Hydroxylation-induced Stabilization of the Collagen Triple Helix: Further characterization of peptides with 4(R)-hydroxyproline in the Xaa position

Kazunori Mizuno, Toshihiko Hayashi‡, and Hans Peter Bächinger§

From the Department of Biochemistry and Molecular Biology, Oregon Health & Science University, and Shriners Hospital for Children, Research Department, Portland, Oregon 97239, USA and ‡Department of Life Sciences, The University of Tokyo, Tokyo 153-8902, Japan

Corresponding author: Hans Peter Bächinger, Ph.D.

Shriners Hospital for Children, Research Department
3101 SW Sam Jackson Park Road
Portland, OR 97239, USA

e-mail: hpb@shcc.org
tel: 503-221-3433
fax: 503-221-3451

Running Title: Characterization of Acetyl-(Gly-4(R)-Hyp-Yaa)10-NH2 peptides
Summary

4(R)-Hydroxyproline in the Yaa position of the -Gly-Xaa-Yaa- repeated sequence of collagen plays a crucial role in the stability of the triple helix. Since the peptide (4(R)-Hyp-Pro-Gly)\(_{10}\) does not form a triple helix, it was generally believed that polypeptides with a -Gly-4(R)-Hyp-Yaa- repeated sequence do not form a triple helix. Recently, we found that acetyl-(Gly-4(R)-Hyp-Thr)\(_{10}\)-NH\(_{2}\) forms a triple helix in aqueous solutions. To further study the role of 4(R)-hydroxyproline in the Xaa position, we made a series of acetyl-(Gly-4(R)-Hyp-Yaa)\(_{10}\)-NH\(_{2}\) peptides, where Yaa was alanine, serine, valine, and allo-threonine. We previously hypothesized that the hydroxyl group of threonine might form a hydrogen bond to the hydroxyl group of 4(R)-hydroxyproline. In water, only the threonine and the valine containing peptides were triple helical. The remaining peptides did not form a triple helix in water. In 1,2- and in 1,3-propanediol at 4 °C all the soluble peptides were triple helical. From the transition temperature of the triple helices, it was found that among the examined residues, threonine was the most stable residue in the acetyl-(Gly-4(R)-Hyp-Yaa)\(_{10}\)-NH\(_{2}\) peptide. The transition temperatures of the valine and allo-threonine containing peptides were 10 degrees lower than that of the threonine peptide. Surprisingly, the serine containing peptide was the least stable. These results indicate that the stability of these peptides depends on the presence of a methyl group as well as the hydroxyl group and that the stereo configuration of the two groups is essential for the stability. In the threonine peptide, we hypothesize that the methyl group shields the interchain hydrogen bond between the glycine and the Xaa residue from water and
that the hydroxyl groups of threonine and 4(R)-hydroxyproline can form direct or water-mediated hydrogen bonds.

**Introduction**

Collagen is the most abundant protein in multicellular animals. Collagens work not only as the scaffold of tissues and organs, but also as regulators of many biological processes including cell attachment, cell proliferation, and gene expression. Although it is one of the most extensively studied proteins, the structure of the collagen triple helix is still not fully understood. Collagen molecules consist of three polyproline II-like left-handed helices (all *trans*) that form a right-handed super helical structure, the triple helix. Formation of a triple helix requires the presence of a repeated -Gly-Xaa-Yaa- sequence. The Xaa and Yaa positions are frequently occupied by proline residues. Almost all prolines in the Yaa position of vertebrate collagens are posttranslationally modified to 4(R)-hydroxyproline by prolyl-4-hydroxylase (E.C. 1.14.11.2). Although there are some exceptions, the transition temperature of the collagen triple helix from various species is correlated to the 4(R)-Hyp content (1,2). Previous studies have shown that the peptide (Pro-4(R)-Hyp-Gly)\(_{10}\) forms a triple helix and has a significantly higher transition temperature than the trimer of the peptide (Pro-Pro-Gly)\(_{10}\) (3). Several mechanisms were proposed for the stabilizing effect of 4(R)-hydroxyproline in the Yaa position (4-9). Neither (Pro-4(S)-Hyp-Gly)\(_{10}\), (4(S)-Hyp-Pro-Gly)\(_{10}\), nor (4(R)-Hyp-Pro-Gly)\(_{10}\) form a stable triple helix in water (10,11). Since prolyl residues seem the most stable among the natural amino acids in the triple helix, it has been believed that (Gly-
4(R)-Hyp-Yaa)\textsubscript{10} peptides (Yaa is any amino acid residue) do not form a triple helix in water, until our report showed that the peptide Ac-(Gly-4(R)-Hyp-Thr)\textsubscript{10}-NH\textsubscript{2} forms a triple helix in water (12).

