Modulation of Peptide−Amphiphile Nanofibers via Phospholipid Inclusions

Sergey E. Paramonov, Ho−Wook Jun, and Jeffrey D. Hartgerink*

Departments of Chemistry and Bioengineering, Rice University, 6100 Main Street, MS60, Houston, Texas 77005

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In this communication, we illustrate a new method to modulate the chemical and mechanical properties of peptide−amphiphile nanofibers. Hydrogels containing a mixture of peptide−amphiphile and phospholipid were prepared and evaluated for their mechanical properties, peptide conformation, and nanostructure. It was found that the storage modulus achieved a maximum at 5 mol % phospholipid and that this coincided with the maximum beta sheet signal as observed by circular dichroism. Throughout the ratios of peptide−amphiphile to phospholipid tested, the storage modulus and peptide secondary structure were closely correlated indicating the coupling between molecular structure and macroscopic properties. The nanostructure of the composite fibers was assessed by vitreous ice cryo-TEM and found to be largely independent of the mixture ratio. These new findings will enhance the versatility of peptide−amphiphiles in nanostructured tissue engineering and drug delivery applications.

Peptide−amphiphiles (PAs) represent an attractive class of bioactive molecules as they self-assemble into a variety of nanostructures, many of which have promising biological activity due to the exposed peptide regions on their outer surface.[1] Among the different PAs, ones containing peptides bound to single alkyl tails are especially interesting since the self-assembly of these molecules leads to a formation of a dense network of nanofibers.[2] Self-assembly of these nanofibrous cylindrical micelles is driven in part by formation of a hydrophobic core composed of closely packed alkyl tails. This is further stabilized by formation of β-sheet type interactions between the peptide chains.[3,4] The self-assembly process is reversible, in many cases, and can be triggered by pH change or addition of multivalent cations. These nanofibers readily form hydrogels, which can be subsequently used in tissue engineering and other applications. The peptide region of the self-assembling system offers a rich opportunity for amino acid modifications and incorporation of different biologically active sequences or peptide segments. This has been utilized in several research efforts aimed at construction of mimics of extracellular matrix.[5,6] The hydrophobic core of the nanofiber represents another promising region for further modifications.[7] These modifications may include introduction of small hydrophobic molecules for drug delivery applications or inclusion of other biologically active amphiphiles into PA nanofibers in order to improve the bioactive properties of PA nanofiber networks. In this communication, we introduce phospholipid inclusions that modulate the mechanical properties of the hydrogel in addition to the peptide secondary structure, while leaving the nanostructure largely unchanged. Phospholipids are the essential component of cell membranes and may play an important role in cell−cell and cell−matrix interactions.[8] By introducing phospholipids into PA nanofibers, one may expect to modulate the mechanical properties of the resulting hydrogels as well as increase the accessibility of peptide bioactive regions for cell receptor proteins.

Hydrogels were prepared containing two components, PA and phospholipid, at a variety of molar ratios. The PA was designed and synthesized as previously described[5] containing a hydrophobic alkyl tail for driving the self-assembly into nanofibers, a matrixmetalloproteinase 2 (MMP2) cleavage site for cell-mediated proteolytic degradation and RGDS as a cell adhesive ligand (Figure 1a). 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine was chosen as a representative example of phospholipids containing only one alkyl tail to maximize the favorable hydrophobic interaction between PAs and lipid molecules.[9] All PA−lipid inclusions formed self-supportive hydrogels upon the addition of Ca^{2+} ions. The mechanical properties of hydrogels were evaluated using rheometry. The storage moduli (Figure 2a) were found to be dependent on the amount of lipid incorporated into the PA nanofiber gel. The ratio of storage modulus to the loss modulus (G'/G'') indicated that only mixtures containing less than 20% of lipid maintained hydrogel structure at 20 Hz (Figure SI-3). It was observed that the value of the storage modulus at the maximum frequency varies nonlinearly with the mol % of lipid in the PA nanofiber gels and experiences a maximum at about 5 mol % of the lipid

