Influence of the Frame Shift and N-terminal Capping Groups on the Assembly of Collagen Triple Helices

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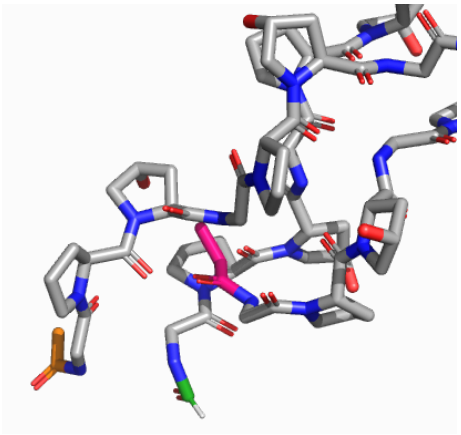
Collagen model peptides (CMPs) are a key tool to study native collagen, the most abundant protein in mammals, which possesses an intriguing triple helical structure.[1] Chemical methods to tune the physico-chemical properties of synthetic collagen triple helices, especially their thermal stability, are important for monitoring collagen crosslinks, which can be related to diseases, or damaged collagen and the design of synthetic collagen-based materials.[2,3,4] This work presents the effect of the CMP frame and N-terminal acyl capping groups on the thermal stability of collagen triple helices as well as the selectivity of collagen heterotrimer formation. Thermal stability studies of collagen homotrimers composed of CMPs with different short N-terminal acyl caps showed a dependence on the N-terminal residue. We hypothesize that these differences originate from a combination of sterics, hydrophobic interactions, and the $K_{trans/cis}$ ratio of the "cap-amino acid" amide bond.



In the second part of the work, we introduce the formyl group as a novel mass-tag for collagen heterotrimer formation. Furthermore, we studied the influence of frame shifts, previously identified for homotrimers, on the thermal stability of heterotrimers. We used 24-meric CMPs in all three collagen frames, containing aspartate and (4*S*)-aminoproline residues which drive selective collagen heterotrimer assembly via salt bridge formation.[5] We show that the trends in relative melting temperatures for the frame-shifted collagen heterotrimers and heterotrimers containing different N-terminal acyl caps are almost identical to those previously observed for homotrimers.[6] Moreover, a Gly residue at the C-terminus led to the same destabilization of the triple helix as identified in homotrimers.[6] These findings aid the design of heterotrimer-based materials and probes with predictable physico-chemical properties.

In the last part of the work, we extended the 24-meric heterotrimers by an amino acid triplet to 27-meric heterotrimers to further increase the relative melting temperature. The 27-meric heterotrimer in the GPO frame is the thermally most stable synthetic collagen heterotrimer constructed by the use of only 3 Amp-Asp salt bridges, reported to date.

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