The thermal stability of the collagen triple helix arises from the interchain hydrogen bonds between the amide group of glycine and the carboxyl group of Xaa, and the restriction of the $\psi$ dihedral of the pyrrolidine ring structure. In addition, several additional factors have been reported to contribute to the stability of the triple helix: solvent water molecule mediated hydrogen bonds (13), the propensity of the pyrrolidine ring puckering down (C$\gamma$-endo pucker) in the Xaa and up (C$\gamma$-exo pucker) in the Yaa position (5,14-16), the inductive effect of the hydroxyl group of 4(R)-Hyp to stabilize the trans X-Hyp conformation and strengthen the hydrogen bond (7,17), and the gauche effect of 4(R)-Hyp in the Yaa to pucker up (7). However, it is still not possible to consistently explain all the experimental results.

Collagens of invertebrate have been shown to exhibit unusual and interesting properties (18-20). An example of this is the cuticle collagen from the deep-sea hydrothermal vent worm \textit{Riftia pachyptila}. This organism lives under extreme conditions (high pressure, low oxygen and steep temperature gradients) but is protected from its environment by a thick cuticle (21,22). The \textit{R. pachyptila} cuticle is mainly composed of a collagen that forms a plywood-like network of fibrils and exhibits a unique amino acid composition (22,23). Characteristic for these collagens is the occurrence of 4(R)-Hyp in the Xaa position of the -Gly-Xaa-Yaa- tripeptide repeat (24,25).

We recently found that the Ac-(Gly-4(R)-Hyp-Thr)\textsubscript{10}-NH\textsubscript{2} peptide forms a triple helix in water, and the addition of galactosyl residue to threonine increases the stability of
the triple helix (12,26). In order to further characterize the mechanism of stabilization of peptides with 4(R)Hyp in the Xaa position, we made a series of polypeptides with the amino acid sequences of acetyl-(Gly-4(R)-Hyp-Yaa)_{10}-NH_{2} (Yaa = Ser, Val, Ala, alloThr) and characterized them by circular dichroism measurements in water, in 1,2- and 1,3-propanediol. The experimental results indicate that the stereo chemical configuration of both hydroxyl and methyl groups of threonine are essential in stabilizing the triple helix in these peptides.

**Experimental Procedures**

*Peptide synthesis and Purification.* Peptides were synthesized either on a Milligen 9050 peptide synthesizer or an ABI 433A (Applied Biosystems, Foster City, CA, USA). Couplings were carried out on PAL-PEG-PS resin (Perseptive Biosystems, 0.16 mmol/g) using Fmoc-amino acids, (Fmoc-Gly-OH, Fmoc-4(R)-Hyp(tBu)-OH, Fmoc-Thr-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ala-OH, Fmoc-Gly-4(R)Hyp (Novabiochem, Darmstadt, Germany), and acetyl glycine (Bachem, CA, USA). Fmoc-Gly-4(R)-Hyp-OH was also synthesized from H-Gly-4(R)-Hyp-OH (Bachem, CA, USA) and (N-(9-Fluorenlymethylxycarbonyloxy)-succinimide) (Bachem, CA, USA). HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, Perseptive Biosystems) (4.0 eq.)/DIPEA mediated peptide couplings. The peptides were cleaved from the resin and purified by semi-preparative high performance liquid chromatography (Vydac® C18, 5µm, 300Å, 250 x 10 mm, W.R. Grace, MD, USA). All synthesized
peptides were characterized by amino acid analysis and matrix-assisted laser desorption/ionization/time of flight mass spectrometry.

