

Structures, function and applications of amphiphilic peptides

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Abstract

Major recent advances: Amphiphilic peptides, in other words, peptides that contain hydrophobic and hydrophilic regions along their lengths, have been molecularly designed and exploited in various ways. Most notable advancements in the past few years are their proposed use for scaffolds for nanometer structures such as molecular wires and mineralization of hydroxyapatite crystals in a particular orientation. Our group has also designed a new class of short and simple surfactant-like peptides that self-assemble into well-defined nanotubes and nanovesicles.

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Keywords: Amino acids; Charged residues; Hydrophobic residues; Non-lipid surfactants; Self-assembly

1. Introduction

Surfactant science and engineering have thrived for decades, with most of their advances fueled by the detergent, soap and lubricant industry. Special attention was, and still is, paid to bulk properties of surfactant molecules such as their ability to reduce surface tension of water and their ability to solubilize lipid and other non-polar organic and biological molecules. Recent progress in the field of nanotechnology, however, has sparked considerable interest in one of surfactant molecules' enabling properties: self-assembly, which is viewed as critical for the creation of

nanometer structures [1,2]. Increasing number of researchers have been turning to biological systems where this bottom-up strategy for manufacturing structurally defined complex assemblies is ubiquitous and becoming more and more tractable [3–5].

Indeed using building blocks of biological origin to produce biologically inspired materials have become possible due to the advancement of chemical synthesis methods of biopolymers and biotechnology in the past decades. Short peptides or polypeptides, for example, can now be chemically synthesized, recombinantly made in cells or plants with little difficulty. When these monomers undergo molecular self-assembly, the structural components formed, such as nanotubes or vesicles, may be further modified and tailored to confer functionality: a nanotube can be coated with metals or semiconducting materials to make nanowires, for example [6]. Since this field is undergoing a rapid growth, this review only focuses

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on one type of biomolecules, amphiphilic peptides that have been engineered and how researchers have exploited them for various applications.

2. Different amphiphilic peptide motifs

This review will focus on three different amphiphilic peptide motifs that are currently being actively investigated for their applications (Fig. 1). The first class has a hydrocarbon tail in the peptide monomer [7,8] (Fig. 1a and b). The second is a heptane bola-amphiphile, which contains a heptane moiety flanked by a glycylglycine group at each end [9,10] (Fig. 1c). The third is a class of polypeptides containing predominantly of natural amino acids [11,12] (Fig. 1d and e). The general feature that ties these three classes together is the presence of both

hydrophobic and hydrophilic region(s) in a molecule. Like most amphiphiles, monomers that belong to all three classes self-assemble in solution into sub-micrometer sized supramolecular structures according to geometrical constraints [13]. Most peptide-amphiphiles reviewed in this article self-assemble into nanotubes or fibrils.

3. Biomineralization

Many applications in biomedical engineering such as tissue engineering call for biocompatible material that can by itself serve as either a substrate for cell growth or be part of a complex composite systems that, like the structures found in the body, are bioactive [14,15]. Such a system will have to be inert biologically and also illicit similar biochemical responses as

