Collagen-Like Triple Helices Incorporating Peptoid Residues†

Murray Goodman,* Giuseppe Melacini, and Yangbo Feng

Department of Chemistry & Biochemistry
University of California, San Diego
La Jolla, California 92093-0343

Received May 20, 1996

Collagen is the most common protein in connective tissues. In all collagens the major structural domain is the triple-helical motif.[1,2] The sequence of the triple-helical part of collagen is characterized by the trimer repeats of Gly-Xaa-Yaa. A high proportion of imino acids are present in the Xaa and Yaa positions.[3−5] Insertion of unnatural residues into collagen sequences can enhance the biostability of synthetic collagen-like biomaterials. However, the incorporation of some proline analogs was found to destabilize the triple-helical conformation.[6−10] Sarcosine was also used as a mimetic of the Yaa residue,[11] but the resulting (Gly-Pro-Sar)$_n$ sequences do not assume triple-helical structures.[12]

We report here the successful incorporation of the peptoid[13] residue *N*-isobutylglycine (Nleu) (Figure 1a) into collagen-like triple-helical structures without disturbing triple-helix stability. This discovery provides insight into the design of novel collagen-like biomaterials used in such applications as drug delivery, wound healing, and ocular devices. It has also been shown that peptoids can result in enhanced resistance to enzyme degradation of peptide-like structures.[13]

The peptoid residue Nleu was introduced in the Yaa position of (Gly-Pro-Yaa)$_n$ sequences. The triple-helical properties of the resulting (Gly-Pro-Nleu)$_n$ sequence were investigated by studying the following acetyl-terminated and template-assembled model compounds: Ac-(Gly-Pro-Nleu)$_9$-NH$_2$ (**I**) (Figure 1b), KTA-[Gly-(Gly-Pro-Nleu)$_9$-NH$_2$]$_3$ (**II**), and the shorter conjugate KTA-[Gly-(Gly-Pro-Nleu)$_6$-NH$_2$]$_3$ (**III**) (Figure 1c) (where KTA represents *cis*,*cis*-1,3,5-trimethylcyclohexane-1,3,5-tricarboxylic acid, known as the Kemp triacid[14]). Our previous publication showed that KTA with a glycine residue as spacer substituted on each carboxyl group and inserted at the amine terminus of three peptide chains significantly enhances triple-helix formation.[15] Compound Ac-(Gly-Pro-Nleu)-NHMe (**IV**) was also prepared for comparison purposes since our NMR data demonstrate that it is not able to form triple-helical conformations.

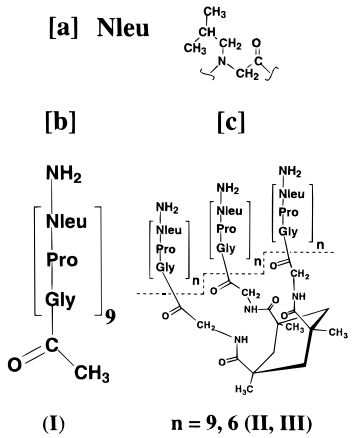

Figure 1. The structures of [**a**] Nleu, [**b**] Ac-(Gly-Pro-Nleu)$_9$-NH$_2$ and [**c**] KTA-[Gly-(Gly-Pro-Nleu)$_n$-NH$_2$]$_3$ (n = 6, 9).

The ^{1}H NMR data for compounds **I**−**III** establish a triple-helical structure for each molecule. From the 2-D ^{1}H NMR TOCSY and NOESY experiments, we obtain the residue specific assignments of analogs **I**−**IV** in D_2O at 27 °C. For compound **IV** (Figure 2a), a set of resonances is observed which is simply explained by tripeptide connectivities (i.e., our non triple helical reference). For compounds **I**−**III** an additional set of resonances is observed (Figure 2b), and several experimental observations indicate that this new set of resonances corresponds to residues in a collagen-like triple helix: (1) For compound **II** in D_2O (5 °C, pH = 2.8), the Gly NH of this set of resonances exhibits a low hydrogen exchange rate ($k < 10^{-5}$ min^{-1}) consistent with the triple-helix hydrogen bond network.[16] (2) As the temperature increases, the relative intensity of the new set of resonances decreases. (3) The chemical shifts of the Gly $C_\alpha H_{r,s}$, Pro $C_\beta H_{r,s}$, and Pro $C_\delta H_{r,s}$ protons of the new set of resonances are similar (within 0.06 ppm) to those of the corresponding resonances in the triple helical set observed for (Gly-Pro-Hyp)$_n$ sequences.[5,15] (4) The new resonances arise from conformations in slow exchange on the NMR time scale. This is typically seen for collagen-like triple helices of other model peptides.[5,15]

