SUPPORTING MATERIALS

Osteogenesis Imperfecta Model Peptides: Incorporation of Residues Replacing Gly within a triple-helix achieved by renucleation and local flexibility

Jianxi Xiao, Balaraman Madhan, Yingjie Li, Barbara Brodsky and Jean Baum

Table S1 Design and thermal stability of collagen OI mutation model peptides.

Peptide name	Peptide sequence*	Tm (^{o}C)†
T1-898	Ac-GPO-GAO-**G**AO-GAO-**G**PV-[G]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	35‡
Gly→Ala		
T1-898[G16A]	Ac-GPO-GAO-**G**AO-GAO-GPV-[A]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	15.5‡
Gly→Ser		
T1-898[G16S]	Ac-GPO-GAO-**G**AO-GAO-GPV-[S]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	14.5§
GPOT1-898[G16S]	Ac-GPO-GPO-**G**PO-GPO-GPV-[S]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	28
Gly→Arg		
T1-898[G16R]	Ac-GPO-GAO-**G**AO-GAO-**G**PV-[R]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	7§
GPOT1-898[G16R]	Ac-GPO-GPO-**G**PO-GPO-GPV-[R]PA-GAR-**G**PO-GPO-GPO-GPO-GY-$CONH_2$	5
GAAT1-898[G16R]	Ac-GPO-GPO-**G**PO-GAO-GAA-[R]PA-GAR-**G**PO-GPO-GPO-GPO-GY-$CONH_2$	13
Gly→Asp		
T1-898[G16D]	Ac-GPO-GAO-**G**AO-GAO-**G**PV-[D]PA-GAR-GPO-GPO-**G**PO-GPO-GY-$CONH_2$	<5§

*^{15}N labeled residues are underlined and bolded. Glycine substitutions are boxed.

†T_m determined from CD thermal transition, as described in Materials and Methods.

‡Hyde et al, 2006 (1).

§Bryan et al, 2011 (2).

Table S2 Translational diffusion coefficients of ^{15}N-labeled residues in model peptides in the disordered states. Diffusion coefficients at 0°C ($D_{0°C}$), diffusion coefficients at 0°C normalized to values at 40°C ($D_{normalized,0°C}$), and diffusion coefficients at 40°C ($D_{40°C}$) are shown in the units of $10^{-7}m^2s^{-1}$. The experiments are performed at 500MHz NMR for all peptides except 600MHz for GPOT1-898[G16S]. $D_{0°C}$ for GPOT1-898[G16S] is obtained at 5°C instead of 0°C, as the peptide has weak disordered peaks at low temperature and satisfactory data could be achieved only at 5°C. Disordered resonances of G13 and G28 in peptides T1-898[G16R] and T1-898[G16D] are overlapped.

Peptide	Residue	$D_{0°C}$	Error	$D_{normalized, 0°C}$	Error	$D_{40°C}$	Error
T1-898	G7	6.12	0.78	19.52	2.49	20.79	0.22
	A18	7.14	0.66	22.77	2.10	20.04	0.42
	G28	6.51	0.13	20.76	0.41	21.73	0.63
T1-898[G16A]	G7	5.69	0.34	18.14	1.08	20.54	0.23
	A16	4.79	0.31	15.27	0.99	20.12	0.16
	G28	6.35	0.38	20.25	1.21	20.57	0.09
T1-898[G16S]	G7	4.48	0.35	14.29	1.12	18.54	0.20
	V15	4.77	0.19	15.21	0.61	20.33	0.19
	A18	4.54	0.18	14.48	0.57	18.25	0.27
	G28	6.12	0.24	19.52	0.77	19.51	0.20
GPOT1-898[G16S]	G7	6.35	0.64	16.74	1.67	16.60	0.21
	V15	6.11	0.31	16.10	0.81	16.40	0.17
	A18	6.58	0.34	17.35	0.90	15.60	0.18
	G22	5.78	0.32	15.22	0.84	15.90	0.12
T1-898[G16R]	G7	3.54	0.08	11.29	0.26	19.02	0.20
	G13	3.70	0.03	11.80	0.10	19.56	0.08
	G28	3.70	0.03	11.80	0.10	19.56	0.08
T1-898[G16D]	G7	3.31	0.06	10.55	0.19	20.91	0.17
	G13	3.71	0.08	11.83	0.26	21.25	0.08
	G28	3.71	0.08	11.83	0.26	21.25	0.08

CD and NMR characterization of T1-898[G16D]

Peptide T1-898[G16D] modeling a Gly to Asp mutation shows very poor thermal stability compared to the control T1-898 and other mutant peptides (Table S1). Peptide T1-898[G16D] could not form fully folded triple helix structure, since the triple helical resonance of G13 is not observed in the HSQC spectrum (Fig. S1A). The triple helical resonances for G7 and G28 are present and have the same chemical shifts as G7 and G28 in other T1-898 series peptides (Fig. 1). It suggests that peptide T1-898[G16D] did not fold through the substitution site to form a fully folded trimer, even though the two ends could be in a triple helical conformation. The peptide might be partially folded at only one end (C or N terminus) or it might be able to form a conformation of two folded ends and an unfolded middle region. Normalized diffusion coefficients $D_{M0°C}$ of all residues are very low and close to the trimer diffusion coefficients (Fig. S1B), indicating that the peptide may predominately form partially folded trimers at the C-terminus (PFT-C), at the N-terminus (PFT-N) or with a break in the middle (PFT-M). However, the measurements of $D_{M0°C}$ may be less accurate for peptide T1-898[G16D] due to overlapping of the disordered resonances.

