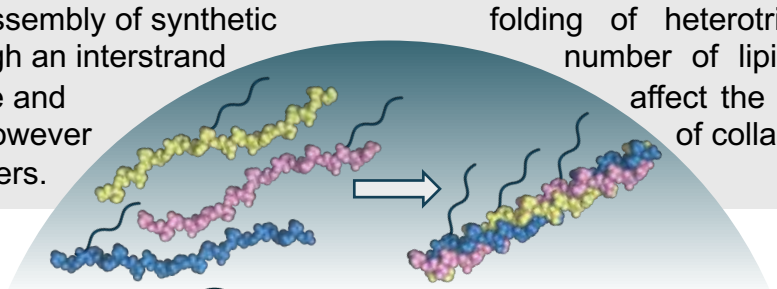
https://doi.org/10.17952/37EPS.2024.P2203

Influence of Hydrophobic Moieties on the Formation and Stability of Collagen Heterotrimers

Maša Stopinšek, Valdrin Islami, Giuseppe Antoniazzi, Philipp Bittner, Renato Zenobi, Helma Wennemers*
Laboratory of Organic Chemistry, ETH Zürich, Switzerland

1 Introduction

- Collagen, the main component of the extracellular matrix, and its triple helical structure provide strength and stability to connective tissues.[1] Synthetic collagen model peptides (CMPs) are useful for studying the structure and stability of natural collagen and exploring the effect of functional groups on the properties and function of collagen. Our group has shown that CMPs bearing hydrophobic moieties form fast-folding and hyperstable homotrimeric triple helices in water.[2,3]
- In nature, most collagens consist of two or three different strands. Our lab showed that the controlled assembly of synthetic heterotrimers can be achieved through an interstrand salt bridge between (4*S*)aminoproline and aspartic acid.[4,5] The folding rate is however slower compared to that of homotrimers.
- Here, we investigate whether lipidation can accelerate the folding of heterotrimeric collagen and assess how the number of lipids and their position within the CMPs affect the thermal stability and formation specificity of collagen heterotrimers.



2 Design of ABC-type Heterotrimers

Unfunctionalized

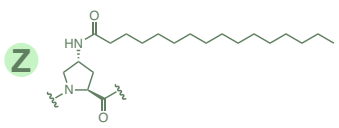
1 POGP**D**GPOGPOG**X**PGPOGPOGPOG
2 POG**X**PGPOGPOGPOGPOGP**D**GPOG
3 POGPOGPOGP**D**GPOGPOG**X**PGPOG

