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Reciprocity of Steric and Stereoelectronic Effects in the Collagen Triple Helix

Matthew D. Shoulders,[†] Jonathan A. Hodges,[†,‡] and Ronald T. Raines*[,†,§]

Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

Received March 23, 2006; E-mail: raines@biochem.wisc.edu

Steric and stereoelectronic effects play a defining role in molecular conformation and reactivity. In small molecules, steric and stereoelectronic effects often have dichotomous consequences. For example, the anomeric effect in glycosides yields axial substituents that are disfavored by sterics.[1] Similarly, replacing the steric effect of a methyl group with the stereoelectronic effect of a fluoro group enables a β-peptide to fold.[2]

Stereoelectronic effects contribute markedly to the conformational stability of an abundant protein: collagen.[3−5] Collagen is a fibrous protein comprising a bundle of three parallel strands folded into polyproline type II helices.[6] Each strand consists of ∼300 repeats of the unit: Xaa−Yaa−Gly, where Xaa is often (2*S*)-proline (Pro) and Yaa is often (2*S*,4*R*)-4-hydroxyproline (Hyp). The pyrrolidine rings in the Xaa and Yaa positions have C$^\gamma$-endo and C$^\gamma$-exo ring puckers, respectively.[7] These puckers can be preordained by a stereoelectronic effect. Specifically, the gauche effect from a 4*S* fluoro group stabilizes the C$^\gamma$-endo pucker; that from a 4*R* fluoro group stabilizes the C$^\gamma$-exo pucker (Figure 1).[3] These stereoelectronic effects can markedly enhance the conformational stability of a collagen triple helix. We reasoned that pyrrolidine ring pucker could instead be fixed and hence collagen stability enhanced by steric rather than stereoelectronic effects. Herein, we report on the bestowal of conformational stability to collagen by steric effects that reiterate stereoelectronic effects.

Density functional theory indicated that the pyrrolidine ring of (2*S*,4*R*)-4-methylproline (mep) has a strong preference (1.4 kcal/mol) for the C$^\gamma$-endo pucker and that of (2*S*,4*S*)-4-methylproline (Mep) has a strong preference (1.7 kcal/mol) for the C$^\gamma$-exo pucker (Figure 1). These conformational preferences are observed in crystalline Ac−mep−NHMe and Ac−Mep−NHMe[8] and follow the trend observed in 4-*tert*-butylprolines.[9] In the preferred conformations, the methyl group of mep and Mep adopts a pseudoequatorial conformation.[10] A methyl group in this conformation should protrude radially from a collagen triple helix and thus not instill any deleterious interstrand steric interactions. Accordingly, we synthesized mepOH and MepOH[11] and incorporated these nonnatural amino acids into collagen strands to yield (mep−Pro−Gly)$_7$, (Pro−Mep−Gly)$_7$, and (mep−Mep−Gly)$_7$. We incubated solutions of each strand at ≤4 °C and then used circular dichroism (CD) spectroscopy to detect formation of triple helices and assess their conformational stability.

(mep−Pro−Gly)$_7$, (Pro−Mep−Gly)$_7$, and (mep−Mep−Gly)$_7$ formed triple helices at 4 °C, as indicated by an ellipticity maximum near 225 nm (Figure 2A). The self-association of (Pro−Mep−Gly)$_7$, (mep−Mep−Gly)$_7$, and, to a lesser extent, (mep−Pro−Gly)$_7$ at 4 °C was confirmed by sedimentation equilibrium experiments.[12] (mep−Pro−Gly)$_7$, (Pro−Mep−Gly)$_7$, and (mep−Mep−Gly)$_7$ triple helices had T_m values of 13, 29, and 36 °C, respectively (Table 1),

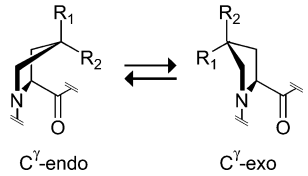

Figure 1. Ring conformations of 4-substituted proline residues. The C$^\gamma$-endo conformation is favored strongly by steric effects when R_1 = Me, R_2 = H (mep) or stereoelectronic effects when R_1 = H, R_2 = F (flp). Similarly, the C$^\gamma$-exo conformation is favored strongly by steric effects when R_1 = H, R_2 = CH_3 (Mep) or stereoelectronic effects when R_1 = OH, R_2 = H (Hyp) or R_1 = F, R_2 = H (Flp).

