J|A|C|S COMMUNICATIONS

Published on Web 09/17/2010

Functionalizable Collagen Model Peptides

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Abstract: The functionalizability and conformational properties of azidoproline (Azp)-containing collagen model peptides (CMPs) were studied. The results show that (4*R*)Azp has a similar stabilizing effect on the collagen triple helix as (4*R*)hydroxyproline and that functionalized CMPs are readily accessible by "click" chemistry. The resulting triazole-functionalized CMPs form stable triple helices, demonstrating that sterically demanding moieties in three symmetry-related positions in all strands are tolerated. The straightforward synthesis and facile functionalizability of the Azp-containing CMPs are intriguing for the development of functional collagen-based materials.

The stability and many functions of collagen, the most abundant protein in mammals, depend largely on functional groups attached to its backbone.[1] Aside from hydroxylations, other modifications (e.g., glycosylations) are known to influence the stability of the collagen triple helix.[1−3] Derivativatized collagens are also becoming increasingly attractive for the development of synthetic functional materials.[4] Thus, collagen model peptides (CMPs) that allow for the facile introduction of desired moieties are important not only for a deeper understanding of the factors that govern the conformational stability of the collagen triple helix but also for the development of functional collagen-based materials. Herein we introduce azidoproline (Azp)-containing CMPs that can easily be functionalized with various groups. In addition, we demonstrate that Azp residues have effects on the stability of collagen that are similar to those of hydroxyproline (Hyp).

The collagen triple helix consists of three polyproline II-like single strands that are composed of repeating Xaa-Yaa-Gly units. Proline (Pro) and (4*R*)Hyp[5] are the most frequent amino acids in the Xaa and Yaa positions, respectively.[1] Imino acids in the Yaa position usually adopt a C(4)-*exo* ring pucker and those in the Xaa position a C(4)-*endo* ring pucker.[1,3] Over the past two decades, many studies have established the importance of Hyp in the Yaa position for the stability of the triple helix.[3,6] Studies with CMPs in which the Hyp residues were replaced by, for example, (4*R*)fluoroproline (Flp) demonstrated that the stabilizing effect is mainly due to stereoelectronic effects.[6,7] We recently found that

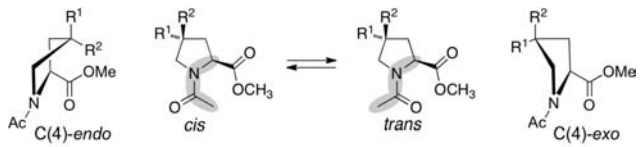


Figure 1. Cis/trans conformer ratios of Ac-Xaa-OCH_3 model compounds in D_2O and their preferred ring puckering [data taken from refs 8 (Azp) and 6a (Hyp)].

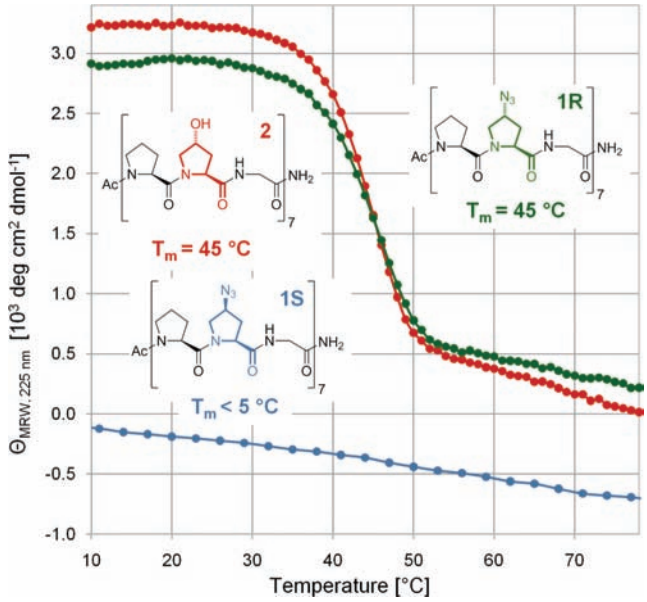
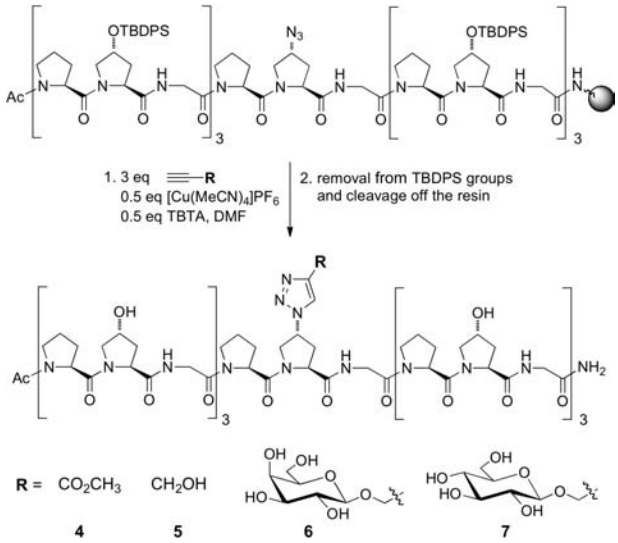


Figure 2. CD thermal transition curves for CMPs **1R**, **1S**, and **2** in 50 mM aqueous AcOH (0.2 mM).[10]

