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Modulating peptide hydrogel strength through halogenation

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Introduction

Peptide-based hydrogels are promising delivery matrices for controlled-release. In such systems, specific peptides can self-assemble into fibers driven by non-covalent interactions (e.g. hydrogen bonding, ionic interactions, pi-pi stacking and Van der Waals interactions), a process followed by fiber entanglement, which eventually leads to the formation of the final hydrogel network. Thanks to the non-covalent nature of the fibers and their entanglements, the resulting gels are injectable (i.e. they present thixotropic or shear-thinning behavior). Fmoc-Phe and related molecules have been widely exploited as self-assembling hydrogelators. It has been found that the assembly properties of these molecules can be profoundly enhanced by the incorporation of various substituents, including halogen atoms, on the side chains. Halogenated Fmoc-Phe derivatives have a much higher propensity for spontaneous self-assembly into hydrogel fibril networks.[1,2] Similarly, halogenation of other peptide-based hydrogels has shown that these modifications can improve peptide hydrogel strength.[3,4]

Results & Discussion

Recently, we designed a new family of short amphipathic peptide-based hydrogels, which form thixotropic injectable hydrogels upon dissolution in aqueous solutions.[5,6] Based on theoretical calculations (i.e. Non-Covalent Interaction calculations[7]), the use of halogenated phenylalanines and tryptophan analogues were suggested to improve the strength of our peptidic hydrogels. The 'parent' structure in this effort is the following sequence: H-FQFQFK-NH_2. To verify whether halogenation would also impact the material properties of this sequence, a library of 14 halogenated phenylalanine-containing peptides was synthesized *via* SPPS using standard Fmoc-chemistry. The initial halogenated hydrogelator sequences were chosen in accordance with the calculated interaction energies of the distinctly substituted phenylalanines. Since meta-halogenated Phe residues (with X = Cl, Br, I), as well as the ortho-para "couple", were calculated to present the highest noncovalent interactions, sequences incorporating such amino acids were prepared. All peptides were made in good yields, whereafter gelation in saline solution and phosphate buffer solution (PBS) could be checked at weight percentages of 1% and 2%.

Beside the use of halogenated phenylalanines, the influence of halogenated tryptophans was also of interest. In contrast with the halogenated phenylalanines, the halo-tryptophans are not commercially available and therefore need to be synthesized. Based on the NCI calculations, we started with the synthesis of 4- and 7-halo-L-tryptophans. The 4-halo indole is synthesized according to the Leimgruber-Batcho method and the 7-halo indole, according to the work of Bartoli and coworkers.[8,9]

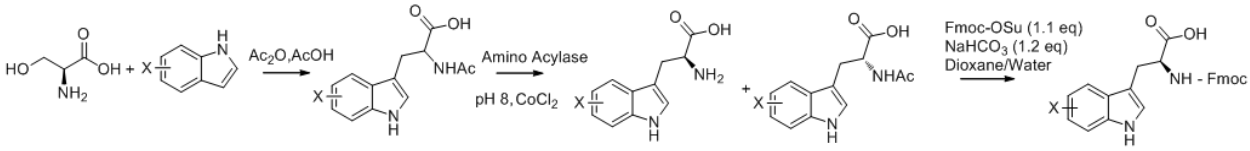

Figure 1: Synthesis of Fmoc-halo-L-tryptophans

Subsequently, the halo-tryptophans are chemically synthesized *via* a described condensation reaction, followed by enzymatic resolution.[10] Finally, the amine is Fmoc-protected in order to obtain a SPPS compatible building block. The 4- and 7-haloindole synthesis proved successful for bromine, chlorine and iodine. The Fmoc-protected brominated and chlorinated tryptophans were synthesized in moderate overall yields (3 steps, 15-29%), whereby enzymatic resolution can only present a maximum yield of 50%. The Fmoc-7-bromo-L-tryptophan and Fmoc-4-chloro-L-tryptophan were introduced into the parent hexapeptide in good yield, and subsequently, the synthesized tryptophan-containing peptide formed a hydrogel both in PBS and saline solution at weight percentages of 1 and 2%.

From a qualitative analysis (i.e. vial inversion test) it was already clear that the halogenated phenylalanines enhanced the rigidity of the hydrogels. Subsequently, quantitative results were needed to validate the material properties of the peptide hydrogels. Therefore, dynamic rheometry was applied. The following procedure was used to determine the G', G" and tan δ parameters. First a pre-shear was performed to disrupt the hydrogel. Followed by a time sweep where the gel can recover over a period of one hour. Subsequently, a frequency sweep is performed to determine if tan δ is independent of the frequency. Finally, a strain sweep determines at which strain the peptide hydrogels breaks.

Table 1: Peptide codes, sequences and average G' of the halo-peptide library.

Name	Sequence	G' (Pa)
CM63	FQFQFK-NH_2	605
WV1	FQFQ**F(3Br)**K-NH_2	12 580
WV4	FQ**F(3Br)**Q**F(3Br)**K-NH_2	3 201
WV5	**F(3Br)**Q**F(3Br)**Q**F(3Br)**K-NH_2	3 775
WV6	FQ**F(3Br)**QFK-NH_2	710
WV7	**F(4Br)**Q**F(4Br)**Q**F(4Br)**K-NH_2	2 829
WV13	**F(2Br)**QFQ**F(4Br)**K-NH_2	48 208
WV14	**F(4Br)**QFQ**F(2Br)**K-NH_2	67 952
WV8	FQFQ**F(3I)**K-NH_2	2 651
WV9	FQ**F(3I)**Q**F(3I)**K-NH_2	5 216
WV16	**F(3I)**Q**F(3I)**Q**F(3I)**K-NH_2	3 014
WV10	**F(4I)**QFQ**F(2I)**K-NH_2	1 936
WV15	**F(2I)**QFQ**F(4I)**K-NH_2	14 419
WV11	**F(4Cl)**Q**F(4Cl)**Q**F(4Cl)**K-NH_2	3 650
WV12	FQFQ**F(4Cl)**K-NH_2	2709
WV18	WQWQ**W(7Br)**K-NH2	N.D
WV19	**W(7Br)**QWQ**W(7Br)**K-NH2	N.D
WV20	WQWQ**W(4Cl)**K-NH2	N.D

These rheometry measurements, confirmed that the halopeptides have a higher rigidity, in comparison with the non-halogenated: the reference peptide (FQFQFK-NH_2) has G' of 650 Pa and the library of halogenated phenylalanine-based peptides give values varying from 917 Pa to 67 952 Pa, which is up to a hundred-fold increase in rigidity. The time sweep suggests that after one hour the hydrogel network is still gaining rigidity. Although the halopeptide hydrogels have a higher rigidity, it is observed that they break more easily because of the lower stress point looking at the strain sweep. The two peptide sequences which stand out are the partially brominated ortho-para "couples" [i.e. F(o-Br)QFQF(p-Br)K-NH_2 and F(p-Br)QFQF(o-Br)K-NH_2]. According to the present study, the theoretically calculated NCI energies give a good indication of which peptides can indeed lead to stronger gels.

Conclusion

Herein, we have conducted material studies on the effect of halogen substitution on the aromatic side chain of Phe and Trp amino acids. It has been discovered that halogen identity and ring position both exert a profound effect on self-assembly rates and on the mechanical properties of the resulting hydrogels. These results clearly demonstrate that subtle, single-atom perturbations amino acids can be used to tune the self-assembly and hydrogelation propensity of these peptide hydrogels.

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