*Circular Dichroism Spectroscopy.* Circular dichroism spectra were recorded on an Aviv 202 spectropolarimeter using a Peltier thermostatted cell holder and a 1-mm (Starna Cells Inc., Atascadero, CA, USA) or a 0.1-mm (Hellma, Germany) path length rectangular cell. Measurements were performed in water, 1,2-propanediol (Sigma-Aldrich), or 1,3-propanediol (Sigma-Aldrich). Molecular sieve type 3 Å 4-8 mesh beads (EM science) were added to both 1,2- and 1,3-propanediol to remove water before use. Peptide concentrations were determined by amino acid analysis. The wavelength spectra represent at least an average of 10 scans with 0.1 nm wavelength steps. Thermal transitions were recorded at a heating rate of 10 °C/h.

*Differential Scanning Calorimetry.* The temperature dependence of the partial heat capacity was measured in a N-DSC II differential scanning calorimeter (Calorimetry Science Corp., UT, USA). The peptide solutions in 1,2-propanediol were centrifuged at 4 °C for 60 min at 100000 x g in a Beckman model L-8 ultracentrifuge before measurement. The heating rate was 15 °C/h, and the data were collected and analyzed using the software provided by the manufacturer.

*Molecular modeling.* The structure was modeled using the structure PDB:1G9W file as a template for the backbone conformation (4,15). The carboxy terminus of (Pro-Pro-Gly)\textsubscript{10} was changed to an amide and the Xaa and Yaa positions replaced by 4(R)-hydroxyproline and threonine. Side chain conformations of the threonine residues were optimized by manually selecting the lowest energy conformer with the Biopolymer program in Insight II (Accelrys, Pharmacopeia Inc., NJ, USA). Subsequent energy minimizations were
performed with the Discover program of Insight II, using the consistent-valence force field. The backbone was tethered in place throughout with a force constant of 100 kcal/Å². The peptides were soaked with a five molecule thick layer of water. The side chains of all amino acids were uncharged, to approximate the effects of solvent shielding (27). The protocol for minimization was as follows: the method of steepest descents was used until a maximum derivative of < 5 kcal/Å was reached, with the charge term included. Next, the method of conjugate gradients was used for 500 iterations until a maximum derivative of < 5.0 kcal/Å was reached, with the charge term included. Next, the method of conjugate gradients was used until a maximum derivative of < 1.0 kcal/Å was reached, with charges and cross-terms energies included. Finally, the va09a quasi Newton-Raphson method was used until a maximum derivative of < 0.01 kcal/Å was achieved, with charges, cross-terms, and a Morse bond potential included.

Results

The far-ultraviolet CD spectra of collagen molecules typically show a negative ellipticity (θ) of about -50000 deg cm²/dmole around 198-200 nm and a maximum ellipticity near 220-225 nm of 7000 deg cm²/dmole, indicating the presence of a left-handed all trans X-Pro bonded polyproline II like secondary structure. Collagen-like peptides are known to show similar spectra when they form a triple helix. Upon denaturation, the positive CD peak around at 225 nm disappears. Figure 1A shows that of the peptides measured in water, only the threonine and the valine peptides showed a positive peak around at 220-225 nm. The serine, the alanine, and the alloThr peptides showed no obvious positive peaks at 220-225 nm. When the temperature dependence of
the CD signal at 221 nm was studied, only the threonine and the valine peptides showed the cooperative transition from triple helix to coil. The other peptides showed a linear decrease of the CD signal with temperature (Fig 1B). It was observed that the valine peptide stock solution (about 1 mM) needed more than one month at 4 ºC after the dissolution of the lyophilized peptide to show a cooperative transition curve. The inability of triple helix formation of the serine peptide indicates that the presence of a hydroxyl group is not sufficient to stabilize a triple helix with 4(R)-Hyp in the Xaa position in water. While the methyl group seems to contribute more to the stability in water, it alone also does not account for the stability found in the threonine peptide. Both groups are necessary to form a stable triple helix. The inability of triple helix formation of the alloThr peptide also clearly indicates that the stereochemical configuration of the two groups of threonine significantly contribute to the stability of the peptide.