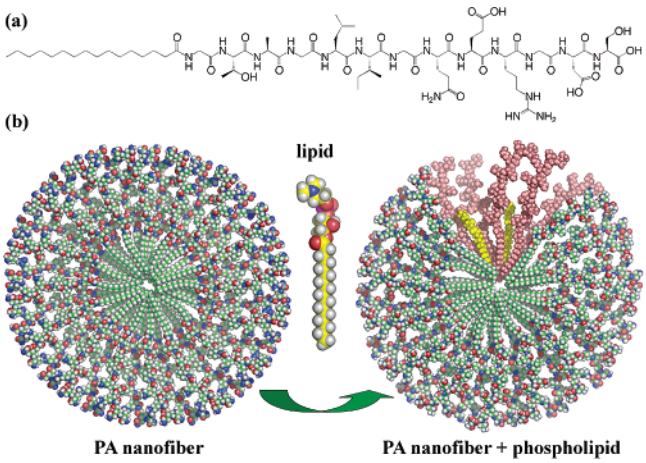

Figure 1. (a) Chemical structure of the PA and (b) cross section of a PA fiber and a PA fiber containing 6.25 mol % of lipid (yellow). Highlighted in pink are the PA molecules situated adjacent to the lipid molecules.

* To whom correspondence should be addressed. E-mail: jdh@rice.edu.

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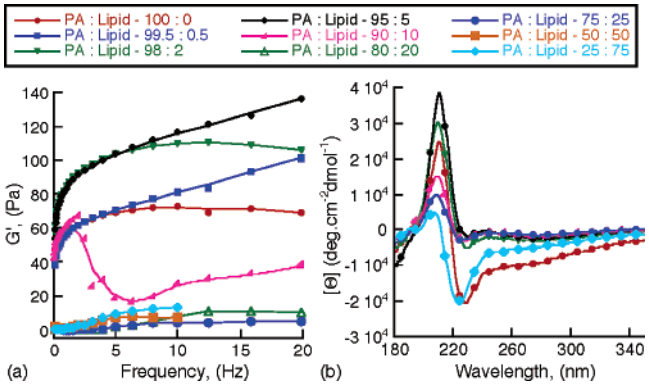

Figure 2. Storage moduli (a) and CD wavelength spectra (b) of prepared PA−lipid gels.

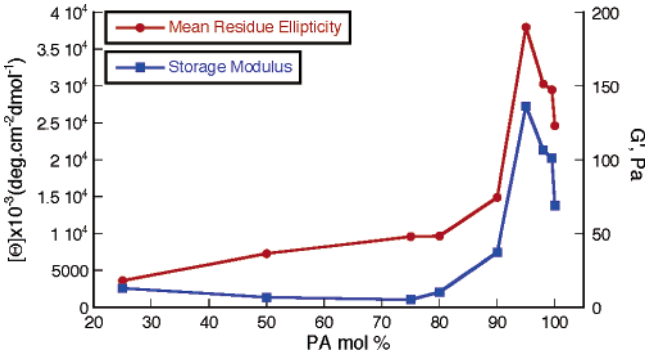

Figure 3. Mean residue ellipticity at 210 nm and storage modulus at 20 Hz. Both properties experience maximum around 5 mol % of the lipid and a rapid decrease at higher lipid concentrations.

followed by a rapid decrease at higher concentration of lipid. This behavior suggests that the lipid molecules incorporate into the PA fiber as opposed to forming phase separated, heterogeneous mixtures. If the lipid molecules were aggregating outside the PA fibers, one may expect the storage modulus to change continuously without a maximum at 5 mol % of the lipid.

The dependence of storage modulus on the mol % of phospholipid led us to believe that the internal organization of the nanofiber may be modified by the phospholipid. This was examined by CD spectroscopy as it is a sensitive technique for peptide secondary structure analysis. CD wavelength spectra (Figure 2b) of prepared PA−lipid gels show a minimum between 220 and 230 nm and a maximum near 210 nm. The shape of the spectra resembles that of the β-sheet structure shifted toward longer wavelengths as has been previously observed for PA systems.[3] The absolute value of the positive and negative peaks varies depending on the amount of lipid in the system. The positive peak increases up to 5 mol % of lipid and then decreases. The same trend is observed in the case of the negative peak. These changes of the CD signal suggest that the introduction of the lipid in the PA system alters to some degree the secondary structure of the PAs and further demonstrates that these lipid molecules are incorporated inside the PA fibers. When mean residue ellipticity at the maximum of the positive peak (210 nm) is plotted together with the storage modulus at the maximum frequency (20 Hz), it reveals a remarkable correlation between the secondary structure of the PAs and the mechanical properties of the hydrogels (Figure 3). Both properties have a maximum around 5 mol % of the lipid followed by a rapid decrease at higher and lower lipid concentrations.