Experiments were performed to determine if the charge repulsion of the side chain of the Asp substitution destabilizes the peptides. The pKa of the side chain acidic group of Asp is 3.86 and hence the peptide T1-898[G16D] was studied at pH 3.5. The ellipticity value of the peptide at pH 3.5 is slightly higher than that in PBS at pH 7. However the observed T_m of the peptide at pH 3.5 is 6^o C, which is not a big rise compared to the same peptide in PBS at pH 7 (Fig. S1C). It indicates that charges do not affect the molecular features of the peptide significantly.

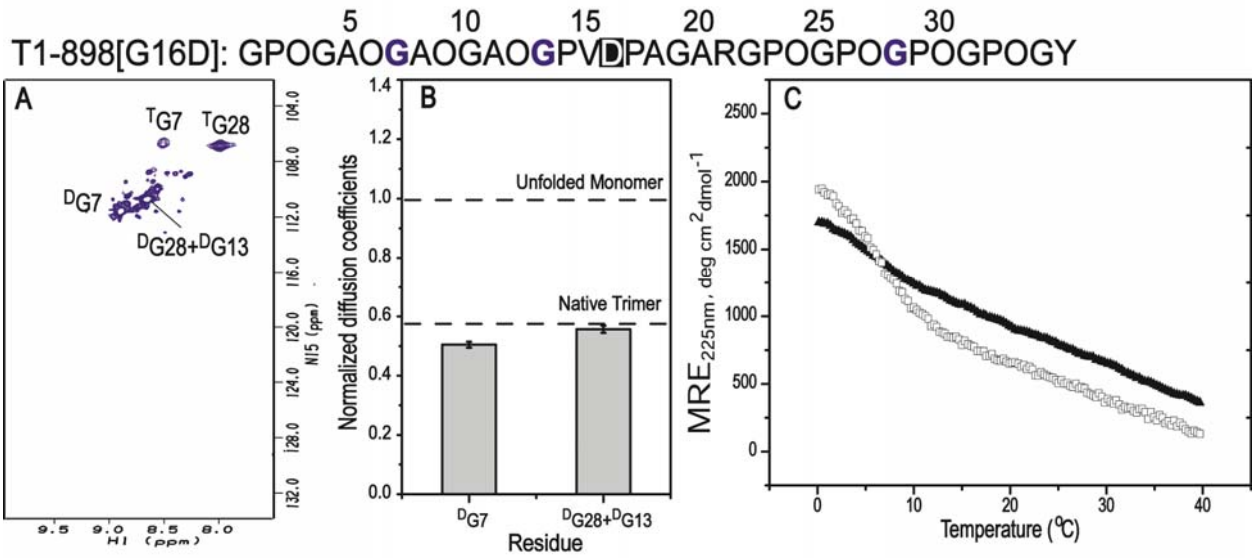

Figure S1 Effect of Gly to Asp substitution on equilibrium conformations of peptide T1-898. ^{1}H-^{15}N HSQC spectra of T1-898[G16D] (A): the peaks corresponding to the disordered and triple helical states are denoted with a superscript D or T, respectively. Minor disordered resonances arise due to cis-trans isomerization in the unfolded state of the Pro/Hyp-rich chains (3). Histogram of the residue-specific translational diffusion coefficients of ^{15}N-labeled residues in disordered states for peptide T1-898[G16D] (B): diffusion coefficients at 0°C are normalized to those at 40°C. The black dashed line represent the normalized diffusion coefficients for unfolded monomer (theoretically with value of 1) and native trimer (with a value of 0.58 for G28 in

peptide T1-898[G16A]), respectively. CD melting transition of T1-898[G16D] (■) in 20mM PBS buffer, pH 7 and T1-898[G16D] at pH 3.5 (◊) (C).

References

1. Hyde, T. J., M. A. Bryan, B. Brodsky, and J. Baum. 2006. Sequence dependence of renucleation after a Gly mutation in model collagen peptides. J Biol Chem 281:36937-36943.
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3. Buevich, A. V., Q. H. Dai, X. Liu, B. Brodsky, and J. Baum. 2000. Site-specific NMR monitoring of cis-trans isomerization in the folding of the proline-rich collagen triple helix. Biochemistry 39:4299-4308.