The formation of a triple-helical structure for compounds **I**−**III** in H_2O at 27 °C is also supported by the NOESY spectra which were analyzed according to a procedure previously used for the NMR study of (Pro-Hyp-Gly)$_{10}$.[5] This approach relies on the distinction between intra- and interchain NOEs on the basis of triple-helical structures modeled for sequences composed of the Gly-Pro-Nleu repeat. In particular, 21 interchain NOEs provide a critical test for the triple-helical array because they are expected to arise uniquely from interchain interactions on the basis of the proposed triple-helical structures (distances smaller than 4.5 Å) and correspond to nonoverlapped resonances. These interchain NOEs (see Supporting Information Table 1) can be sorted into three main classes: 3 NOEs between Gly and Pro, 5 NOEs between Gly and Nleu, and 13 NOEs between Pro and Nleu. These observations indicate that in each cross section of the triple helix, the residues Gly, Pro, and Nleu are closely packed supporting the register shift typically found in collagen-like triple helices. A full report on the NOE analysis appears in a forthcoming paper.[17]

The circular dichroism (CD) spectra of compounds **I**−**III** in H_2O at 20 °C closely resemble the CD spectra (the positive

† This is paper 4 of our series on collagen-based structures.

(1) Van Der Rest, M.; Garrone, R. *FASEB J.* **1991**, *5*, 2814−2823.
(2) Linsenmayer, T.; Hay, E. D. *Cell Biology of Extracellular Matrix*; Plenum Press: New York, 1991; pp 7−44.
(3) Fietzek, P. P.; Kuhn, K. *Mol. Cell Biochem.* **1975**, *8*, 141−157.
(4) Fraser, R. D. B.; McRae, T. P. *Conformation in Fibrous Proteins*; Academic Press: New York, 1973.
(5) Li, M.; Fan, P.; Brodsky, B.; Baum, J. *Biochemistry* **1993**, *32*, 7377−7387.
(6) Lane, J. M.; Parkes, L. J.; Prockop, D. J. *Biochim. Biophys. Acta* **1971**, *236*, 528−541.
(7) Takeuchi, T.; Prockop, D. J. *Biochim. Biophys. Acta* **1969**, *175*, 142−155.
(8) Bertoluzza, A.; Bonora, S.; Fini, G.; Morelli, M. A.; Verdini, A. S. *Proc. Eur. Conf. Spectrosc. Biol. Mol. 1st* **1985**, 401−403.
(9) Bertoluzza, A.; Bonora, S.; Fini, G.; Morelli, M. A.; Verdini, A. S. *Proc. Int. Conf. Laser Scattering Spectrosc. Biol. Objects* **1986**, 317−326.
(10) Prockop, D. J.; Berg, R. A.; Kivirikko, K. I.; Uitto, J. *Biochemistry of Collagen*; Ramachandran, G. N., Reddi, A. H., Eds.; Plenum Press: New York, 1976; pp 163−273.
(11) Ananthanarayanan, V. S.; Brahmachari, S. K. *Biopolymers* **1976**, *15*, 707−716.
(12) Feng, Y.; Melacini, G.; Taulane, J. P.; Goodman, M. *Biopolymers* In press. This is paper 5 of our series on collagen-based structures.
(13) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367−9371.
(14) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140−5143.
(15) Goodman, M.; Feng, Y.; Melacini, G.; Taulane, J. P. *J. Am. Chem. Soc.* **1996**, *118* (21), 5156−5157.
(16) Miller, M. H.; Nemethy, G.; Scheraga, H. A. *Macromolecules* **1980**, *13*, 910−913.
(17) Melacini, G.; Feng, Y.; Goodman, M. *J. Am. Chem. Soc.* **1996**, *118*, 10725−10732. This is paper 6 of our series on collagen-based structures.