(4*S*)Azp and (4*R*)Azp have similar conformational properties as the respective Hyp and Flp derivatives.[8] This is due to the fact that the strength of the stereoelectronic azido-gauche effect is comparable to those exerted by fluorine and hydroxy groups.[8] Both (4*R*)Azp and (4*R*)Hyp derivatives adopt C(4)-*exo* ring puckers and have a higher population of the amide trans conformer than does (4*S*)Azp, which adopts a C(4)-*endo* ring pucker (Figure 1). We therefore hypothesized that CMPs with (4*R*)Azp in place of (4*R*)Hyp residues should have comparable stabilities, whereas CMPs with (4*S*)Azp in place of (4*R*)Hyp should have significantly reduced stabilities. In addition, we envisioned that the azido group should allow for facile further functionalization by, for example, "click" chemistry.[9]

To test the influence of Azp on the stability of the triple helix, we prepared CMPs with the general structure Ac-(Pro-Yaa-Gly)$_7$-NH_2 bearing either (4*R*)Azp (**1R**) or (4*S*)Azp (**1S**) in the Yaa position. CMP **2** with (4*R*)Hyp residues in the Yaa position was prepared for comparison. The relative stabilities of the triple helices of these CMPs were investigated by thermal denaturation using circular dichroism (CD) spectroscopy as a monitoring tool (Figure 2). As expected, in the CD spectra of CMPs **1R** and **2**, a maximum at 225 nm that is typical of the collagen triple helix was observed.[1] In contrast, this maximum was missing in the spectrum of **1S**. This indicates that (4*R*)Azp but not (4*S*)Azp allows for the formation of a collagen triple helix. When solutions of **1R** and **2** were heated, midpoints of the thermal transition (T_m values) of 45 °C were observed. These results demonstrate that (4*R*)Azp stabilizes the collagen triple helix equally as well as (4*R*)Hyp despite the lack of

Scheme 1. Synthesis of Functionalized CMPs **4**−**7**



H-bonding sites and the different steric demands of the azido group in comparison with the hydroxy group. The experiments show that the conformational preference for the C(4) ring pucker and the amide cis/trans conformer ratios, which were comparable for (4*R*)Azp and (4*R*)Hyp (Figure 1), are critical for the stability of the collagen triple helix. Thus, the results demonstrate that the conformational properties observed in the monomer Ac-(4*R*)Azp-OMe are reflected in the respective CMP. Most importantly, they further highlight the importance of stereoelectronic effects for the conformational stability of the collagen triple helix.

To evaluate the functionalizability of CMPs containing Azp, Ac-(Pro-Hyp-Gly)$_3$-(Pro-(4*R*)Azp-Gly)-(Pro-Hyp-Gly)$_3$-NH$_2$ (**3**) with an azido moiety in the middle was prepared.[11] CMP **3** was readily synthesized by couplings of the trimeric building blocks Fmoc-Pro-Hyp(TBDPS)-Gly-OH[12] and Fmoc-Pro-(4*R*)Azp-Gly-OH using standard solid-phase peptide synthesis. Functionalization of solid-phase-bound CMP **3** using Huisgen's 1,3-dipolar cycloaddition ("click" reaction) was straightforward.[13] Reaction of the azido group with alkynes bearing unprotected hydroxy, ester, and sugar moieties proceeded smoothly in the presence of substoichiometric amounts of Cu(I)/TBTA.[14] After removal of the silyl protecting groups and cleavage from the resin, the crude triazole-functionalized CMPs **4**−**7** were obtained in purities of ≥80% (after 17 synthesis steps) and easily purified by preparative HPLC (Scheme 1).

To evaluate the effect of the triazole moieties on the conformational stability of the collagen triple helix, thermal denaturation studies as described above were performed. All of the functionalized CMPs formed triple helices, as indicated by the maxima observed at 225 nm. Midpoints of thermal transitions of 37 and 38 °C were observed upon heating solutions of **4**−**7** (Table 1). These are lower than those observed for CMPs **1R**, **2**, and **3** but demonstrate that CMPs with moieties as sterically demanding as the galactosylated triazole in three symmetry-related positions still form stable collagen triple helices.[15]

Table 1. T_m Values of Functionalized CMPs **3**−**7** Determined by Heating Solutions of **3**−**7** in 50 mM Aqueous AcOH Solutions (0.2 mM)[10]

CMP	**3**	**4**	**5**	**6**	**7**
T_m (°C)	45	38	37	38	38

In summary, we have shown that (4*R*)Azp has a similar stabilizing effect on the collagen triple helix as (4*R*)Hyp in the Yaa position. This is consistent with the importance of stereoelectronic effects for the conformational stability of the collagen triple helix.[6] Furthermore, we have shown that functionalized CMPs with various moieties are readily accessible from Azp-containing CMPs by click chemistry. The presented functionalized CMPs bear moieties as sterically demanding as monosaccharides attached to triazole units in three symmetry-related positions but still form stable triple helices. The straightforward synthesis and facile functionalizability of the Azp-containing CMPs render them particularly intriguing for the development of functional collagen-based materials. Research toward this goal is in progress and will be reported in due time.

Acknowledgment. This work was supported by the NCCR NANO and Bachem. Dr. M. Kümin is gratefully acknowledged for his contributions to the synthesis of the Pro monomers. H.W. is grateful to Bachem for an endowed professorship.

Supporting Information Available: Details of the synthesis of CMPs **1**−**7** and the determination of their T_m values. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA103392T