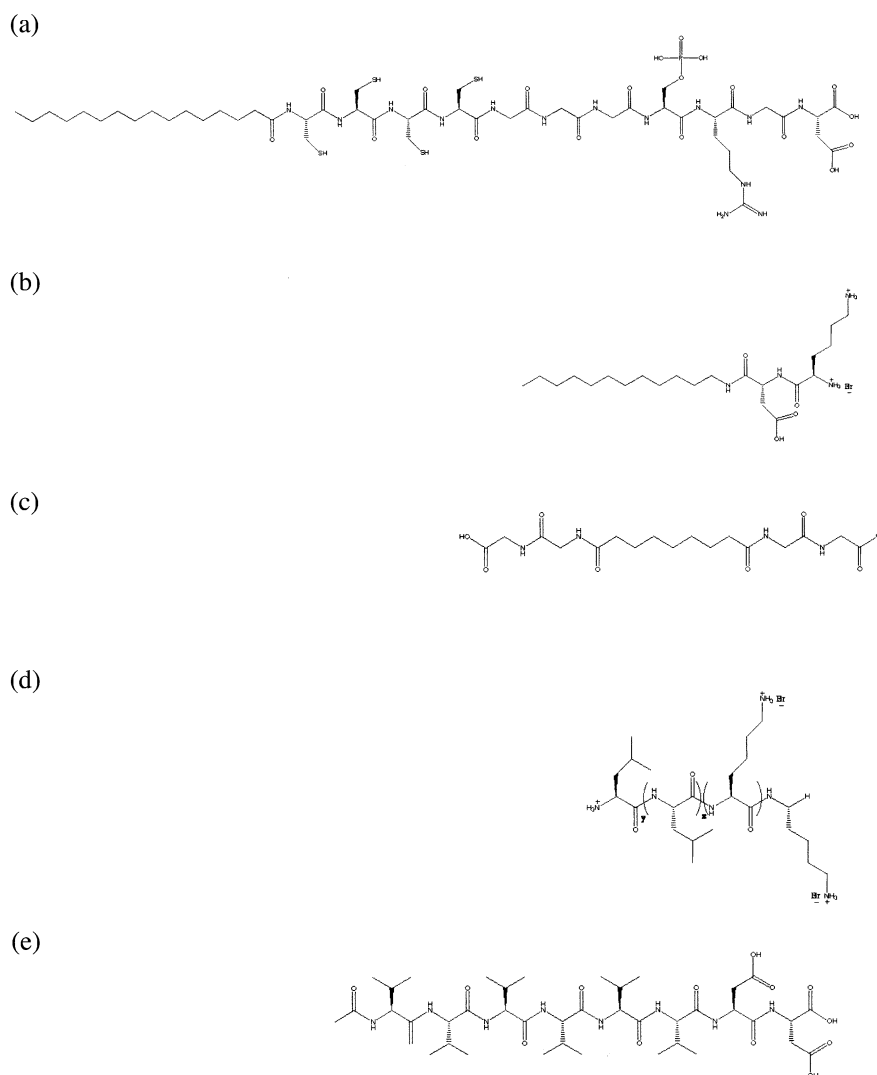


Fig. 1. Chemical structures of various amphiphilic peptides: (a) and (b) are peptides that contain alkane chains as their hydrophobic tail; (c) bis(*N*- α -amido-glycylglycine)-1,7-heptane dicarboxylate, an example of dicarboxylic oligopeptide bola-amphiphiles; (d) KxLy as indicated in the text; and (e) V6D2, are polypeptides consisting of natural L-form amino acids.

the parts it is replacing. Synthetic systems also need to support cell growth and resemble the environment of extracellular matrix [16]. Even though the exact molecular structures that promote cell proliferation are not known, researchers are vigorously studying different materials that may serve as scaffolds for tissue implantations [15].

One such system that has been advanced recently and relevant to this review is bone tissue. Bone tissue contains both an organic and inorganic component and at the lowest structural level is an organization of hydroxyapatite crystals inside grooves and channels of type I collagen fibrils [17,18]. The complexity of arranging the two organic and inorganic phases in a way that resembles bone has made bone engineering a challenging problem to solve and many different materials have been proposed to accomplish such feat. Hartgerink et al. used a self-assembling peptide-amphiphile to serve as a template for the crystallization of hydroxyapatite crystals in the correct crystallographic orientation [8,19]. The power in using a self-assembling peptide based system lies in the researchers' ability to rationally design functional groups on each peptide and to also control the assembly and disassembly process through pH adjustments. Of particular importance in biomineralization is the positioning of negatively charged surfaces that induce crystal formation by supersaturating ionic concentration locally [18]. The researchers also attached a three amino acid moiety of arginine–glycine–aspartic acid (RGD) to the carboxy-terminal of the peptide. This sequence has been found to be important in cell adhesion and its incorporation into the system may promote cellular adhesion and overall biocompatibility [20].

