Thermally and Photochemically Triggered Self-Assembly of Peptide Hydrogels

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Self-assembling systems present attractive platforms for engineering stimulus-responsive materials with controlled nano- and microstructures. Recent efforts to design such systems include synthetic block copolymers,[1a] proteins that self-assemble into hydrogels via leucine zipper motifs,[1b] cyclic peptides that self-assemble into nanotubes,[1c−e] and peptides that form fibrillar β-sheet networks.[1f−i] Here we demonstrate systems that self-assemble from fluid precursors into highly cross-linked peptide hydrogels in response to thermal or photochemical triggering. Our system takes advantage of stimuli-responsive release of encapsulated salts from liposomes to trigger the self-assembly of a 16-amino acid peptide. We show that the induction of self-assembly by gentle warming or by exposure to near-infrared light results in rapid gelation of peptide/liposome suspensions into highly cross-linked, fibrillar, β-sheet hydrogels. The ability to trigger rapid sol−gel transformations of peptide solutions via physiologically benign stimuli (temperature, light) may lead to the development of new injectable materials for drug delivery, wound healing, and tissue engineering applications.

The 16-amino acid peptide consists of alternating hydrophobic and hydrophilic residues: H_2N-$(FEFEFKFK)_2$-COOH, (FEK16). We chose to investigate FEK16 because several related peptides with alternating hydrophobic−hydrophilic residues are known to self-assemble into β-sheet structures, often in an ionic-strength-dependent manner.[1f,2] β-sheet structure is favored by the strictly alternating hydrophobic−hydrophilic primary structure of the peptide, which positions all hydrophobic side chains on one side of the β-sheet and all hydrophilic side chains on the other.[3] Salt-induced self-assembly of this peptide may be driven by the shielding of electrostatic repulsive forces with increasing ion concentrations, allowing attractive hydrophobic and van der Waals forces to dominate.[2d]

The self-assembly characteristics of FEK16 were found to resemble those of other peptides with similar alternating structures.[1f,1i,2b] FEK16 was found to be highly soluble in pure H_2O but formed self-assembled aggregates (gels at FEK16 concentrations >10 mg/mL) in the presence of mM concentrations of NaCl, KCl, and $CaCl_2$. FTIR spectroscopy of FEK16 (30 mg/mL) in D_2O and $CaCl_2/D_2O$ revealed strong amide I absorption peaks at 1622 and 1694 cm^{-1} and an amide II peak at 1525 cm^{-1} (see Supporting Information for IR spectra), indicating a high content of β-sheet structure.[4a−b] A weak band at 1694 cm^{-1} suggested that the β-sheet was antiparallel, although it is also possible for this peak to arise from β-turn or disordered structures.[4b] More detailed structural analysis such as solid-state NMR spectroscopy will be necessary to confirm the antiparallel conformation.[4c] The IR spectra of self-assembled FEK16 induced by $CaCl_2$ addition was nearly identical to that of FEK16 in pure D_2O, suggesting that FEK16 exists predominantly as small β-sheet multimers in the liquid state and that gel formation involves coalescence/aggregation of these preformed β-sheet domains. CD was utilized to assess the concentration dependence of β-sheet formation in H_2O. It was confirmed that FEK16 adopted a β-sheet conformation at concentrations above 10 μM, whereas below this concentration the peptide adopted an α-helical conformation. At low peptide concentrations (<10 μM), addition of $CaCl_2$ induced an α-helix-to-β-sheet transition in FEK16. (See Supporting Information for CD spectra).

When concentrated aqueous solutions of FEK16 (40 mg/mL) were extruded from a micropipet into Dulbecco's phosphate buffered saline (PBS) (Gibco), aqueous NaCl (200 mM) or aqueous $CaCl_2$ (7.5−560 mM), hydrogels were formed within a few minutes. These assemblies maintained the shape of the extruded filament and were strongly stained by Congo red, an indicator of aggregated β-sheet structure.[5] Furthermore, once assembled, these structures did not disassemble upon the addition of excess buffer or upon the exchange of buffer with water. In contrast to its behavior in salt solutions, FEK16 did not form hydrogels or stain with Congo red in water or aqueous sucrose.