D aspartic acid, **X** (4*S*)aminoproline

Functionalized with palmitic acid

At the same position

4P POGP**D**G**Z**OGPOG**X**PGPOGPOGPOG
5P POG**X**PG**Z**OGPOGPOGPOGP**D**GPOG
6P POGPOG**Z**OGP**D**GPOGPOG**X**PGPOG

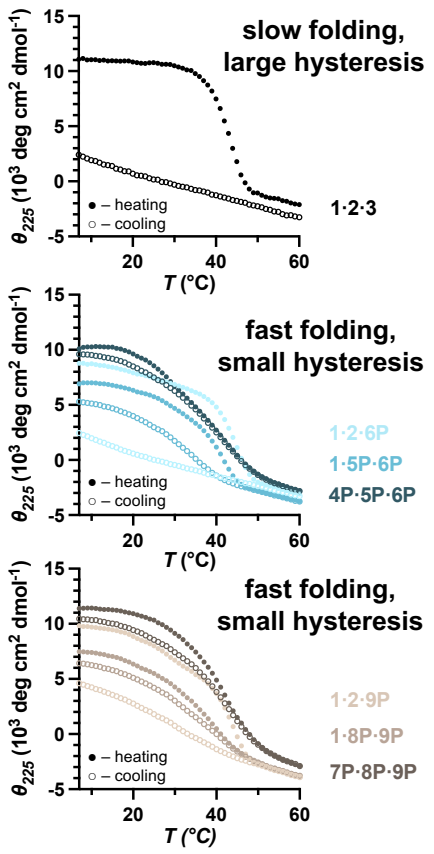


Distributed along the triple helix

7P POGP**D**GPOGPOG**X**PGPOG**Z**OGPOG
8P POG**X**PGPOG**Z**OGPOGPOGP**D**GPOG
9P POG**Z**OGPOGP**D**GPOGPOG**X**PGPOG

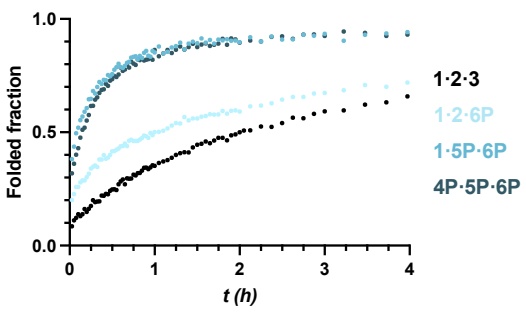
3 Thermal Stability Studies with CD Spectroscopy

		T_m [°C]
unfunctionalized	**1·2·3**	42
one functional moiety	**4P·2·3**	45
	1·5P·3	43
	1·2·6P	44
two functional moieties	**1·5P·6P**	40
	4P·2·6P	36
	4P·5P·3	39
three functional moieties	**4P·5P·6P**	34
one functional moiety	**7P·2·3**	43
	1·8P·3	43
	1·2·9P	44
two functional moieties	**1·8P·9P**	37
	7P·2·9P	38
	7P·8P·3	41
three functional moieties	**7P·8P·9P**	40



- Slight decrease of thermal stability and **reduction of the hysteresis** by increasing the number of fatty acid appendages
- The position and the relative distance between lipidated residues does not affect thermal stability and folding rate of triple helices significantly

4 Temperature Jump Experiments



monitored by CD spectroscopy

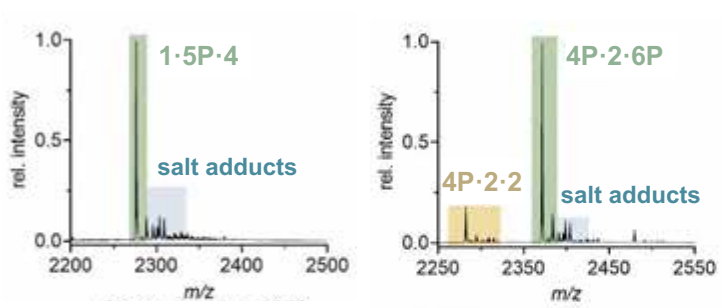
		$t_{1/2}$ [h]
unfunctionalized	**1·2·3**	16
one functional moiety	**1·2·6P**	1.8
two functional moieties	**1·5P·6P**	0.4
three functional moieties	**4P·5P·6P**	0.7

40-fold decrease

- Shortest $t_{1/2}$ and fastest folding for the **difunctionalized** triple helix

5 Native ESI-MS Measurements

Example of analysis of two different samples



- Confirmed formation of all desired heterotrimers
- All mixtures except **4P·2·6P** and **7P·8P·3** assembled with high selectivity

6 Conclusion

- Functionalization of CMPs with palmitic acid does not increase the thermal stability of collagen heterotrimers.
- Addition of more than one palmitic acid appendage allows for a faster assembly of individual strands into a triple helix.
- Thermal stability and folding rate of triple helices are independent of the position of the lipidated residue within the triple helix.

References

1. M. D. Shoulders, R. T. Raines, *Annu. Rev. Biochem.* **2009**, *78*, 929.
2. J. Egli, C. Siebler, M. Köhler, R. Zenobi, H. Wennemers, *J. Am. Chem. Soc.* **2019**, *141*, 5607.
3. J. Egli, C. Esposito, M. Muri, S. Riniker, H. Wennemers, *J. Am. Chem. Soc.* **2021**, *143*, 5937.
4. N. B. Hentzen, V. Islami, M. Köhler, R. Zenobi, H. Wennemers, *J. Am. Chem. Soc.* **2020**, *142*, 2208.
5. V. Islami, P. Bittner, T. Fiala, N. B. Hentzen, R. Zenobi, H. Wennemers, *J. Am. Chem. Soc.* **2024**, *146*, 1789.

Swiss National Science Foundation

Let's connect!