S0002-7863(96)01717-9 CCC: $12.00

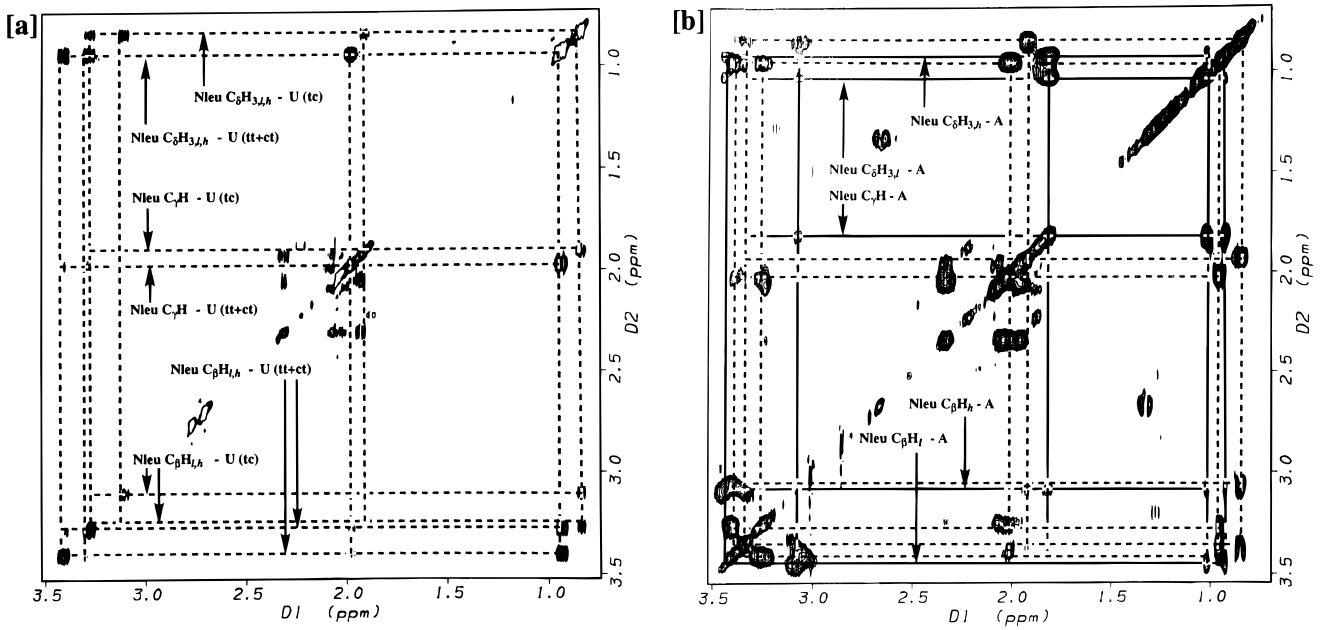

Figure 2. (a) Expanded region of the TOCSY spectrum of Ac-Gly-Pro-Nleu-NHMe (**IV**) in H_2O (pH 2.8, 27 °C) showing the Nleu side chain connectivities. Only non-triple-helical, unassembled (**U**) resonances are observed, including (trans, trans) and (cis, trans) structures (labeled as **U**(**tt**+**ct**)) and (trans, cis) structures (labeled as **U**(**tc**)). (b) Expanded region of the TOCSY spectrum of KTA-[Gly-(Gly-Pro-Nleu)$_6$-NH_2]$_3$ (**III**) under the same experimental conditions as part a. An additional set of resonances (solid lines) is observed, and it is assigned to assembled (**A**) triple-helical structures.

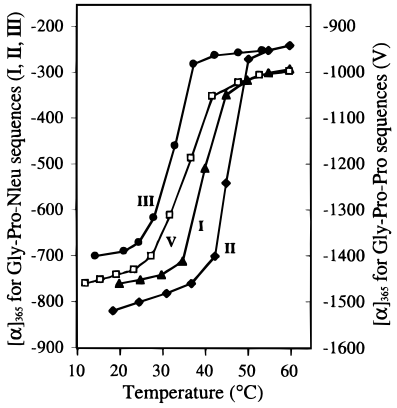

Figure 3. The thermal melting curves of Ac-(Gly-Pro-Nleu)$_9$-NH_2 (**I**) (0.9 mg/mL), KTA-[Gly-(Gly-Pro-Nleu)$_9$-NH_2]$_3$ (**II**) (0.04 mg/mL), KTA-[Gly-(Gly-Pro-Nleu)$_6$-NH_2]$_3$ (**III**) (0.2 mg/mL), and Ac-(Gly-Pro-Pro)$_{10}$ (**V**) (0.2 mg/mL) in H_2O determined by optical rotation measurements. The melting temperature of Ac-(Gly-Pro-Nleu)$_9$-NH_2 (**I**) is 39 °C while the melting temperature of Ac-(Gly-Pro-Pro)$_{10}$ (**V**) is 35 °C.

and negative peaks and the crossover) of collagen and triple-helical model peptides as reported in the literature.[15,18,19] The presence of triple-helical conformations for compounds **I**−**III** is also clearly seen in melting curve measurements. As demonstrated in Figure 3, optical rotation measurements showed that compounds **I**−**III** in H_2O exhibit cooperative transition curves, supporting the presence of ordered conformations.

On the basis of the NMR spectroscopy experiments and the chirooptical measurements discussed above, we conclude that the structures composed of (Gly-Pro-Nleu)$_n$ sequences (compounds **I**−**III**) form triple-helical conformations with thermal stability comparable to that of Gly-Pro-Pro triple helices (Figure 3).[12] The peptoid residue Nleu is therefore an excellent surrogate for Pro in the Yaa position of Gly-Pro-Yaa collagen sequences. These structures represent the first clearly established peptoid-containing collagen-like triple helices. These findings open new opportunities in the design of collagen mimetics.

Acknowledgment. This project is funded by grants from the National Science Foundation (NSFDMR-9201133) and Ciba-Vision Inc.

Supporting Information Available: Synthesis, HPLC profiles, CD spectra, mass spectra, NMR methods, and additional NMR spectra used for the collagen-based peptide-peptoids composed of Gly-Pro-Nleu sequences (16 pages). See any current masthead page for ordering and Internet access instructions.

JA961717I

(18) Brown, F. R., III; Di Corato, A.; Lorenzi, G. P.; Blout, E. R. *J. Mol. Biol.* **1972**, *63*, 85−99.

(19) Brown, F. R., III; Carver, J. P.; Blout, E. R. *J. Mol. Biol.* **1969**, *39*, 307−313.