Design and Synthesis of Self-Assembling Peptides for Hydrogel Formation

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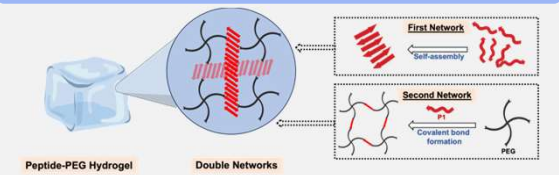
Abstract

Peptides are adaptable structures that can be used to create a variety of nanostructures, which can then be used to create hydrogels and nanoparticles. The characteristics of peptide-based hydrogels are outstanding qualities such as tissue-like flexibility, biodegradability, high water absorption capacity, injectability, and mechanical stability. Peptide-based hydrogels are therefore the most intriguing for biological applications and diagnostic research, such as drug transport, cellular engineering, regenerative medicine, and biomedicine. Many self-assembling peptides, however, are unable to create strong hydrogels that are appropriate for use in biomedicine. In this work, we present the synthesis and design of a self-assembling peptide that can conjugate with PEG to generate a stable hydrogel. The peptide has an amphiphilic region that facilitates self-assembly into β-sheet nanofibers and contains lysine residues to improve solubility in aqueous solutions. A solid-phase peptide synthesizer was used to create the peptides, and HPLC was used to purify the final product. The β-sheet secondary structure of the peptide was revealed by CD spectroscopy, and MALDI was used to confirm the successful synthesis. These peptides will be employed in further studies to generate PEG-peptide conjugates, which will facilitate the production of strong hydrogels appropriate for a variety of biological uses.

Introduction



Scheme 1. Schematic representation of peptide-PEG hydrogel formation.

Peptide-based hydrogels showcase a variety of applications as a result of their incredible qualities such as biocompatibility, biodegradability, injectability, and tunable mechanical stability. With potential biological applications in cellular engineering, drug transport, regenerative medicine, and more, the successful design and synthesis of self-assembling peptides capable of forming hydrogels compatible with biomedical applications becomes imperative. Previously, peptide-based hydrogels relied on non-covalent networks at high concentrations; however, the storage moduli for these hydrogels were relatively low. In response, assemblies with high storage moduli were developed and required significant acidity, rendering them incompatible with biomedical applications. Therefore, this experiment incorporated a double network formation by employing covalent N-hydroxysuccinimide (NHS) coupling reactions to generate peptide-PEG conjugates, thus enhancing the storage moduli of the hydrogels while ensuring biological compatibility. This approach is anticipated to open avenues for developing peptide-based hydrogels with customized rheological characteristics and varied biological capabilities, suitable for a wide array of biomedical uses.

Synthesis and Purification

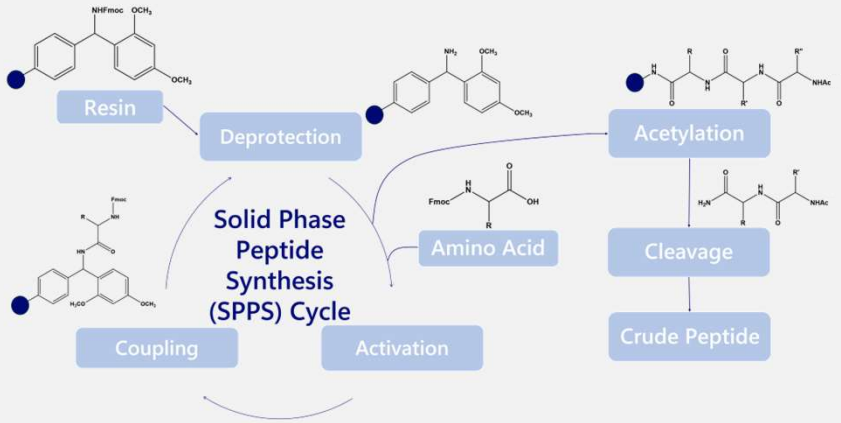
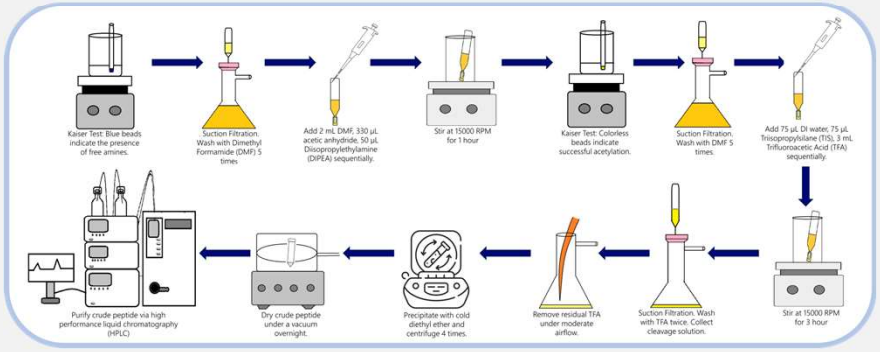


Figure 4. Standard Fmoc-Solid Phase Peptide Synthesis (SPPS) Cycle procedure used for this study.



