Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *http://doi.dx.org/10.17952/36EPS.2022.001*

Synthetic Collagen Peptides

Helma Wennemers
Laboratory of Organic Chemistry, ETH Zurich, D-CHAB, 8093 Zurich, Switzerland

Introduction

Collagen, the most abundant protein in mammals, is a key contributor for the strength and stability of skin, bones, and connective tissue [1]. Collagen formation is thus vital for the integrity of skin, tendons, and the tissue in essentially any organ. Excessive collagen formation is, however, characteristic of fibrotic and malignant diseases, which include major global health issues.

The Wennemers group has used collagen model peptides (CMPs) to understand the stability of collagen at the molecular level and to establish functional synthetic collagen triple helices [2]. These include pH-responsive synthetic collagen [3-6], hyperstable triple helices [7-9], and heterotrimeric collagen [10]. Building on these data, we designed and synthesized a chemical probe for the simultaneous monitoring and targeting of lysyl oxidase (LOX)-mediated collagen cross-linking [11]. The probe allows for the detection of LOX activity *in vivo* and in tissue sections.

Results and Discussion

Functionalizable and pH responsive collagen triple helices. C^{γ}-substituted proline derivatives are valuable tools for developing functionalized collagen peptides for biological and materials investigations [1,2], yet the stereochemistry at C^{γ} can produce undesired steric or stereoelectronic constraints. We mitigated this drawback by using γ-azaproline (γ-azPro) as a proline mimetic that lacks a stereogenic center at the γ-position of the ring and can thus utilize the invertibility of nitrogen to adapt its conformation (Figure 1) [5,6]. NMR spectroscopic analyses and DFT calculations highlight how alkylated γ-azPro and alkylated derivatives thereof are conformationally dynamic and adopt conformational preferences through ring pucker flip along with nitrogen inversion. Incorporation of alkylated γ-azPro into collagen peptides produced functionalized pH-responsive triple helices with similar thermal stabilities, regardless of their placement in the Xaa or Yaa position within the characteristic Xaa-Yaa-Gly repeating unit of collagen peptides.

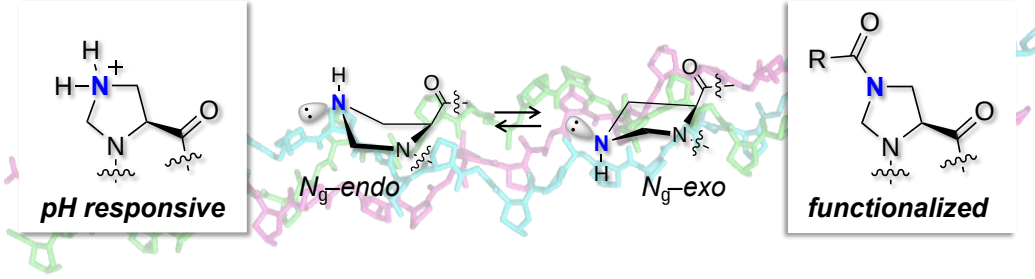
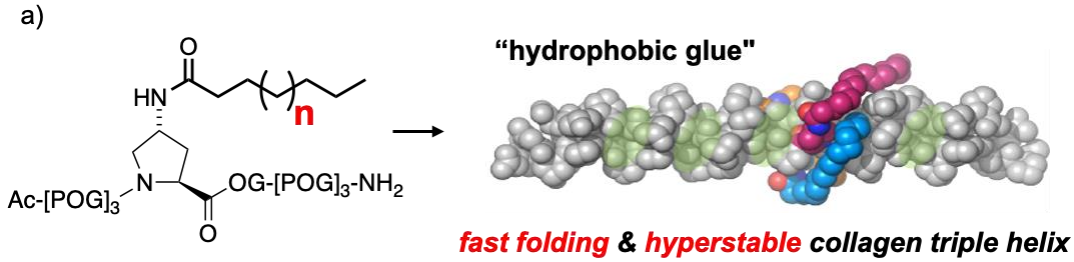


Fig. 1. pH-responsive and functionalized collagen triple helices using γ-azaPro

Lipidation bestows hyperstability and fast-folding on collagen triple helices. Cross-linking of collagen single strands by oxime ligation creates hyperstable collagen triple helices [7]. Through the study of the *trans*/*cis* ratio of Xaa-Pro amide bonds and their *trans*/*cis* isomerization speed in different solvents [12] we hypothesized that a hydrophobic environment should bestow collagen triple helices with hyperstability and fast-folding. Indeed, pendant hydrophobic moieties endow triple helical collagen with hyperstability and accelerate the *cis-trans* isomerization to an extent that thermally induced unfolding and folding of collagen triple helices take place at the same speed (Figure 2a) [8,9]. A systematic study with CMPs bearing different hydrophobic moieties even revealed a direct correlation between the length of the fatty acid and the stability and the folding speed of the triple helix (Figure 2b) [9]. Our experimental data and MD simulations revealed that lipidation enhances the

thermal stability and folding rate of triple-helical collagen by: *(i)* an increase of the *trans*/*cis* ratio of Xaa-Pro bonds, *(ii)* an acceleration of *trans*-*cis* isomerization, and *(iii)* van der Waals interactions with the grooves of the collagen triple helix [9].