4. Templates for nanotechnology

Excitement in nanoscale sciences led to the development of many new materials and methods to generate functional aggregates in the sub-micrometer regime. In the field of nanometer-sized electronics, many researchers are looking at various materials for constructing nanowires [21–24]. Some of these materials require several steps in order to form conducting nanowires: first a self-assembling system forms a template and second a metallization process deposits a conductive metal around the nanotubes. The self-assembling monomers may then be digested or dissolved, leaving behind the metallic coat. Since the diameter of the wire may be varied according to the application at hand, the self-assembling system must be also able to form different sized tubes according to the environment or specific chemical modifications.

Recently, Matsui and MacCuspie reported the for-

mation of metalloporphyrin coating on nanotubes made of peptide bola-amphiphile [25]. These bola-amphiphiles self-assemble in solution to form tubular structures with diameters ranging between 20 nm and 1 μm [26]. Furthermore, they have previously shown that these nanotubes contain free amide and carboxylic groups that can intercalate metal ions such as platinum, copper, and nickel, making them suitable as templates for nanowires [26,27]. In this case porphyrins were used for photonics devices due to their efficiency in electron transfer. In their article, the authors showed that the metalloporphyrin (namely protoporphyrin IX Zn (II)) was able to bind to the peptide nanotube and to themselves by hydrogen bonding. They are now turning towards formation of more complicated networks of these derivatized nanotubes.

In a similar study, Matsui and Douberly were able to grow and immobilize these peptide nanotubes on gold substrate through functionalization of a self-assembled monolayer of 4-mercaptobenzoic acid [28]. They proceeded to coat the nanotube with a layer of nickel by immersing it in a nickel bath. This preliminary result will be valuable in creating and optimizing circuitry made from nanowires.

One can also envision the linkage of a gold or platinum nanocrystal to the peptide itself. Upon self-assembly of the peptides into nanotubes, these conductive metals will cover the surface and serve as the conductive coat for nanowires. It will be interesting to see whether one can conduct a current through these derivatized peptide nanotubes.

5. Peptides consisting mostly of natural amino acids

Another class of peptide-amphiphiles contains predominantly natural amino acids and is also analogous to biological lipids in having a polar region and a non-polar segment. Using a transition metal mediated α -amino-acid *N*-carboxyanhydride polymerization, Nowak et al. synthesized 100–200 residue-long polypeptides with a string of positively charged lysines (K) on one end and a string of hydrophobic amino acid such as leucine (L) or valine (V) at the other end [11]. The chemical structure of a polypeptide containing lysines and valines is depicted in Fig. 1d. These diblock polypeptides form remarkably strong hydrogels in aqueous solution, even at low concentrations (0.25–2.0 wt.%). It was discovered that in order for the polypeptides to efficiently form hydrogels, the hydrophobic tails of the monomers need to adopt well-ordered conformations, namely an α -helix for leucine and a β -sheet for valine. An interesting property of the diblock polypeptide hydrogel is its ability to withstand temperatures up to 90 °C and to

recover rapidly after being stressed. This is remarkable considering the polypeptides are present at low quantities.

Our laboratory has been working on a very simple amphiphilic peptide motif that resembles a lipid molecule [12]. Each peptide has a polar head group containing charged amino acids such as aspartic acid or lysine and a short hydrophobic tail consisting of a string of hydrophobic amino acids such as alanine, glycine, or valine. Our peptides in general have lengths of less than ten amino acids, comparable to the length of biological phospholipids that make up the cellular membrane. Upon dissolving these peptides in water and adjusting the pH to neutrality, we observed the formation of tubules with diameters approximately 50 nm using a transmission electron microscope (TEM). The peptide V6D2 (containing six hydrophobic valines and one hydrophilic aspartic acid at the carboxy terminus of the peptide, Fig. 1e), in particular, self-assembled into tubes of homogeneous diameter as revealed by both dynamic light scattering and TEM (Figs. 2 and 3). In general the size distribution of structures becomes more polydisperse as the hydrophobic tail is lengthened, as the longer monomers have more freedom to pack in different arrangements [29].

