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Research review paper

From short peptides to nanofibers to macromolecular assemblies in biomedicine

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ABSTRACT

In the last few years, a variety of self-assembling short peptides that consist exclusively of simple amino acids have been designed and modified. These peptides exhibit self-assembling dynamic behaviors. At the molecular structural level, they form α-helical, β-sheet and β-hairpins structures in water. These structures further undergo spontaneous assembly to form nanofibers which aggregate into supramolecular scaffolds that entrap large volumes of water. Furthermore, nanostructures and supramolecular structures that self-organized from these short peptides also have a broad spectrum of biotechnological applications. They are useful as biological materials for 2D and 3D tissue cell cultures, regenerative and reparative medicine, tissue engineering as well as injectable drug delivery matrices that gel *in situ*. We have endeavored to do a comprehensive review of short peptides that form nanofibrous hydrogels. In particular, we have focused on recent advances in peptide assembly motifs and applications.

Contents

1. Introduction

Peptides are versatile building blocks for fabricating supramolecular architectures. Their ability to adopt specific secondary structures, as prescribed by amino acid sequence, provides a unique platform for the design of self-assembling biomaterials with hierarchical three-dimensional (3D) macromolecular architectures, nanoscale features and tunable physical properties. To date, synthetic membranes, multilamellar structures, amphiphilic micelles, tubules and fibrillar networks have been obtained from the self-assembly of various peptide motifs (Zhang, 2003). In this review, we will focus on recent advances in the design of short peptides that self-assemble into nanofibrous networks capable of entrapping water—hydrogels.

Through probing various protein motifs found in nature, scientists have been able to elucidate the molecular interactions that govern peptide self-assembly. Peptide self-assembly is highly specific—the intermolecular interactions such as hydrogen bonding, ionic, electrostatic, hydrophobic and van der Waals interactions are mediated by molecular recognition. This understanding of molecular and structural biology has inspired the design and synthesis of increasingly complex self-assembled biomaterials for biomedicine and bionanotechnology. By engineering the amino acid sequence, the secondary structure of peptides

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(β-sheets, β-hairpins and α-helices) can be manipulated to optimize the interactions between adjacent peptides. Long-range organization of peptide monomers produces nanofibrils which aggregate into 3D fibrous networks.

From self-assembly motifs derived from naturally occurring proteins, scientists have moved towards designing *de novo* short self-assembling peptides that are amendable to functionalization. Functionalized hydrogels have been developed for various biomedical applications. In the latter part of this review, we have highlighted recent progress made in applying short self-assembling peptide hydrogels to the delivery of bioactive therapeutics and as biological scaffolds in regenerative medicine.

2. Peptide motifs that favor self-assembly to nanofibrous hydrogels

2.1. β-Sheet peptides

Pioneering work by Zhang in the early 1990s, a serendipitous discovery of a natural protein motif that self-assembled in water, utilized β-sheet peptide motifs as peptide scaffolds. The first member of this class of soft biomaterials, AEAK16-II (AEAEAKAKAEAEAKAK), was serendipitously discovered in a yeast protein, Zuotin (Zhang et al., 1993). Subsequently designed members are characterized by periodic repeats of ionic hydrophilic and hydrophobic amino acids. This motif causes the peptides to fold into β-sheet secondary structures with distinct hydrophobic and hydrophilic surfaces (Zhang et al., 1993) (Fig. 1A). During assembly in aqueous conditions, the hydrophobic alanines form overlapping hydrophobic interactions, while on the hydrophilic aspect, positive and negative charges of adjacent peptides pack together through intermolecular ionic interactions in a checkerboard-like manner. Consequently, the β-sheets stack to form nanofibers of approximately 10 nm in diameter as illustrated in Fig. 1B. The nanofibers aggregate into scaffolds that are extremely hydrated, containing more than 99% water (5 to 10 mg/mL w/v of peptide in water). The propensity for self-assembly into nanofibers is retained when the L-amino acids are replaced with the corresponding D-chiral isoform, although peptides consisting of hetero-chiral amino acids can only form non-structured nano-aggregates (Luo et al., 2008a, 2010). This suggests that β-sheet self-assembly requires homo-chirality. Hydrogel formation is also influenced by peptide sequence, concentration, and salt concentration. In the 16-amino acid peptides RADA16-I (Ac-RADARADARADARADA-NH_2) and RADA16-II (Ac-RARADADARARADADA-NH_2), arginine and aspartate residues (substituting lysine and glutamate in AEAK16) facilitate nanofiber scaffold formation in the presence of salts. By substituting alanine with isoleucine (in IKIE), valine (in VKVE) or phenylalanine (in FKFE), peptides with more hydrophobic residues are formed, and required lower critical concentrations for β-sheet formation. Reducing the number of repeats from 4 to 2, as demonstrated by comparing 16-amino-acid peptides with 8-mers, also lowers the critical gelation concentration (Ulijn and Smith, 2008). High salt concentrations inhibit gelation by masking the charges on the β-sheet while low salt conditions enhance gelation by limiting random interactions at low peptide concentrations. In general, the resulting β-sheet structures are stable across a broad range of temperature, wide pH ranges in high concentration of denaturing agent urea and guanidium hydrochloride. Interestingly, mechanical disruption by sonication disrupts the macromolecular structure temporarily but not the supramolecular β-sheet structures. The longer micron-length $RADA_4$ fibers reassemble after 2 hours, demonstrating