Triggered assembly of peptide gels was accomplished by utilizing stimuli-responsive liposomes designed to release salts such as $CaCl_2$ at a specific temperature[6] or in response to near-infrared (NIR) light exposure.[7] $CaCl_2$-containing temperature-sensitive liposomes were prepared by the interdigitation−fusion technique[8] from 9:1 dimyristoylphosphatidylcholine (DMPC): dipalmitoylphosphatidylcholine (DPPC) as described.[6] Light-sensitive liposomes were prepared from 6:4 distearoylphosphatidylcholine (DSPC):diplasmenylcholine (DPPlsCho) containing bacteriochlorophyll as described.[7b] Both light- and temperature-sensitive liposomes were stable for long periods of time at room temperature. Extravesicular sucrose was used to osmotically balance the $CaCl_2$-containing liposomes. DMPC/DPPC liposomes containing 50 mM $CaCl_2$ exhibited a sharp Ca^{2+} release profile when heated to 37 °C (see Supporting Information), a consequence of the increased permeability of entrapped solutes at the gel-to-

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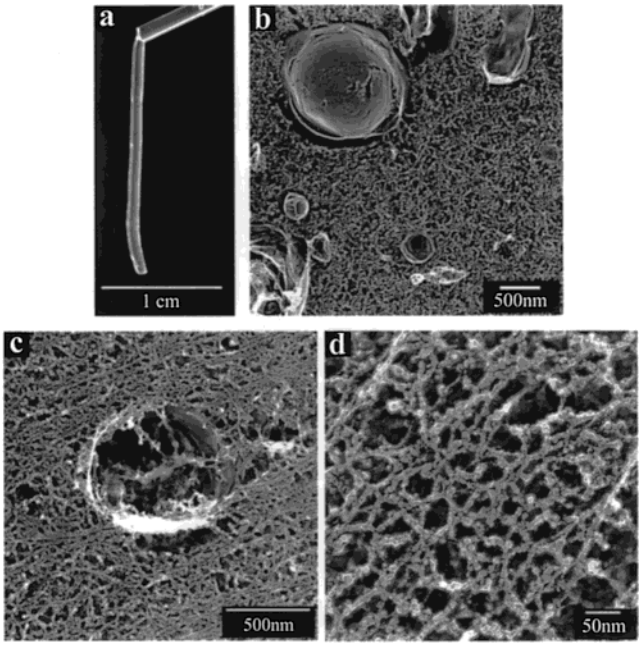

Figure 1. Macroscopic (a) and freeze-fracture TEM (b−d) images of FEK16 gels formed by thermally triggered release of $CaCl_2$ from DPPC/DMPC liposomes.

fluid phase transition temperature of this lipid system.[9] Similarly, light-sensitive liposomes released encapsulated $CaCl_2$ when exposed to NIR excitation (800 nm) under aerobic conditions.[7b]

When $CaCl_2$-loaded DMPC/DPPC liposomes were suspended in aqueous solutions of FEK16 (12−30 vol % liposomes @ 20 mg lipid/mL, 30 mg/mL FEK16 in 57 mM aqueous sucrose) and subsequently warmed from ambient temperature to 37 °C, hydrogels were formed, as seen by macroscopic photography and freeze-fracture TEM (Figure 1). Macroscopically, the formed gels were self-supporting and adopted the shape of their containers (Figure 1a). Microscopically, the hydrogels consisted of a randomly cross-linked network of extended peptide nanofibrils that entrapped the liposomes (Figure 1b,c). Under high magnification, the fibrils appeared to be formed from coalesced globular domains, with approximately 10−50 nm between fibril cross-link points (Figure 1d). Also, the fibrils had diameters of approximately 10 nm, which is similar to the dimensions of β-sheet fibrillar assemblies of related peptides.[1f,i,10] The peptide fibrils entrapped the liposomes within the network (Figure 1b), and in some cases the peptide fibrils appeared to penetrate the liposomal wall (Figure 1c).

