Biomaterials 30 (2009) 1339–1347

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Macromolecular diffusion and release from self-assembled β-hairpin peptide hydrogels

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ARTICLE INFO

Article history:
Received 25 August 2008
Accepted 9 November 2008
Available online 18 December 2008

Keywords:
Hydrogel
Delivery
Self-assembly
Peptide

ABSTRACT

Self-assembling peptide hydrogels are used to directly encapsulate and controllably release model FITC–dextran macromolecules of varying size and hydrodynamic diameters. MAX1 and MAX8 are two peptide sequences with different charge states that have been designed to intramolecularly fold and self assemble into hydrogels at physiological buffer conditions (pH 7.4, 150 mM NaCl). When self-assembly is initiated in the presence of dextran or protein probes, these macromolecules are directly encapsulated in the gel. Self-diffusion studies using fluorescence recovery after photobleaching (FRAP) and bulk release studies indicate that macromolecule mobility within, and release out of, these gels can be modulated by varying the hydrogel mesh size. The average mesh size can be modulated by simply varying the concentration of a given peptide used to construct the gel or by altering the peptide sequence. In addition, results suggest that electrostatic interactions between the macromolecules and the peptide network influence mobility and release. Depending on probe size, release half-lives can be varied from 8 h to over a month.

1. Introduction

Advances in genomics, proteomics, and cell biology have led to the rapid development of small protein and antibody therapeutics [1–5]. Growth factors, hormones, enzymes, cytokines, and monoclonal antibodies have been developed for a range of ailments such as cancer, autoimmune diseases, and metabolic disorders [1,3,6–8]. Although the number of biopharmaceuticals approved and in advanced clinical testing continues to expand, several limitations remain in the preparation and administration of these drugs [9–11]. For example, the susceptibility of proteins to physical and chemical degradation during storage and proteolytic degradation upon administration can severely limit the therapeutic efficacy; the half-lives, $t_{1/2}$, of many protein-based therapeutics range from only 2 to 100 min [12]. To address this problem, hydrogel materials have been used as carriers and delivery devices for protein-based drugs [13–16]. Some hydrophilic gels provide a suitable environment to enhance macromolecule stability and help limit enzymatic degradation during delivery. Encapsulation of the protein therapeutic within the hydrogel can also enable prolonged activity if the rate of release of the drug can be controlled.

Hydrogel matrices for protein drug delivery have been prepared from both naturally derived (collagen, hyaluronic acid, chitosan, alginate) and synthetic (polyethylene oxide, polyacrylamide, polyvinyl alcohol) polymers [17–19]. A promising approach for the design of hydrogels is the use of self-assembling materials in which non-covalent interactions between molecules drive the assembly and formation of supramolecular networks that define the material [20,21]. Using peptides as building blocks for self-assembly allows one to make sequence specific modifications at the molecular level that ultimately influence the bulk properties of the self-assembled hydrogel. Therefore, changes to the peptide sequence, which are accomplished easily by solid phase methodology, can be used to quickly fine tune material properties, such as crosslink density, mesh size, hydrophilicity/electrostatics, and degradation rate [22–24]. All of these factors influence the release profile of a therapeutic that has been encapsulated into the gel. An additional benefit of using self-assembled gels is realized during the formulation of the drug-loaded material. In principle, precise concentrations of therapeutics can be directly encapsulated into the gel network during the self-assembly process. This is in contrast to methods that diffuse the drug into a preformed gel where the ultimate concentration of encapsulated therapeutic may be difficult to control. Herein, we report the application of self-assembled peptide hydrogels to encapsulate and controllably release model biomacromolecules. Experiments indicate that macromolecule

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doi:10.1016/j.biomaterials.2008.11.019

mobility within and release out of these gels can be modulated by varying the hydrogel mesh size. The average mesh size can be modulated by simply varying the concentration of peptide used to construct the gel or by making changes to the peptide sequence. In addition to mesh size, results show that electrostatic interactions between the macromolecule and the peptide network influence the release profile.

We have designed a class of self-assembling peptides that undergo triggered hydrogelation in response to physiological pH and salt conditions (pH 7.4, 150 mM NaCl) to form mechanically rigid, viscoelastic gels [25–28]. The parent peptide, MAX1, consists of 20 amino acids, eight of which are lysine residues. When dissolved in low ionic strength ($\leq$10 mM NaCl) aqueous solutions at pH 7.4, MAX1 remains unfolded and soluble mainly due to electrostatic repulsion between the positively charged lysine side chains. However, when a physiological relevant concentration of NaCl (150 mM) is added, this charge is effectively screened and the peptide folds into an amphiphilic β-hairpin. The hairpin is composed of two β-strands of alternating lysine and valine residues connected by a four residue type II′ β-turn, [25,29,30] Fig. 1. The lysine-rich face of the hairpin is hydrophilic and the valine-rich face is hydrophobic. The folded hairpin is highly amenable to self-assembly, ultimately forming a network of β-sheet rich fibrils. MAX1 self-assembles laterally forming a network of hydrogen bonds along the long axis of a given fibril. The peptide also assembles facially, burying the hydrophobic valine-rich face from water, forming a bilayer. Thus, each fibril is composed of a bilayer of hairpins where the surface of the fibril displays positively charged lysine side chains. Transmission electron microscopy (TEM) and small angle neutron scattering (SANS) show that in the long portions of the fibrils, hairpins facially assemble in a manner in which all of their β-strands are in register with each other and the maximal amount of valine side chain surface area is shielded from water [27,29,31]. However, imperfections in this mechanism where the face of one hairpin is rotated relative to its partner in the bilayer can occur. This results in a site for nascent fibril growth in a new three-dimensional direction and constitutes the formation of an interfibril crosslink, Fig. 1. In addition to the interfibril crosslinks, at sufficient peptide concentrations, entanglements of fibrils also contribute to the mechanical rigidity of the gel.

