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Multifunctional thermoresponsive designer peptide hydrogels

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ABSTRACT

We report the synthesis and characterization of multifunctional peptides comprised of a hydrogel forming β-sheet peptide segment and a matrix metalloproteinase 2 substrate containing a propargylglycinyl linker that is further derivatized with an RGD peptide sequence *via* "click" chemistry. In contrast to currently known systems, these multifunctional peptides formed gels that are stiffer than those formed by their respective precursors. All the peptides showed reversible thermoresponsive properties, which render them as suitable lead systems for a variety of possible biomedical applications.

Statement of Significance

In general, it has been frequently observed that chemical biofunctionalization of peptide hydrogels adversely affects peptide assembly, hydrogel formation or mechanical properties, which severely compromises their application. A functionalization protocol that allows to generate peptide hydrogels that display significantly improved mechanical properties over their unfunctionalized counterparts is reported in this work. These peptides also showed thermoresponsive viscoelastic characteristics, including an example of a peptide hydrogel that displays lower critical solution temperature behaviour.

1. Introduction

The potential application of designed peptide hydrogels as scaffolds in biomedicine has recently attracted much attention [1–3]. However, in order to be able to emulate the characteristics of native matrices, e.g. collagen, peptide hydrogels should elaborate features that could regulate key molecular interactions at the cell-material interface [4]. Thus a designed hydrogel should potentially promote interactions such as that involved in: 1) receptor-ligand complex formation that mediates cell adhesion and migration, 2) molecular interactions that facilitate proteolytic biodegradation or processes that regulate cell differentiation and 3) mechanical signalling with cells [5].

Most reported peptide hydrogels are mechanically soft (G' < 1 kPa) at non-cytotoxic concentrations (<3 wt%) [6]. Furthermore, it is known that the biofunctionalization of peptide hydrogels (e.g. typically through extension at the peptide C-terminus of β-sheet peptide hydrogels) [7] has a detrimental effect on peptide assembly, hydrogel formation and mechanical properties [8,9]. While several reports indicate that matching the mechanical properties of a peptide hydrogel to those of the corresponding natural matrix might not be required for cell support applications [6,10], it is important to have systems which at least retain their mechanical properties upon biological functionalization. Two approaches have been followed when trying to accomplish this task. These involve mixing unmodified peptides with their corresponding biofunctionalized derivatives so that the former functions as a template for hydrogel formation [10–12], and the design of peptide hydrogels with bioactive sequences that do not disrupt but rather integrate into the assembled peptide structure [13,14]. The first of these two approaches has shown promise but material inhomogeneity presents a major drawback. The

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applicability of the second strategy is limited by the bioactive peptide sequence e.g. it is rare to find a β-sheet-inducing bioactive sequence that can be inserted into a β-sheet peptide hydrogel. Therefore, further peptide derivatization strategies need to be developed.

Designed peptide hydrogels comprise a peptide sequence which self-assembles into a defined secondary structure (typically β-sheets). This assembly leads to the formation of fibrils/fibres that further entangle to form a supramolecular network, which entraps large amounts of water [15]. Peptide assembly is typically triggered by external stimuli such as ions, pH and temperature [16,17]. The design of temperature triggered (also called responsive) hydrogels is of interest in biomedicine as they can be applied for thermally-controlled drug delivery [18] and for *in situ* gelling scaffolds for tissue regeneration [19]. Thermoresponsive hydrogels include two categories, positively and negatively responsive systems, which are identified by having an upper critical solution temperature (UCST) or a lower critical solution temperature (LCST), respectively [20]. Examples of these types of thermoresponsive peptide hydrogels can be found in the literature. For instance, elastine type gels, inspired by the amino acid sequence of natural elastin (composed of repeats of the pentapeptide sequence Val-Pro-Gly-X-Gly, where X is any amino acid except Pro), exhibit a transition from solution to an aggregated state when the temperature exceeds a critical limit [21]. The thermoresponsive properties and biomedical applications of hydrogels composed of block copolymers of polyethylene glycol (PEG) and poly(Ala, Ala/Phe, Ala/Leu, etc.) have been also extensively studied [22–25]. These types of systems present a sol-to-gel transition as the temperature increases, which is attributed to micellar aggregation due to conformational changes of the peptide from random coils to β-sheets. However, reports of thermoresponsive designer peptide hydrogels that maintain their mechanical properties upon functionalisation are lacking.

An understanding of the correlation between the structure and the mechanical properties of designer multifunctional peptide hydrogels is relevant because there is a dearth in the literature of methodologies that allow one to generate multifunctional peptide constructs that maintain the mechanical properties of the parent hydrogel peptide.