Temperature- and light-activated gelation of FEK16/liposome suspensions was detected using oscillating rheometry. As shown in Figure 2, light-sensitive formulations remained fluid in the dark, but gelled rapidly when exposed to NIR light for 5 min, as indicated by a substantial increase in the dynamic storage modulus (G'). Temperature-sensitive formulations exhibited G' values typical of fluids ($\leq$1Pa) for extended periods of time at ambient temperature; however; G' increased similarly by 2−3 orders of magnitude upon heating to 37 °C. Gelation was rapid, occurring within a few minutes for temperature-triggered gelation and within 10−20 min for light-triggered gelation, and G' continued to increase over the course of several hours for both gels. Control experiments indicated that gel formation in both cases was due

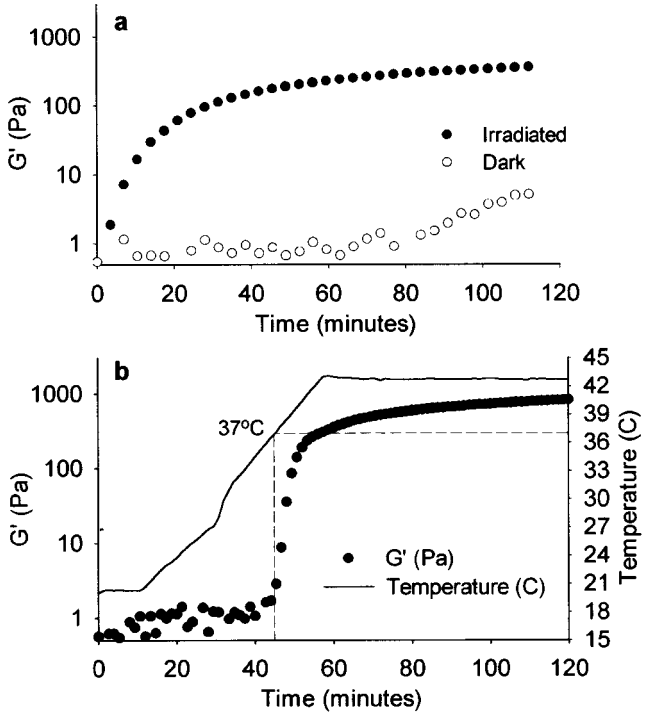

Figure 2. Rheological detection of (a) light- and (b) temperature-triggered gelation of FEK16/liposome precursor fluid (12 vol % $CaCl_2$-loaded liposomes in a sucrose solution of FEK16 (30 mg/mL)).

to triggered release of $CaCl_2$ from the liposomes and subsequent self-assembly of FEK16.

Other self-assembling peptide systems have been investigated recently, some based upon related β-sheet-forming peptides.[1f−i,10,11] Whereas similar peptides such as $(AEAEAKAK)_2$ formed membranous assemblies,[1f] the formation of coherent three-dimensional hydrogels of FEK16 is perhaps due to the more homogeneous introduction of ions in our studies, the use of divalent salts, differing peptide concentrations, or the effect of phenylalanine (vs alanine) on β-sheet structure. By illustrating a new strategy for the rapid induction of peptide gelation by warming to body temperature or by exposure to tissue-penetrating NIR wavelengths, our results may lead to useful new medical materials based on self-assembling peptides. Such an approach may be useful in a broad range of biomedical applications, including injectable in situ gelling materials for controlled drug release, tissue repair, scaffolds for cell delivery, and tissue engineering. FEK16 is an attractive candidate for such applications because similar peptide assemblies appear to be noncytotoxic, cell-adhesive, and noninflammatory.[10,12]

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Supporting Information Available: Materials, detailed experimental methods, FTIR and CD spectra of FEK16, and temperature release profiles of DMPC/DPPC liposomes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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