A convenient way to control mesh size of the gel is to simply increase the peptide concentration used to prepare the gel. The additional peptide leads to the formation of more fibrils that entangle and crosslink into the network, providing gels with smaller mesh sizes.

A second method to control mesh size involves modulating the rate of gel formation. In general, the kinetics of peptide folding and self-assembly dictates the number of crosslinks that are formed during the material formation process [25,27]. In turn, the number of crosslinks directly influences the mechanical rigidity and mesh size of the gel. Slow kinetics of assembly lead to less rigid gels with fewer crosslinks and a larger average mesh size. Fast kinetics lead to a more rigid gel containing more crosslinks and a smaller average mesh size. Smaller mesh sizes will more effectively hinder macromolecular diffusion during delivery.

For the β-hairpin peptides described herein, it is possible to control the folding and self-assembly kinetics to form hydrogels with different mesh sizes. For example, making changes to the peptide sequence can directly influence the rate of peptide folding and self-assembly. MAX8, a design descendent of MAX1, contains a point substitution on the hydrophilic face of the hairpin where one lysine at position 15 is replaced with a glutamic acid [27]. This simple substitution reduces the overall charge state of the peptide to about +7 at physiological pH. For comparison, MAX1 contains eight lysine residues and an N-terminal ammonium group leading to a charge state of about +9 at pH 7.4. Since MAX8 has a lower amount of positive charge to be screened, it folds and self-assembles much faster than MAX1 in response to similar buffer conditions [27]. Therefore, at identical peptide concentrations and solution conditions, MAX8 will form more rigid gels that contain more crosslinks, resulting in smaller mesh sizes. As will be shown, one can vary either peptide sequence or peptide concentration or a combination of both to modulate hydrogel mesh size, which directly influences the release profiles of encapsulated therapeutics.

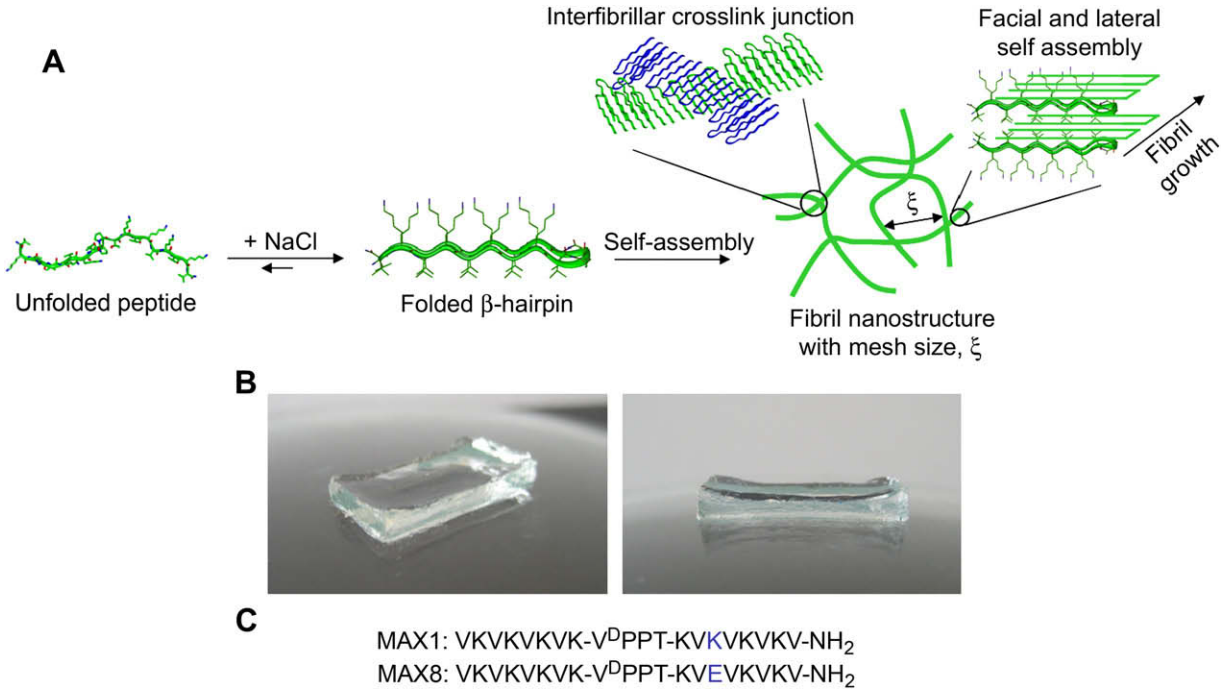

Fig. 1. (A) Proposed mechanism for the folding and self-assembly of MAX hydrogels. (B) Images of 1 wt% MAX8 hydrogels. (C) Peptide sequences of MAX1 and MAX8.