Scheme 2. Detailed methodology containing acetylation, cleavage, and purification of synthesized peptide.

Purification: A preparative reverse-phase C4 column with a linear gradient of water/acetonitrile containing 0.05% TFA ran for 35 minutes. Elution was monitored by UV-Vis absorbance measurements at 230 nm and 280 nm with an expected peptide retention time of 16 minutes. Desired peaks were collected and analyzed with MALDI-TOF to confirm successful synthesis and purification. Purified samples were lyophilized for a minimum of two nights before hydrogel formation.

Results

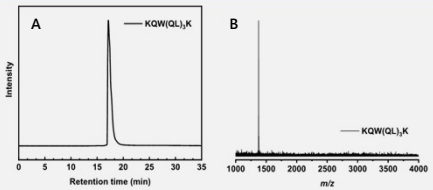


Figure 5. HPLC chromatogram and MALDI mass spectrophotometry showing the successful synthesis (sequence: KQW$(QL)_3$K).

Figure 6. Vial inversion to assess hydrogel strength. **(a)** Synthesized peptide sequence in PBS 1x. **(b)** Synthesized peptide with PEG in PBS 1x.

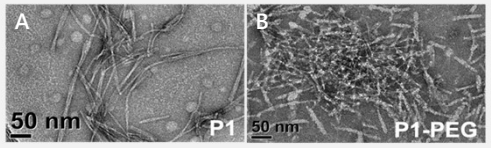


Figure 7. Transmission electron microscope (TEM) images to showcase **(a)** peptide nanofibers of synthesized protein and **(b)** double cross-linking between synthesized protein and PEG to form the hydrogel.

Conclusion

- We have effectively developed a self-assembling peptide sequence suitable for synthesizing peptide-based hydrogels.
- NHS-amine coupling chemistry is employed to fabricate a double network hydrogel, offering vast applications in various fields.
- Key Components for Hydrogel Formation:
  - Lysine-rich self-assembling peptide featuring an alternating hydrophobic-hydrophilic sequence to establish a sandwich-like β-sheet structure.
  - NHS-activated PEG polymer, which interacts with the amine side chains of the peptide.

Future Work

- Assessment of the strength and antimicrobial efficacy of the hydrogel for biological applications.
- Investigation of the potential for extending this method to create double network hydrogels using other amine-containing self-assembling peptides.

Peptide Design

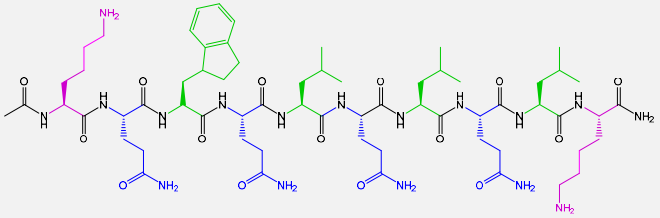


Figure 1. Chemical structure of synthesized peptide (P1 sequence: KQW$(QL)_3$K)

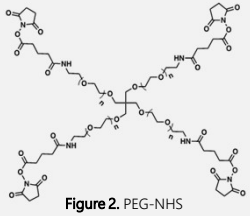


Figure 2. PEG-NHS

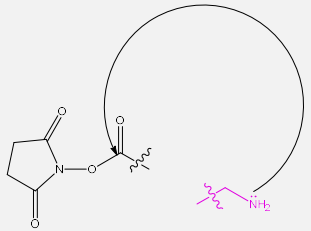


Figure 3. NHS-amine coupling reaction between PEG-NHS and lysine containing peptide sequence..

The primary peptide sequence (P1) was proposed based on the prior investigation to formulate the KQW$(QL)_3$K sequence (Figure 1). The alternating hydrophobic-hydrophilic pattern promotes the formation of sandwich-like nanofibers for self-assembly. Leucine and tryptophan embed within the hydrophobic core due to their hydrophobic properties, while glutamine remains on the outer surface, creating a balance between intermolecular hydrogen bonding and hydrophobic interactions among the repeating units. The presence of lysine improves the solubility of the sequence for hydrogel formation, while tryptophan allows for accurate UV-Vis-based concentration measurements. The second network involves the NHS-activated PEG polymer containing the NHS group that reacts with the lysine sidechains on the peptide sequence to generate the covalent N-hydroxysuccinimide (NHS) coupling reaction (Figure 3).

Acknowledgments

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References

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