To compare the thermal stability of all the peptides, we had to use a solvent where a stable triple helix could be obtained for all peptides. 1,2-propanediol was shown to increase the melting temperature of collagen-like peptides, we therefore used this solvent to compare the thermal stability of these peptides (3). Previous studies have shown that solvents with more than one hydroxyl group, such as ethylene glycol, glycerol, erythritol, xylitol, sorbitol, sucrose, lactose, maltose, and glucose, increase the stability for type I collagen at pH 4.0 (28). Similar results were obtained for galactose with Ac-(Gly-4(R)-Hyp-Thr)10-NH2 and Ac-(Gly-Pro-Thr)10-NH2 peptides (26), 1,2-propanediol for (Pro-Pro-Gly)10 and (Pro-4(R)-Hyp-Gly)10 (3), and ethylene glycol for Ac-(Gly-Pro-Nleu)6 and 9, or (Gly-Nleu-Pro)6-NH2 peptides (29). In 1,2-propanediol the peptides that do not form triple helix in water formed a triple helix, with the exception of the alanine peptide that
was not soluble in 1,2-propanediol. Figure 2A shows the CD spectra of Ac-(Gly-4(R)Hyp-Yaa)_{10}-NH_{2} peptides in 1,2-propanediol. All the soluble peptides show positive peaks around 220-225 nm and also showed a cooperative transition when the temperature was increased (Fig. 2B). In 1,2-propanediol, the threonine peptide is still most stable. The order of transition temperatures in 1,2-propanediol is Thr \( (T_m = 48 \, ^{\circ}\text{C}) > allo\text{Thr} \ (T_m = 37 \, ^{\circ}\text{C}) > \text{Val} \ (T_m = 33 \, ^{\circ}\text{C}) > \text{Ser} \ (T_m = 27 \, ^{\circ}\text{C}) \). We also measured Ac-(Gly-Pro-Thr)_{10}-NH_{2}, that does not form a triple helix in water (26). The midpoint of the thermal transition is at 33 °C. This value is a little higher than the \( T_m \) of 27 °C determined for H-(Gly-Pro-Thr)_{10}-Gly-Pro-Cys-Cys (23). These data indicate that 1,2-propanediol is a more stabilizing solvent not only for (Pro-Pro-Gly)_{10} and (Pro-4(R)-Hyp-Gly)_{10} but also for the (Gly-4(R)-Hyp-Yaa) peptides. Table 1 lists the thermodynamic data determined from the transition curves of the Ac-(Gly-4(R)-Hyp-Yaa)_{10}-NH_{2} peptides in 1,2-propanediol.

Figure 3A shows the CD spectra of the peptides in 1,3-propanediol. Again, all peptides showed a positive peak around 220 to 225 nm and the corresponding transition curves are shown in Figure 3B. The peptides are more stable in 1,3-propanediol than in 1,2-propanediol. The serine peptide was not soluble in this solvent and limited solubility was observed for the valine peptide. The threonine containing peptide was again the most stable one \( (T_m = 56 \, ^{\circ}\text{C}) \), followed by the valine \( (T_m = 47 \, ^{\circ}\text{C}) \), the alanine \( (T_m = 44 \, ^{\circ}\text{C}) \), and the \textit{allo}threonine containing peptide \( (T_m = 43 \, ^{\circ}\text{C}) \).