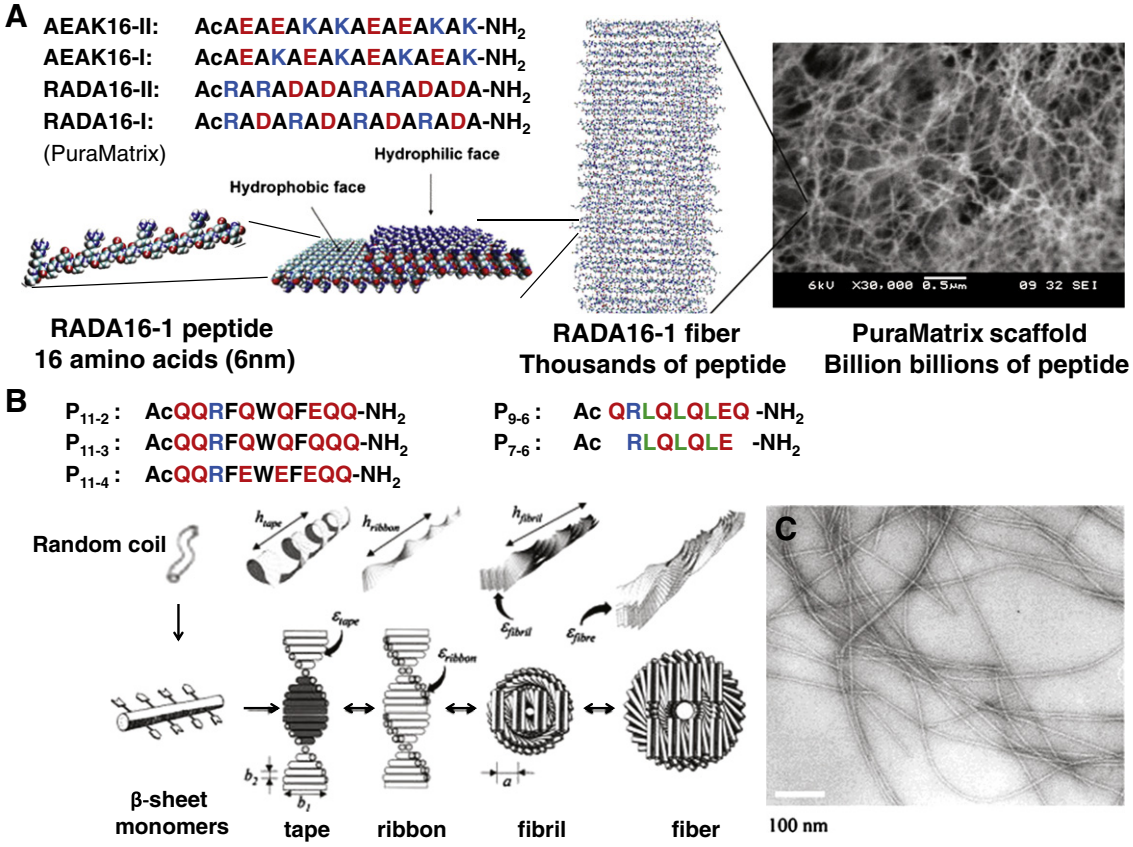

Fig. 1. (A) β-Sheet forming short peptides with alternating ionic complementary properties: peptide sequences of 4 β-sheet 16-mer peptides, including the commercially available RADA16-I (PuraMatrix™). Structure and assembly of RADA16-1 peptide into fibers and nanofibrous scaffolds (electron microscopy image of RADA16-I is shown). (B) Short amphiphilic β-sheet peptides that self-assemble into anti-parallel nanotapes and further aggregate into ribbons and higher order structures. In a recent paper, shorter sequences (P_{9-6} and P_{7-6}) with aliphatic hydrophobic resides (in green) were demonstrated to form fibrillar structures. (Reproduced with permission from reference (Aggeli et al., 2001).) (C) Transmission electron micrograph of a P_{11-4} gel in water (6.3 mM, pH 3) showing semirigid fibrils and fibers. (Reproduced with permission from reference (Aggeli et al., 2003). Copyright 2003 American Chemical Society.)