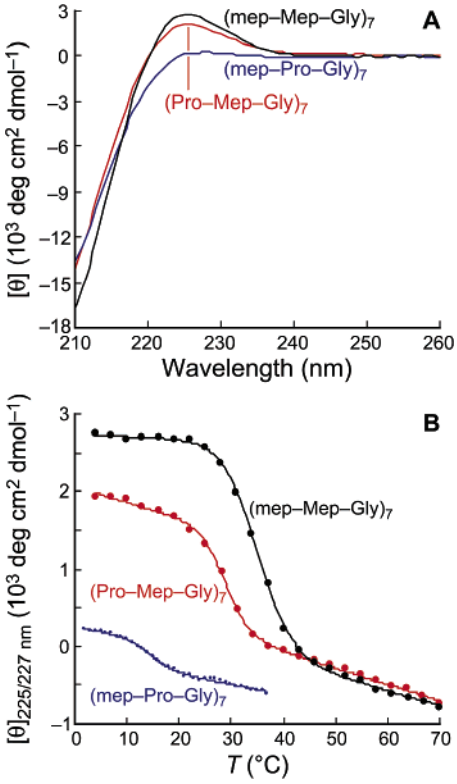

Figure 2. Conformational analysis of (mep−Pro−Gly)$_7$, (Pro−Mep−Gly)$_7$, and (mep−Mep−Gly)$_7$ by CD spectroscopy. (A) Spectra of peptide solutions (0.2 mM in 50 mM acetic acid) incubated at ≤4 °C for ≥24 h. (B) Effect of temperature on the molar ellipticity at 225 nm [(Pro−Mep−Gly)$_7$ and (mep−Mep−Gly)$_7$] or 227 nm [(mep−Pro−Gly)$_7$]. Data were recorded at intervals of 1 or 3 °C after equilibration for ≥5 min.

which are much greater than that of (Pro−Pro−Gly)$_7$. Thus, we conclude that steric effects can indeed stabilize the collagen triple helix.[13]

Mep in the Yaa position confers more stability to a triple helix than does mep in the Xaa position (Table 1). Likewise, (2*S*,4*R*)-4-fluoroproline (Flp) in the Yaa position increases triple-helical propensity more than does (2*S*,4*S*)-4-fluoroproline (flp) in the Xaa position.[3,14] We suspected that this dichotomy could arise from the

[†] Department of Chemistry.
[‡] Present address: Affinergy, Inc., 21 Davis Dr., Research Triangle Park, NC 27709.
[§] Department of Biochemistry.

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Table 1. Effect of 4-Methylproline and 4-Fluoroproline Diastereomers on the Conformational Stability of the Collagen Triple Helix

peptide	T_m (±1 °C)	ref
(Pro−Flp−Gly)$_7$	45	3a
(mep−Mep−Gly)$_7$	36	this work
(Pro−Hyp−Gly)$_7$	36	3a
(flp−Pro−Gly)$_7$	33	3c
(Pro−Mep−Gly)$_7$	29	this work
(mep−Pro−Gly)$_7$	13	this work
(flp−Flp−Gly)$_7$	8[a]	17b
(Pro−Pro−Gly)$_7$	−6[a]	17b

[a] Based on the extrapolation of data from solutions containing TMAO.

effect of the steric and stereoelectronic effects on the peptide bond itself. The trans:cis ratio of Ac−Pro−OMe in D_2O is only $K_{trans/cis} = 4.6$.[3a] Yet, all peptide bonds in the collagen triple helix are in the trans conformation ($\omega = 180°$).[6]

To determine the effect of a 4-methyl group on the value of $K_{trans/cis}$, we synthesized [$^{13}CH_3$]Ac−mep−OMe and [$^{13}CH_3$]Ac−Mep−OMe and evaluated $K_{trans/cis}$ with ^{13}C NMR spectroscopy. The trans:cis ratio was 2-fold greater for Ac−Mep−OMe ($K_{trans/cis} = 7.4$) than for Ac−mep−OMe ($K_{trans/cis} = 3.6$). These data provide an explanation for triple helices formed by (Pro−Mep−Gly)$_7$ being more stable than those formed by (mep−Pro−Gly)$_7$.[15] Apparently, a balance exists between preorganization of the proper ring pucker and stabilization of a trans peptide bond.[16]

Our findings have numerous implications. Only recently were stereoelectronic effects found to contribute to the conformational stability of a protein.[3] Herein, steric effects are shown to reiterate those same stereoelectronic effects. The stability of a non-natural (mep−Mep−Gly)$_7$ triple helix is indistinguishable from that of the "natural" (Pro−Hyp−Gly)$_7$ triple helix (Table 1), indicating that side-chain heteroatoms (and hence side-chain solvation) are not necessary for the formation of a stable triple helix. The stereoelectronic effects induced by heteroatoms are not additive in collagen. A (flp−Flp−Gly)$_7$ triple helix is less stable than is a (flp−Pro−Gly)$_7$ or (Pro−Flp−Gly)$_7$ triple helix (Table 1), presumably because of an unfavorable steric interaction between fluoro groups on adjacent strands.[17] In contrast, the steric effects are additive, as a (mep−Mep−Gly)$_7$ triple helix is more stable than is a (mep−Pro−Gly)$_7$ or (Pro−Mep−Gly)$_7$ triple helix (Table 1). The methyl groups of mep and Mep in synthetic collagen can likely be elaborated to larger functionalities without undesirable encumbrance. We imagine the creation of a new class of hyperstable collagen mimetics by the judicious integration of stereoelectronic and steric effects. The application of these venerable principles coupled with recent advances in the self-assembly of collagen fragments[18] provides the means to create sturdy synthetic collagens for applications in biomedicine and biotechnology.

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Supporting Information Available: Procedures and additional data for syntheses and analyses reported herein (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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