In the energy-minimized structure of the threonine peptide shows that the methyl group of threonine faces towards the inside of the triple helix between the peptide bond of Thr-Gly of the adjacent chain and the glycine carbonyl of the same chain (Fig. 4). The
methyl group covers the interchain hydrogen bond between the carbonyl group of hydroxyproline in the Xaa position of the adjacent chain and the amino group of the next glycine residue in the same chain. The hydroxyl group points toward the outer surface of the triple helix. The pyrrolidine ring of hydroxyproline in the Xaa position is puckering down (Cγ-endo), which brings the Cγ atom closer to the threonine residue. The distance between the oxygen atom of the hydroxyl group of threonine and the oxygen atom of the hydroxyl group of hydroxyproline is 3.7 Å, which is longer than the distance found in typical hydrogen bonds (2.90-3.20 Å).

Discussion

We have recently shown that Ac-(Gly-4(R)-Hyp-Thr)_{10}-NH_{2} forms a stable triple helix in water. This was a surprise because it was assumed that 4(R)-hydroxyproline in the Xaa position was believed to prevent the formation of a triple helix, because it was shown that (4(R)-Hyp-Pro-Gly)_{10} was unable to form this structure. On the other hand, for *Lumbricus terrestris* cuticle collagen, it is reported that the sequence -Gly-Hyp-Ser- accounts for 4 to 5% of the total hydroxyproline content and that the cuticle collagen of *Riftia pachyptila* has a very low proline and hydroxyproline content (5%). The stabilizing factor in the cuticle collagen of *R. pachyptila* is the galactosylation of threonines occurring in the Yaa position. However, sequencing revealed the presence of 4(R)-hydroxyproline in the Xaa position. Our results presented here with peptides containing 4(R)-hydroxyproline in the Xaa position and threonine, serine, valine, alanine, and allothreonine shed some insight into the stabilizing forces of these peptides. In our
previous studies with the threonine containing peptide, we concluded that there could be an additional hydrogen bond in this peptide, as indicated by an increase in the $\Delta H^\circ$ between the proline and 4(R)-hydroxyproline containing peptides. However, the serine containing peptide in this study does not form a stable triple helix in water. In 1,2-propanediol we find a lower increase in $\Delta H^\circ$ for the serine compared to the threonine peptide, and this together with a slightly larger $\Delta S^\circ$ makes the serine containing peptide significantly less stable. The conclusion therefore is that the hydroxyl group alone does not account for the increased stability.

The valine containing peptide is nearly as stable as the threonine containing peptide in water, indicating that the methyl group plays an important role in stabilizing the triple helix through van der Waals interactions as well. The allothreonine containing peptide shows that the stereochemistry of the hydroxyl and methyl group is important in this stabilization. In water, the allothreonine containing peptide does not form a triple helix and the $\Delta H^\circ$ in 1,2-propanediol is significantly lower than that of the threonine containing peptide.

The effect of both 1,2- and 1,3-propanediol on thermal stability of the Ac-(Gly-4(R)-Hyp-Yaa)$_{10}$-NH$_2$ are shown in Figures 2 and 3. Ethylene glycol also has the similar effect on some of the peptides used in this study (data not shown). Glycerol is also known to increase the melting temperature of type I collagen (28,30). In contrast, the melting temperature of type II collagen is decreased by the addition of 1,2-propanediol, 2-propanol, 1-propanol (31). The stabilizing effect of 1,2-propanediol on the (Pro-Pro-Gly)$_{10}$, and (Pro-$4R_Hyp$-Gly)$_{10}$, were reported (3). The detailed mechanism of stabilization by these solvents is still unclear. Recent synchrotron radiation analysis of
the [(Pro-Pro-Gly)\textsubscript{10}]\textsubscript{3} crystal grown in micro gravity by Berisio et al. (16), which diffracted up to 1.3 Å indicates, that many water molecules interact with the peptide. These authors suggest that the unusually high percentage of exposed unsaturated carbonyl groups, together with the triple helix peculiar rod-like shape are the main reasons of the observation. It is still controversial how water or other solvent molecules contribute to the stability of the triple helix (32). However, previous studies and our experimental results indicate both 1,2- and 1,3- propanediol significantly contribute to the stability of the triple helix. Generally, the peptides are more stable in 1,3-propanediol. This is especially true for the valine peptide, which shows a T\textsubscript{m} value that is 14 °C higher than in 1,2-propanediol. We hypothesize that 1,2-propanediol and 1,3-propanediol molecules might act as three or four hydrogen-bonded water molecules, which interact with the surface of the triple helix. The increase in stability in 1,3-propanediol over 1,2-propanediol probably results from the difference in the spacing of the hydroxyl groups. 1,3-propanediol can form longer connections than 1,2-propanediol and potentially can also provide van der Waals contacts with the central CH\textsubscript{2} group. This could explain the increased stability of the valine peptide in 1,3-propanediol. The common effect on the examined peptides implies propanediol molecules preferentially interact with the main chain carbonyl groups of Gly, or the Yaa position residues.