Most of the structures observed are of tubular nature that can be up to microns in length. We also have observed vesicles in our preparations and able to see a vesicle budding off or into another structure [12]. This suggests the dynamic nature of the self-assembly and disassembly processes even over relatively long time scales (weeks and months). The lengths of these polypeptide surfactants can be easily varied and many chemical modifications on the

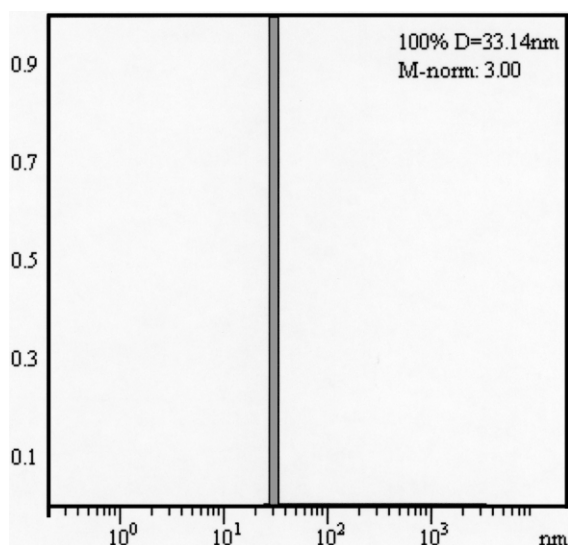


Fig. 2. Dynamic light scattering of solution containing 5 mM V6D2.

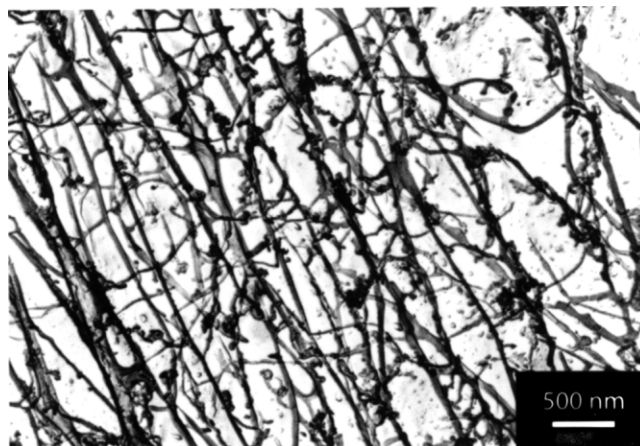


Fig. 3. TEM image of a replica of V6D2. The sample was prepared using the quick-freeze/deep-etch method to preserve tubular structures formed in solution.

monomer level are made known by the peptide chemist. Such system can be very cost effective.

6. Conclusions

The advancement of nanotechnology necessitates not only improvements of current methods but also the discovery of novel materials. These new materials have to be relatively inexpensive to synthesize and need to be amenable to chemical modifications that can confer various functionalities. As outlined in this review, the class of amphiphilic peptides can prove to be a promising starting material for many applications or it may serve as a complementary substrate for existing methods. The following years will no doubt reveal the utility of these molecules in the material scientist's toolbox.

Acknowledgements

We were benefited by early stimulating discussions with Michael Altman and Daniel Hu, both of whom were undergraduate students in our laboratory. We also thank the members of our laboratory for support and helpful discussions. This work was supported in part by grants from NSF CCR-0122419 to the Center for Bits and Atoms of the Media Laboratory at M.I.T. and Naval Research Labs/Defense Advanced Research Project Agency.

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