N_{55}O_{73}S_3$ 4863.2, found 4864.6.

Hyp-α1α2(*o*-Npys)-PEGA. Hyp-α1α2(*o*-Npys)-PEGA was prepared as described for Pro-α1α2(*o*-Npys)-PEGA starting with Hyp-α1α2(Acm)-PEGA (8.3 μmol): MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{212}H_{301}N_{56}O_{74}S_4$ 4946.2, found 4948.6.

Fragment 2 (Hyp-α1α2α1′). Fragment **2** was prepared and purified as described for fragment **1**, starting with Hyp-α1α2(*o*-Npys)-PEGA (8.3 μmol) and Hyp-α1 (13 μmol) to give **2** (16.5 mg, 28% overall): MS (MALDI–TOF) $[M+H]^+$ (average) calculated for $C_{308}H_{443}N_{80}O_{107}S_4$ 7106.5, found 7109.0; HPLC t_R 13.88 min [Aligent C8 column, 90:10–10:90 H_2O/CH_3CN containing TFA (0.1% vol/vol) over 60 min].

Estimation of Assembly Length from R_h

The lengths of assemblies $(\mathbf{1})_n$ and $(\mathbf{2})_n$ were estimated from the R_h values measured with DLS by using the Broersma relations (Eq. **1**) (2–4) and Tirado and Garcia de la Torre relations (Eq. **2**) (5, 6), as described for collagen (7).

Broersma (2–4) relations:

$$0 = 2R_h[\delta - (^1/_2)(\gamma_{\parallel} + \gamma_{\square})] - L \quad \textbf{[1]}$$

$$\delta = \ln(2L/d)$$

$$\gamma_{ll} = 0.807 + 0.15/\delta + 13.5/\delta^2 - 37/\delta^3 + 22/\delta^4$$

$$\gamma_{\perp} = -0.193 + 0.15/\delta + 8.1/\delta^2 - 18/\delta^3 + 9/\delta^4.$$

Tirado and Garcia de la Torre (5, 6) relations:

$$0 = 2R_h[(\ln(p) + v)] - L \quad \textbf{[2]}$$

$$p = L/d$$

$$v = 0.312 + 0.565p^{-1} - 0.1000p^{-2}.$$

In Eqs. **1** and **2**, L is the rod length and d is the rod diameter. After assuming that $d = 1.36$ nm for collagen (8, 9), Eqs. **1** and **2** were solved for L with the FINDROOTS algorithm of IGOR PRO (Wavemetrics).

References

1. Jenkins, C. L., Vasbinder, M. M., Miller, S. J. & Raines, R. T. (2005) *Org. Lett.* **7,** 2619–2622.
2. Broersma, S. (1960) *J. Chem. Phys.* **32,** 1626–1631.
3. Broersma, S. (1960) *J. Chem. Phys.* **32,** 1632–1635.
4. Broersma, S. (1981) *J. Chem. Phys.* **74,** 6989–6990.
5. Tirado, M. M. & Garcia de la Torre, J. (1979) *J. Chem. Phys.* **71,** 2581–2587.
6. Tirado, M. M. & Garcia de la Torre, J. (1980) *J. Chem. Phys.* **73,** 1986–1993.
7. Claire, K. & Pecora, R. (1997) *J. Phys. Chem. B* **101,** 746–753.
8. Boedtker, H. & Doty, P. (1956) *J. Am. Chem. Soc.* **78,** 4267–4280.
9. Boudko, S., Frank, S., Kammerer, R. A., Stetefeld, J., Schulthess, T., Landwehr, R., Lustig, A., Bächinger, H. P. & Engel, J. (2002) *J. Mol. Biol.* **317,** 459–470.