The order of stability of the threonine peptide, followed by the valine, 
\textit{allo}threonine, and serine peptides suggests that the stereochemical configuration of both the methyl and the hydroxyl group is important for the stability. The energy minimization calculations suggest that the methyl group of threonine shields the interchain hydrogen bond between the amino group of glycine and the carboxyl group of
hydroxyproline from solvent molecules. This interchain hydrogen bond is a major source of the thermal stability of the collagen triple helix. Exclusion of solvent molecules has also been observed for the galactosyl-threonine containing peptides (33). The increased stability of the threonine peptide compared to the valine peptide suggests the possibility of a contribution by hydrogen bond formation of the hydroxyl group of threonine. In the energy-minimized structure the formation of a direct hydrogen bond between the hydroxyl groups of hydroxyproline and threonine seems unlikely, however a water-mediated hydrogen bond cannot be excluded. The stability of the allothreonine peptide suggests that such a contribution exists and that it is stereospecific.

**Footnotes**

This work was supported by a grant from Shriners Hospital for Children. The authors thank Eric Steel for expert technical assistance and Dr. Kerry Maddox for amino acid analyses.

**Abbreviations:**

*allo*Thr; (2S,3S)-2-amino-3-hydroxybutyric acid; L-*allo*-threonine, CD; circular dichroism

Fmoc; N-(9-fluorenyle) methoxycarbonyl
References

1. Burjanadze, T. V. (1979) *Biopolymers* **18**, 931-938
2. Burjanadze, T. V., and Veis, A. (1997) *Connect Tissue Res* **36**, 347-365
3. Engel, J., Chen, H. T., Prockop, D. J., and Klump, H. (1977) *Biopolymers* **16**, 601-622
4. Vitagliano, L., Berisio, R., Mazzarella, L., and Zagari, A. (2001) *Biopolymers* **58**, 459-464
5. Vitagliano, L., Berisio, R., Mastrangelo, A., Mazzarella, L., and Zagari, A. (2001) *Protein Sci* **10**, 2627-2632
6. Kramer, R. Z., Bella, J., Brodsky, B., and Berman, H. M. (2001) *J Mol Biol* **311**, 131-147
7. Jenkins, C. L., and Raines, R. T. (2002) *Nat Prod Rep* **19**, 49-59
8. Gough, C. A., Anderson, R. W., and Bhatnagar, R. S. (1998) *J Biomol Struct Dyn* **15**, 1029-1037
9. Brodsky, B., and Ramshaw, J. A. (1997) *Matrix Biol* **15**, 545-554
10. Inouye, K., Sakakibara, S., and Prockop, D. J. (1976) *Biochim Biophys Acta* **420**, 133-141
11. Inouye, K., Kobayashi, Y., Kyogoku, Y., Kishida, Y., Sakakibara, S., and Prockop, D. J. (1982) *Arch Biochem Biophys* **219**, 198-203
12. Bann, J. G., and Bächinger, H. P. (2000) *J Biol Chem* **275**, 24466-24469
13. Bella, J., Brodsky, B., and Berman, H. M. (1995) *Structure* **3**, 893-906
14. Berisio, R., Vitagliano, L., Mazzarella, L., and Zagari, A. (2000) *Biopolymers* **56**, 8-13
15. Berisio, R., Vitagliano, L., Sorrentino, G., Carotenuto, L., Piccolo, C., Mazzarella, L., and Zagari, A. (2000) *Acta Crystallogr D Biol Crystallogr* **56** (Pt 1), 55-61

