A Macrocyclic Scaffold for the Collagen Triple Helix

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General Experimental. Chemical reagents were obtained from Aldrich Chemical (Milwaukee, WI) or Fisher Scientific (Hanover Park, IL), and used without further purification. Amino acids and their derivatives were obtained from Novabiochem (San Diego, CA) or Chem-Impex International (Wood Dale, IL). DMF and CH$_2$Cl$_2$ were drawn from a Baker CYCLE-TAINER. Fmoc-Pro–Pro–Gly trimer was synthesized in solution as described previously.$^1$ NMR spectra were recorded with a Bruker DMX-400 NMR spectrometer in the National Magnetic Resonance Facility at Madison (NMRFAM). CD experiments were performed with an Aviv Model 202SF CD spectrometer at the University of Wisconsin–Madison Biophysics Instrumentation Facility. Mass spectrometry was performed with a Perkin-Elmer Voyager matrix-assisted laser desorption ionization time-of-flight (MALDI–TOF) mass spectrometer in the Biophysics Instrumentation Facility.

Synthesis and Purification of Gly(ProProGly)$_7$NH$_2$. The peptide Gly(ProProGly)$_7$NH$_2$ was synthesized on a 0.1-mmol scale by solid-phase methods using the Fmoc-Pro–Pro–Gly tripeptide and Fmoc-protected amino acids, HBTU-mediated coupling, and standard reaction cycles on Applied Biosystems Pioneer instrument at the University of Wisconsin–Madison Biotechnology Center. Use of a resin with a PAL linker generated an amidated C-terminus following cleavage from the resin with trifluoroacetic acid (TFA)/triisopropylsilane/H$_2$O (38:1:1). The peptide was purified by reverse-phase HPLC with a Varian semipreparative C18 column and elution with an H$_2$O/acetonitrile gradient containing TFA (0.1% v/v). The peptide product was judged to be >90% pure based on HPLC analysis. MALDI–TOF m/z calcld for C$_{86}$H$_{125}$N$_{23}$O$_{22}$ (M) 1831.94, obsd 1833.38 (M + H$^+$).

Synthesis of macrocycle 2. To a solution of macrocycle 1$^2$ (7.8 mg, 8.5 × 10$^{-3}$ mmol) in CH$_2$Cl$_2$ (10 mL) was added DIEA (35.0 μL, 0.206 mmol) and 3-mecaptopropionic acid (9.0 μL, 0.103 mmol). The resulting mixture was stirred under Ar(g) at room temperature for 2 h and then concentrated by rotary evaporation to an oil-like residue. The product was purified by reverse-phase HPLC with a Varian semipreparative C18 column and elution with an H$_2$O/acetonitrile gradient. The macrocycle 2 product (3.0 mg, 35%) was judged to be >90% pure based on HPLC analysis. MALDI–TOF m/z calcld for C$_{42}$H$_{60}$O$_{21}$S$_3$ (M) 996.28, obsd 1019.89 (M + Na$^+$).

Synthesis of Tethered [Gly(ProProGly)$_7$NH$_2$]$_3$ (3). To a solution of macrocycle 2 (1.5 mg, 1.5 × 10$^{-3}$ mmol) in DMF (10 mL) was added DIEA (6.3 μL, 0.036 mmol) and PyBOP (9.4 mg, 0.018 mmol). The resulting mixture was stirred under Ar(g) at room temperature for 30 min. The peptide Gly(ProProGly)$_7$NH$_2$ (33.0 mg, 0.018 mmol) was added to the solution after 30 min, and the reaction mixture was then stirred under Ar(g) at room temperature. After 3 days, the reaction mixture was purified by reverse-phase HPLC with an Agilent semipreparative C8 column and elution with an H$_2$O/acetonitrile gradient containing TFA (0.1% v/v). The macrocycle 3 product (3.0 mg, 31%) was judged to be >90% pure based on HPLC analysis. MALDI–TOF m/z calcld for C$_{300}$H$_{429}$N$_{69}$O$_{84}$S$_3$ (M) 6442.22, obsd 6449.05 (M + H$^+$).

Circular Dichroism (CD) Spectroscopy. Far-UV CD spectra were obtained at 4 °C in 50 mM HOAc at pH 2.9 using a 1-mm pathlength quartz cuvette and a spectrometer bandwidth of 1 nm. The concentrations of Gly(ProProGly)$_7$NH$_2$ and tethered [Gly(ProProGly)$_7$NH$_2$]$_3$ were 0.4–1.2 mg/mL. Thermal denaturation experiments were conducted by monitoring molar ellipticity at 226 or 227 nm in a 1-mm pathlength quartz cuvette with a 5-min equilibration at each temperature. For cation-association experiments, solutions of tethered

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[Gly(ProProGly)₇NH₂]₃ contained NaCl, KCl, or CsCl (0.10 M). All samples were incubated at 4 °C for at least 24 h before measurements.

Values of $T_m$ were determined by fitting the molar ellipticity at 226 or 227 nm to a two-state model defined by the equation:

$$
\Theta = \frac{(a_n + b_n T) + (a_d + b_d T) \exp(-\frac{\Delta G_U^0(T)}{RT})}{1 + \exp(-\frac{\Delta G_U^0(T)}{RT})}
$$

(1)

The temperature dependence of $\Delta G_U^0$ is described by the Gibbs–Helmholtz equation:

$$
\Delta G_U^0(T) = \Delta H^0(T_m)(1 - T/T_m) - \Delta C_p^0[(T_m-T) + T\ln(T/T_m)]
$$

(2)

where $\Delta G_U^0(T)$ is the free energy of unfolding, $T_m$ is the temperature at the midpoint of the unfolding transition, $\Delta H^0(T_m)$ is the enthalpy change at $T_m$, and $\Delta C_p^0$ is the heat capacity change between the folded and unfolded states. The parameters $a_n$, $b_n$, $a_d$, and $b_d$ define the signals of folded state (F) and unfolded state (U) as a function of the absolute temperature ($T$). The value of $\Delta C_p^0$ was set to 0 in the fit. (The exact value of $T_m$ is not sensitive to the choice of $\Delta C_p^0$.)
Figure S1. HPLC traces of macrocycles 2 (top) and 3 (bottom) in H$_2$O/acetonitrile gradients containing TFA (0.1% v/v).

Figure S2. Thermal denaturation curves of tethered [Gly(ProProGly)$_7$NH$_2$]$_3$ (3) in 50 mM HOAc containing salt (0 or 0.10 M).