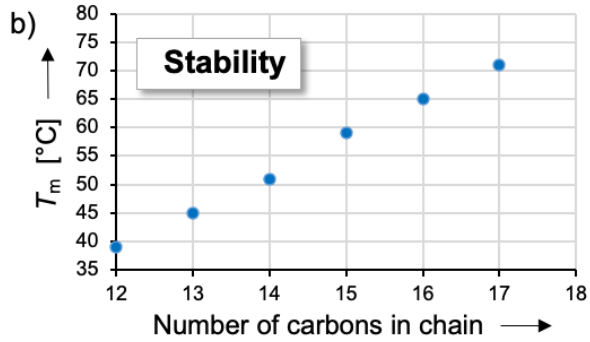


Fig. 2. a) Example of lipidated collagen triple helices. b) Correlation between lipid chain lengths and T_m values.

Heterotrimeric collagen triple helices. Heterotrimeric collagen triple helices are most common in nature. Synthetic heterotrimeric ABC-type collagen triple helices are difficult to access since a total of $3^3 = 27$ trimers that differ in their composition and register can form in a mixture of three different strands. We used our knowledge from pH-responsive (4*S*)-aminoproline (Amp) containing collagen model peptides (CMPs) [3,4] and covalently cross-linked CMPs [7] to create synthetic collagen hetero-trimers by placing (4*S*)-aminoproline (Amp) and aspartic acid (Asp) residues in coplanar Xaa and Yaa positions of neighboring strands (Fig. 3a) [10]. This geometrically well-defined Amp–Asp salt bridge enabled the design of strands that assemble exclusively into the target heterotrimers. An important feature for this selective assembly is that unpaired (4*S*)Amp and Asp residues disfavor triple helix formation and preclude undesired assemblies [10]. Thus, specific heterotrimer formation only occurs when the Amp and Asp residues are paired in coplanar Xaa and Yaa positions. As little as three of the lateral Amp–Asp salt bridges sufficed to assemble 24-mers into tailored AB_2 or ABC-type heterotrimers with control over their composition and register (Figure 3b).

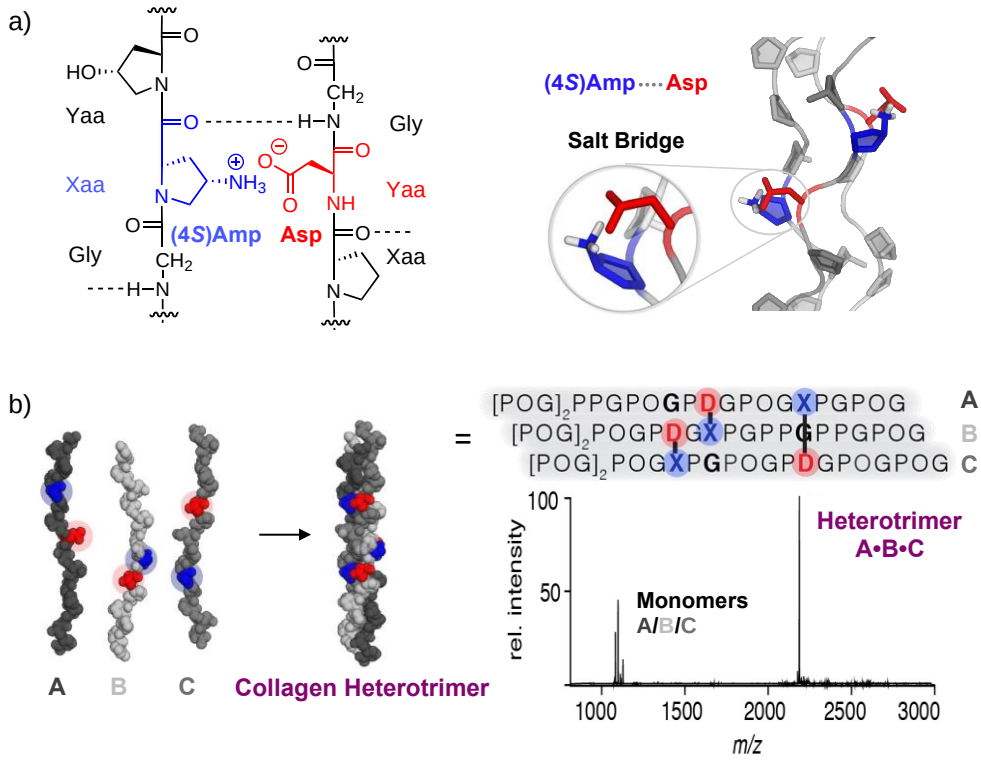


Fig. 3. Collagen heterotrimer formed by the Amp–Asp salt bridge.

For studying collagen heterotrimer formation, we established a native ESI-MS method [13]. This powerful tool allows for simultaneously monitoring the composition and thermal stability of non-covalently assembled triple helices. In contrast to CD-spectroscopy, an analysis tool that only provides data averaged over all species in a mixture, native ESI-MS allows for distinguishing different triple helices in a mixture of CMPs. A temperature-controlled native ESI source enabled the measurement of their thermal denaturation profiles in a single experiment.

Acknowledgments

This research was supported by the Swiss National Science Foundation (2000020_178805).

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