16. Berisio, R., Vitagliano, L., Mazzarella, L., and Zagari, A. (2002) *Protein Sci* **11**, 262-270

17. Holmgren, S. K., Taylor, K. M., Bretscher, L. E., and Raines, R. T. (1998) *Nature* **392**, 666-667

18. Coyne, K. J., Qin, X. X., and Waite, J. H. (1997) *Science* **277**, 1830-1832

19. Qin, X. X., Coyne, K. J., and Waite, J. H. (1997) *J Biol Chem* **272**, 32623-32627

20. Waite, J. H., Qin, X. X., and Coyne, K. J. (1998) *Matrix Biol* **17**, 93-106

21. Gaill, F., Mann, K., Wiedemann, H., Engel, J., and Timpl, R. (1995) *J Mol Biol* **246**, 284-294

22. Gaill, F., Wiedemann, H., Mann, K., Kühn, K., Timpl, R., and Engel, J. (1991) *J Mol Biol* **221**, 209-223

23. Mann, K., Mechling, D. E., Bächinger, H. P., Eckerskorn, C., Gaill, F., and Timpl, R. (1996) *J Mol Biol* **261**, 255-266

24. Goldstein, A., and Adams, E. (1970) *J Biol Chem* **245**, 5478-5483

25. Muir, L., and Lee, Y. C. (1970) *J Biol Chem* **245**, 502-509

26. Bann, J. G., Peyton, D. H., and Bächinger, H. P. (2000) *FEBS Lett* **473**, 237-240

27. Vitagliano, L., Nemethy, G., Zagari, A., and Scheraga, H. A. (1993) *Biochemistry* **32**, 7354-7359

28. Gekko, K., and Koga, S. (1983) *J Biochem (Tokyo)* **94**, 199-205

29. Feng, Y., Melacini, G., and Goodman, M. (1997) *Biochemistry* **36**, 8716-8724
30. Leikina, E., Mertts, M. V., Kuznetsova, N., and Leikin, S. (2002) *Proc Natl Acad Sci U S A* **99**, 1314-1318

31. Bächinger, H. P., and Morris, N. P. (1990) *Matrix* **10**, 331-338

32. Engel, J., and Prockop, D. J. (1998) *Matrix Biol* **17**, 679-680

33. Bann, J. G., Bächinger, H. P., and Peyton, D. H. (2003) *Biochemistry* **42**, 4042-4048

**Figure legends**

Figure 1. *Circular dichroism spectra and thermal transition curves of Ac-(Gly-4(R)-Hyp-Yaa)10-NH2 peptides in water.* The peptides were measured in water at a concentration of 100 µM. (A) CD spectra measured at 4 °C. Yaa = Thr (diamond), Ser (down triangle), Val (circle), Ala (up triangle), and alloThr (square). (B) Thermal transition curves of the peptides in water monitored by circular dichroism at 221 nm with a heating rate of 10 °C/h. Yaa = Thr (diamond), Ser (down triangle), Val (circle), Ala (up triangle), and alloThr (square).