In this work, we describe the synthesis, biofunctionalization *via* "click" chemistry and characterization of multifunctional peptide hydrogels. These peptides consist of a β-sheet peptide hydrogel forming unit (HβP), a matrix metalloproteinase (MMP) 2 peptide sequence substrate (MMP2s) and an RGD adhesion moiety (Scheme 1). Interestingly, the fully biofunctionalized peptides formed gels that are stiffer than those formed by their precursors and in addition were thermoresponsive. A model for the molecular structure of the peptides that is consistent with these properties is proposed.

2. Experimental

2.1. Materials and general protocols

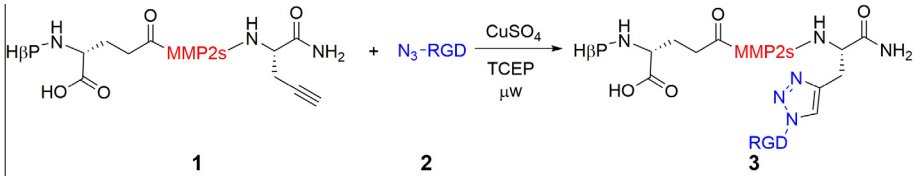
All solvents and reagents were used as received without further purification. Conventional Fmoc-protected amino acids, Fmoc-rink amide linker and O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) were purchased from GL Biochem (Shanghai, China). *N*,*N*′-Dimethylformamide (DMF) (AR grade) and acetonitrile (HPLC grade) were purchased from Scharlau (Barcelona, Spain). Trifluoroacetic acid (TFA) was purchased from Oakwood chemicals (West Columbia, SC). *N*,*N*′-Dii sopropylcarbodiimide (DIC), *N*,*N*-diisopropylethylamine, *N*-methylmorpholine (NMM), piperidine, triisopropylsilane (TIPS), Brij-35 and 4-aminophenylmercuric acetate were purchased from Aldrich (St Louis, USA). Tris(2-carboxyethyl)phosphine was purchased from AK Scientific, Inc (California, USA). The aminome thyl-chemmatrix®resin was purchased from PCAs BioMatrix, Inc. (Quebec, Canada) and the aminomethyl polystyrene resin was purchased from Rapp Polymer GmbH (Tübingen, Germany). The MMP2 protein was purchased from Acrobiosystems (Newark, USA). TCNB buffer pH 7.4 was prepared fresh and consists of Tris (50 mM), $CaCl_2$ (10 mM), NaCl (100 mM) and Brij-35 (0.05%, w/v).

Microwave-assisted reactions were performed using a CEM Discovery System (NC, USA). Peptide characterization was done by HPLC-UV-ESI-MS in positive mode (Agilent 1120 compact LC system equipped with Agilent 6120 Quadrupole MS and a UV detector at 214 nm (Palo Alto, CA, USA) using an Eclipse XDB-C8 column (5 μm; 4.6 × 150 mm; Agilent), unless otherwise indicated, at a flow rate of 0.3 mL·min^{-1} and a linear gradient (ESI). The solvent system consists of A (0.1% formic acid in H_2O) and B (0.1% formic acid in MeCN), unless otherwise indicated. Peptide purification was performed by semi-preparative RP-HPLC (Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector (Milford, MA, USA)) using an X-Terra Prep MS C18 ODB (10 μm, 19 × 300 mm), a 0.5% B·min^{-1} linear gradient from 5% B to 85% B and a 10 mL·min^{-1} flow rate at room temperature. The solvent system consisted of A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN). TEM images were analyzed with ImageJ [26].

2.2. Peptide synthesis

2.2.1. β-Sheet peptide hydrogel-forming segment fused with the MMP2 substrate sequence

Peptide synthesis was performed *via* the Fmoc/tBu strategy on an aminomethyl-Chemmatrix resin functionalized (0.62 mmol·g^{-1}) with a Fmoc-Rink amide linker using a PS3 Synthesiser (Tucson, AZ, USA) on either a 0.1 or 0.05 mmol scale. The Fmoc group was deprotected with 20% v/v piperidine in DMF 2 × 5 min at room temperature. Amino acid couplings were



HβP = H-Pro-Glu-Glu-Leu-Xaa^5-Leu-Glu-Leu-Xaa^9-Leu-Glu-Pro-
MMP2s = –Ile-Pro-Glu-Ser-Leu-Arg-Ala-Gly-
RGD = –Gly-Arg-Gly-Asp-Ser-Pro-NH_2

	Xaa^5	Xaa^9	Hydrogel
1a	Glu	Glu	No
1b	Glu	Lys	Yes
1c	Lys	Lys	Yes
3b	Glu	Lys	Yes
3c	Lys	Lys	Yes

Scheme 1. Structure and biofunctionalization of the multifunctional peptide discussed in this paper.

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