Molecular models of the nanofiber were prepared illustrating the internal organization of the nanofiber without phosolipid

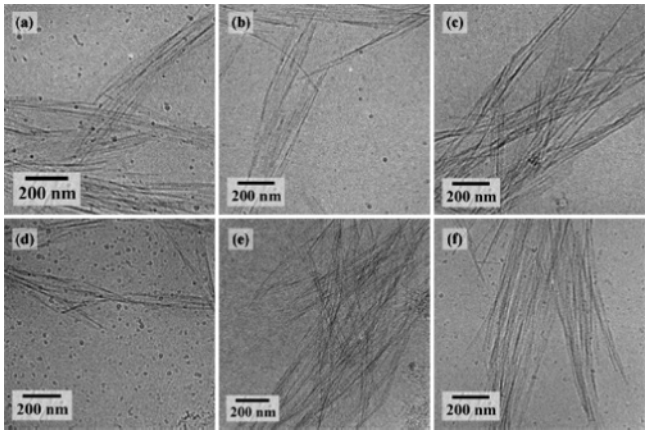

Figure 4. Vitreous ice cryo-TEM images of PA-lipid inclusions: (a) 98:2, (b) 95:5, (c) 90:10, (d) 75:25, (e) 50:50, and (f) 25:75. All materials maintained fibrous nanostructure (for other PA−lipid ratios see the Supporting Information).

and with 5 mol % phospholipid, which corresponds to one lipid molecule per 16 PA molecules in one section of a nanofiber (Figure 1b). The inclusion of lipid molecules affects the geometry of adjacent PA molecules providing additional flexibility for PAs to find their optimal packing and hydrogen bonding conformation. The increase of the storage modulus at 5 mol % may be attributed to the optimization of hydrogen bonding as well as molecular packing of the PA molecules constituting the nanofibers. This can be seen in the CD spectra where in case of 5 mol % inclusion the positive peak reaches its maximum which may occur due to the formation of a more stable β-sheet type structure. Addition of more lipid molecules appears to result in a disruption of the β-sheet network and a dramatic decrease in the storage modulus of the hydrogels. Nevertheless all prepared inclusions form fibers as shown by cryo-TEM (Figure 4). Careful analyses of the obtained images showed no significant dependence of the fiber diameter on the molar ratio of the prepared inclusions. The average diameter was found to be 10.8 $\pm$ 0.8 nm as expected from modeling (Figure 1b). Fiber formation is observed in the entire range of lipid concentrations, though inclusion of more than 20 mol % of lipid destabilizes the hydrogels.

It should be noted that the prepared hydrogels are made out of a supramolecular polymer where the nanofiber is composed of noncovalently bonded molecules brought together by hydrophobic interactions and further stabilized by hydrogen bonding. The strength of a given fiber is thus closer to the energy of the fiber cross-links as compared to the strength of a covalent fiber and its cross-links. Unlike a conventional polymeric hydrogel, the strength of the self-assembled gel depends not only on the number and strength of the cross-links but also on the strength of a nanofiber itself. Since the hydrogen bonding in the peptide region stabilizes the nanofiber, an optimization of the secondary structure enhances the fiber strength and therefore improves the mechanical properties. As previously reported, optimization of the concentration of Ca^{2+} ions also improves the strength of the gel as it creates more cross-links and stabilizes the supramolecular nanofibers.[5]

In conclusion, we have described a novel peptide−amphiphile/lipid system that is capable of forming hydrogels up to 20 mol % of lipid. The mechanical properties of this system strongly correlate with the secondary structure of the PA showing the direct correlation between molecular structure and macroscopic properties. This composite system allows us to easily optimize the mechanical and chemical properties of the

hydrogel to a particular application simply by adjusting the ratio of included components. We expect that other phospholipids and related small molecules may provide similar results.

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Supporting Information Available. Detailed methods, additional TEM images, CD, and mechanical characterization are available free of charge via the Internet at http://pubs.acs.org.

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(9) For a detailed procedure of preparation of PA-lipid inclusions see the Supporting Information.

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