Figure 2. *Circular dichroism spectra and thermal transition curves of Ac-(Gly-4(R)-Hyp-Yaa)10-NH2 peptides in 1,2-propanediol.* (A) CD spectra measured at 5 °C at a concentration of 100 µM. Yaa = Thr (diamond), Ser (down triangle), Val (circle), Ala (up triangle), and alloThr (square). (B) Thermal transition curves of the peptides in 1,2-propanediol measured with a heating rate of 10 °C/h. Yaa = Thr (diamond), Ser (down triangle), Val (circle), Ala (up triangle), and alloThr (square).
triangle), Val (circle), \textit{allo}Thr (square), and for comparison Ac-(Gly-Pro-Thr)$_{10}$-NH$_2$ (up triangle).

Figure 3. \textit{Circular dichroism spectra and thermal transition curves of Ac-(Gly-4(R)-Hyp-Yaa)$_{10}$-NH$_2$ peptides in 1,3-propanediol.} (A) CD spectra measured at 5 °C at a concentration of 100 µM. Yaa = Thr (diamond), Ser (down triangle), Val (circle), Ala (up triangle), and \textit{allo}Thr (square). (B) Thermal transition curves of the peptides in 1,3-propanediol measured with a heating rate of 10 °C/h. Yaa = Thr (diamond), Val (circle), Ala (up triangle), and \textit{allo}Thr (square).

Figure 4. \textit{Structure of the energy-minimized Gly-4(R)-Hyp-Thr peptide.} The structure was modeled using the PDB:1G9W file as a template for the backbone conformation. A2, A5, A8, B32, B35, C62, and C65 are replaced by Thr. A1, A4, A7, B31, B34, C61, and C64 are replaced by 4(R)-Hyp. (A) The residues A5 (Thr) and B34 (Hyp) are shown as CPK models, the other residues are shown as ball-and-stick models. (B) The view from the other side. The hydrogen bond between GlyNH...OCHyp is covered by the methyl group of threonine. The residues A5 (Thr) and B34 (Hyp) except the carbonyl group of Hyp are shown as CPK model. The N-terminus is at the bottom.
Table 1

Thermodynamic data of the triple helix coil transition of the Ac-(Gly-4(R)-Hyp-Yaa)-NH₂ peptides in 1,2-propanediol. The thermodynamic parameters are expressed per mol of tripeptide units in a triple helix.

| Peptide                     | Tₘ ᵃ  | ΔH°_VH | ΔS°  | Tₘ ᵇ  | ΔH°_cal | c  |
|-----------------------------|-------|--------|------|-------|---------|----|
|                             | °C    | kJ/mol | J/mol/K | °C    | kJ/mol  | mM |
| Ac-(Gly-4(R)-Hyp-Thr)₁₀⁻NH₂ | 48.2  | -10.6  | -29   | 50.1  | -15.1   | 1.0|
| Ac-(Gly-4(R)-Hyp-Val)₁₀⁻NH₂ | 34.9  | -6.3   | -18   | 37.8  | -7.0    | 1.9|
| Ac-(Gly-4(R)-Hyp-Ser)₁₀⁻NH₂ | 26.4  | -10.4  | -30   | c     | -       | -  |
| Ac-(Gly-4(R)-Hyp-alloThr)₁₀⁻NH₂ | 37.6  | -7.9   | -22   | 39.6  | -7.2    | 2.2|

ᵃ Melting temperatures Tₘ are concentration dependent and are determined from the CD measurements (100 µM), together with ΔH°_VH and ΔS°, using equation 2 in reference 3.
ᵇ Melting temperatures Tₘ determined by differential scanning calorimetry at the indicated concentration c.
ᶜ The serine peptide was not soluble at the higher concentrations needed for differential scanning calorimetry.
Figure 1
Figure 2
Figure 3

A

Wavelength (nm)

$\theta$ (deg cm$^2$ dmol$^{-1}$) x10$^{-4}$

B

Degree of conversion

Temperature (°C)
Figure 4
Hydroxylation-induced stabilization of the collagen triple helix: Further characterization of peptides with 4(R)-hydroxyproline in the Xaa position
Kazunori Mizuno, Toshihiko Hayashi and Hans Peter Bächinger

J. Biol. Chem. published online June 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M304741200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts