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Self-assembly of Surfactant-like Peptides with Variable Glycine Tails to Form Nanotubes and Nanovesicles

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ABSTRACT

The self-assembly of surfactant-like peptides containing 4–10 glycines as the component of the hydrophobic tails and aspartic acids as the hydrophilic heads is described. The peptide monomers form nanotubes and nanovesicles in water at neutral pH. These nanostructures become more polydisperse as the length of the glycine tails increases. These unique structures may serve not only as scaffolds for constructing diverse nanodevices but also as enclosures to encapsulate rudimentary enzymes for studying prebiotic molecular evolution.

Introduction. The design and fabrication of nanoscale devices require the discovery and development of novel materials. Traditional top-down approaches of processing materials to produce micrometer-sized structures may not scale down efficiently to produce finer material in the few nanometer regimes. On the other hand, the bottom-up approach of building up from the molecular level can be complementary to the traditional material processing methods. One avenue to construct nanometer-sized materials is to learn from biology where all the tools and devices are built at the molecular scale through self-assembly and genetically and biochemically programmed assembly. These nanomaterials and molecular machines have inspired us to design new materials using the individual building blocks having dimensions in a few nanometers range.

Similar to the construction of diverse architectural and complex structures using simple bricks, we are interested in using simple "molecular bricks", namely the building blocks of sophisticated biological systems: amino acids, nucleic acids, lipids, and sugars, to design and fabricate diverse molecular scaffolds, molecular devices, and molecular machines.

We have developed several types of biomaterials by designing various classes of self-assembling peptides.[1−9] These include nanofibers,[1−7] hydrogels,[1−5] nanocoatings,[8] and other nanostructures.[9] Other investigators have also employed the biological building blocks to construct nanomaterials, from peptide and protein that form filaments and fibrils,[10−18] to nanolayers at the water−air interface,[19−23] to diacetylenic lipids that form nanotubules.[24−26]

We previously reported a set of short peptides that self-assemble into nanotubes in aqueous solution at pH 7. These peptides consist of at least one charged amino acid at their polar "heads" and a string of six hydrophobic amino acids at their "tails". Each monomer has a length of approximately 2 nm when extended, but the self-assembled tubes are approximately 30−50 nanometers in diameter and microns in length, as visualized by transmission electron microscopy (TEM).[9] We proposed the use of these peptides for scaffolds and nanomaterials science research.

Here we further extended our previous analysis of peptide sequences and lengths that have the ability to form supramolecular structures. We selected two simple amino acids, glycine and aspartic acid, for two reasons. First, glycine is the simplest member of the 20 amino acids with only one hydrogen on its side chain, and it is achiral. Despite this simplicity, glycine has been found to be a major building unit in many strong structural proteins including all types of collagen,[27] several types of spider silk,[28] silkworm silk,[29] and marine mussel water-based bioadhesives.[30] Likewise, aspartic acid is the simplest charged amino acid with a carboxylic group as its side chain, therefore conferring a total of two negative charges to a peptide when located at the C-terminus. Aspartic acid clusters in biomineral proteins have been found to facilitate and to promote biomineralization by attracting and organizing inorganic ions.[31] Second, both glycine and aspartic acid have been found to form in the presumed prebiotic conditions.[32]

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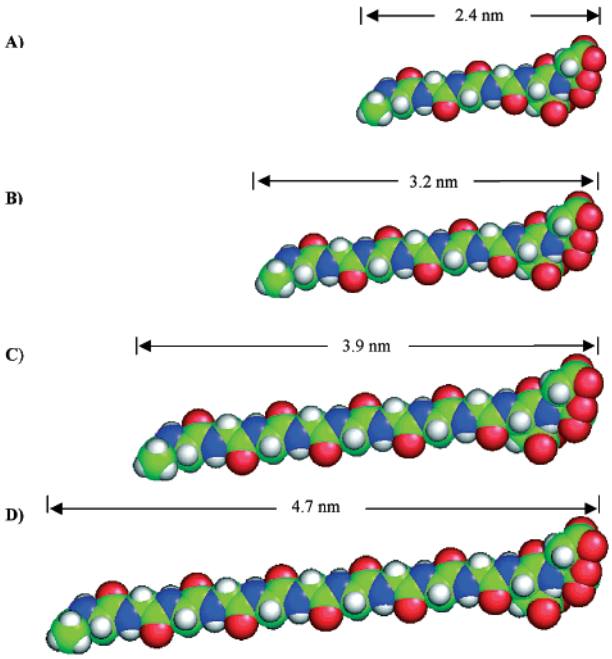

Figure 1. Molecular structures of individual glycine tail-based surfactant peptides. (A) G_4D_2, (B) G_6D_2, (C) G_8D_2, and (D) $G_{10}D_2$. Color code: carbon, green; hydrogen, white; red, oxygen; and blue, nitrogen. The tail length of glycines varies depending on the number of glycine residues. The lengths of these molecules in the extended conformation range from 2.4 nm of G_4D_2 to 4.7 nm of $G_{10}D_2$.

Experimental. All designed glycine-based peptides were custom synthesized at SynPep (www.synpep.com, Dublin, CA) and made up to 5 mM in deionized water, neutralized with 1 N and 0.1 N NaOH, and sonicated in a water bath for 10 min to get clear solutions. Dynamic light scattering was carried out using a PDDLS/batch light scattering instrument (Precision Detectors, Franklin, MA), and the data were mass normalized for a proteinaceous solution and analyzed by the PRECISION DECONVOLVE software package. Carbon coated platinum replicas were prepared using a quick-freeze/deep-etch method as described previously[8,9] and examined using either the Phillips EM-410 or the JEOL 200CX transmission electron microscope.[9]

Result and Discussion. Figure 1 shows the atomic structures of the peptides used in this study. Briefly, each peptide consisted of two aspartic acids at their C-terminal ends and a string of glycines. The N-terminus of each peptide was acetylated, abolishing the extra positive charge, but the C-terminus had a free carboxyl group, yielding a total of three negative charges per peptide monomer at neutral pH. Four peptides were investigated: G_nD_2, where $n = 4$, 6, 8, 10 glycines, and two aspartic acids, having lengths of 2.4, 3.2, 3.9, 4.7 nm in their extended conformations, respectively. Solubility became compromised as the hydrophobic tail was lengthened, and thus no peptide with a tail longer than 10 glycines was investigated. All the peptide solutions remained visibly clear in the duration of the study.

Size distribution obtained by dynamic light scattering revealed the presence of structures having dimensions in the order of 40−80 nm (Figure 2). Interestingly, a second peak having dimensions around 100 to 200 nm started to emerge in G_6D_2 and became more predominant as the length of the glycine tail increased to 8 and 10. Although it is difficult to determine the exact molecular identity of this structure, it is apparent that the size distribution of the structures becomes broader as the glycine tail becomes longer. This is expected due to the increased flexibility of the longer glycine tails, which may have prompted different size structures to arise simultaneously ab initio due to the freedom of the hydrophobic chain to pack in different conformations. It should also be noted that our instrument has an effective range between 10 nm to 1000 nm, thus any structures beyond the limit may not be detected.

We used the quick-freeze/deep-etch technique to prepare the surfactant-like glycine peptide samples for visualization under transmission electron microscope (TEM). This technique essentially preserved structures formed in solution by preventing ice formation. Figure 3 shows the presence of a network of nanopipes and nanotubes. These structures were highly dependent on the quality of sample preparation but seemed to be quite homogeneous throughout the replica. G_4D_2 consisted of mostly nanotubes with diameter around 40 nm (Figure 3A). We observed more vesicles formed in the G_6D_2 solution along with similar nanotubes (Figure 3B), whereas, G_8D_2 and $G_{10}D_2$ had more occurrence of entangled nanotubes (Figures 3C and 3D, respectively). Figure 3D also revealed the presence of woven membranous structures bridging adjacent nanotubes and more heterogeneity in the $G_{10}D_2$ solution. These observations are correlated to results obtained by dynamic light scattering, with structures from $G_{10}D_2$ peptides having a very broad size distribution. As the length of the glycine tail increases, one would expect membranes to emerge as the monomers pack closely to one another and prevent the formation of curvature required for vesicles and tubes. We speculate that this membrane is of laminar nature having a thickness of approximate 10 nm, corresponding to a bilayer of peptide monomers arranged tail to tail with one another. It should be noted that the thickness might vary depending on how much overlap the hydrophobic tails of closely located monomers have with their neighbors. Additional studies such as solution neutron and small-angle X-ray scattering will be carried out to determine this structure.

We previously observed that the self-assembly and disassembly processes of peptide nanotubes and vesicles are very dynamic over time. On close inspection (Figure 4), the TEM images show different structures that may have formed due to this dynamic behavior of the G_6D_2 system. Figures 4A and 4B show a finger-like structure akin to a three-way junction. The two short ends may have been two nanotubes in the process of elongation. The red arrows in Figure 4C reveal the presence of opening on one side of a nanotube, suggesting the existence of the tubular defect that may initiate branch birth and growth as observed in Figure 4A. Nanovesicles are also observed in Figure 4D. The red arrows point to a "pinched" structure that may have formed when two vesicles divided from one another. This is akin to the "stalk intermediate" described by Brownian dynamics simulations

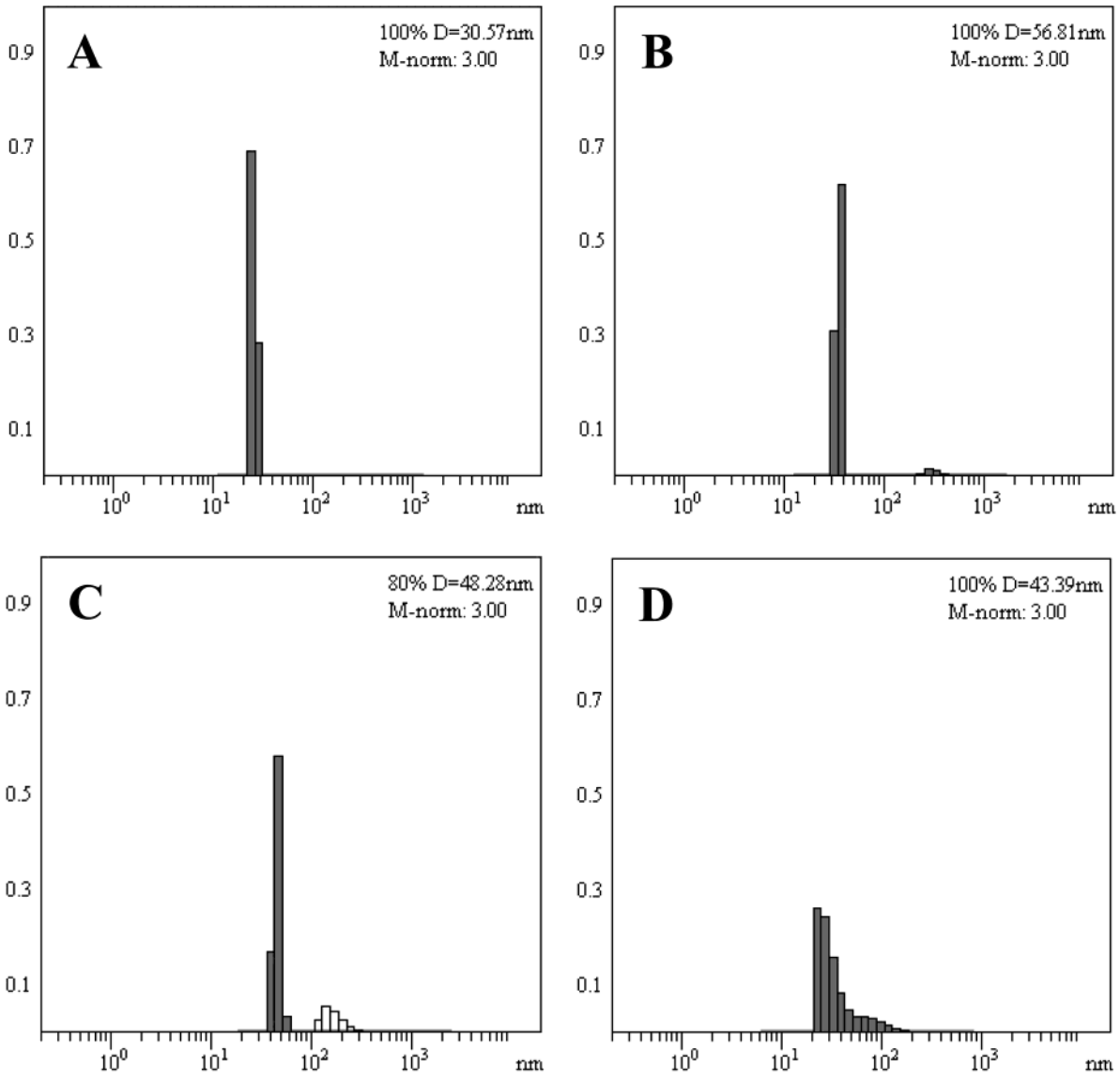

Figure 2. Size distribution of peptide solutions obtained by dynamic light scattering (DLS). (A) G_4D_2, (B) G_6D_2, (C) G_8D_2, and (D) $G_{10}D_2$. Note the discrete distributions in G_4D_2 and G_6D_2, and the more heterogeneous forms in G_8D_2 and $G_{10}D_2$. Only the diameters of the nanostructures are measured. The lengths of the nanotubes in tens of microns are beyond the instrument measurement.

of vesicle fusion.[33] Such phenomenon of vesicle division has also been reported in phospholipids.[34] The ability of peptide nanovesicles to spontaneously divide may have important implications because they may serve as enclosures for rudimentary prebiotic enzymes. Similar as in lipid surfactants, the addition of monomers to an existing vesicle may induce it to grow and ultimately divide into similar entities. This might represent the earliest form of prebiotic molecular division and might have allowed for some rudimentary enzymes to undergo prebiotic molecular evolution in an enclosed environment.

Two simplest proposed molecular models for these nanotubes and nanovesicles are presented in Figure 5. In our models the peptides form a bilayer, similar to a biological phospholipid, to sequester the glycine tails from the aqueous environment. The proposed nanotubes and nanovesicles would consist of a presumed bilayer structure. However, unlike a lipid, packed peptides would likely form hydrogen bonds with one another on the backbone. At the present time the complexity of the self-assembly process is beyond a real-time simulation of the formation of a supramolecular structure from a large monomer population.

We also observed that sequences of different lengths and composition could form similar supramolecular structures, suggesting that the surfactant-like self-assembly rules for this class of peptides are generally applicable. Interestingly, some other sequences such as V_6K and A_6K (six valines for the hydrophobic chain and six alanines followed by a positively charged lysine, respectively) formed similar type structures with varying degrees of homogeneity (von Maltzahn et al., in preparation). These observations suggest that there is not necessarily a sequence specific requirement for the formation of the nanostructures. In other words, many combinations of amino acid lengths and sequence can potentially serve the same purpose as long as they have a hydrophilic head and a hydrophobic tail. This resembles some chemicals and

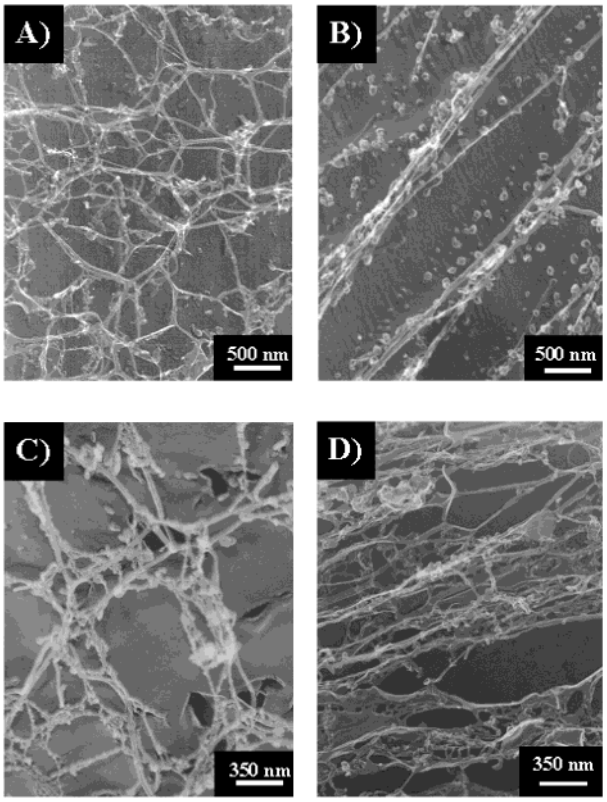

Figure 3. TEM images of platinum coated replica of (A) G_4D_2, (B) G_6D_2, (C) G_8D_2, and (D) $G_{10}D_2$. Samples were quick-frozen in liquid propane (−180 °C) immediately after DLS data collection. TEM images of G_6D_2 show the presence of nanovesicles and nanotubes. The scales are marked in each panel.

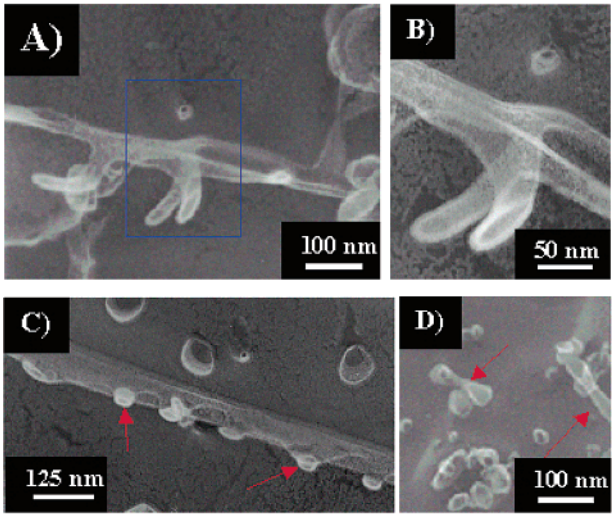

Figure 4. High resolution of TEM images of G_6D_2 showing different structures and dynamic behaviors of these structures. (A) A pair of finger-like structures branching off from the stem. (B) Enlargement of the box in (A), the detail opening structures are clearly visible. (C) The openings (arrows) from the nanotube where may resulted in the growth of the finger-like structures. Some nanovesicles are also visible. (D) The nanovesicles may undergo fission (arrows).

lipid surfactant molecules, where the precise sequence is not a prerequisite for vesicle and micelle formation, but rather the chemistry and structure play a more important role.

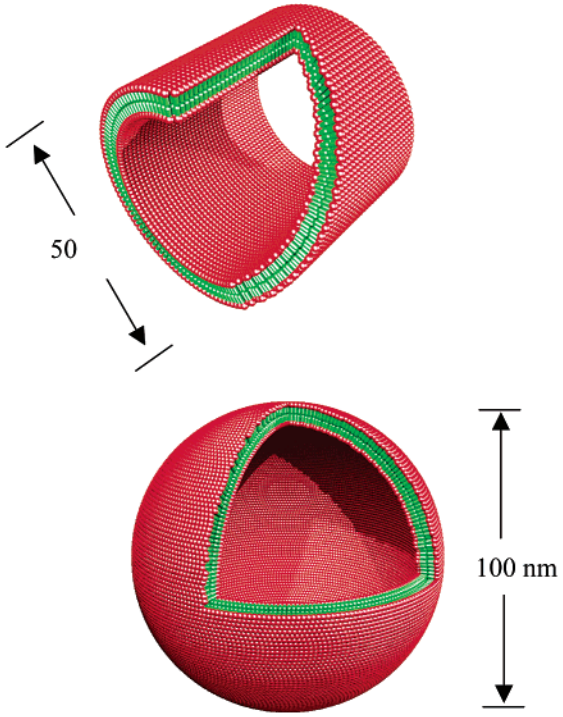

Figure 5. Molecular modeling of cut-away structures formed from the peptides with negatively charged heads and glycine tail. (A) Peptide nanotube with an area sliced away. (B) Peptide nanovesicle. Color code: red, negatively charged aspartic acid heads; green, nonpolar glycine tail. The glycines are packed inside of the bilayer away from water and the aspartic acids are exposed to water, much like other surfactants. The modeled dimension is 50−100 nm in diameter.

It should be noted that while peptide formation may have been possible in the environment of prebiotic molecular evolution, one would expect that without complicated catalytic machinery there would be randomness in both the lengths and amino acid sequences of these peptides. It would thus be advantageous for prebiotic molecular evolution if there were no strict sequence or length requirement for the formation of enclosures.

The other aspect of studying glycine surfactant peptide is the report that glycine, aspartic acid, and alanine are of particular interest to prebiotic molecular evolution due to their presumed presence in the prebiotic environment of early Earth and in CI-type carbonaceous chondrites Orgueil and Ivuna.[35] Furthermore, glycine is the simplest of the 20 naturally occurring amino acids and most likely to be the predominant amino acid several billion years ago. It has indeed been experimentally demonstrated that these amino acids or their derivatives can form polypeptides when subjected to repeated hydration−dehydration cycles[36] under microwave heating as well as when heated on dried clays.[37] If peptides consisting of any combination of these amino acids can self-assemble into nanotubes or vesicles, they would have the potential to provide a primitive enclosure for the earliest RNA-based or peptide enzymes, facilitating catalysis and prebiotic molecular evolution by sequestering the rudimentary enzymes in an enclosed or semi-isolated environment.

It is presumed that in the prebiotic world, on one hand, peptides of various lengths might self-assemble into distinct vesicles that could enclose prebiotic rudimentary enzymes to isolate them from the environment. On the other hand, a diverse population of peptides and RNA might condense into more complex structures to evolve different functions. These simple amino acids may have facilitated the formation of various supramolecular structures that can enclose the primordial ribozymes and rudimentary peptide enzymes, allowing them to eventually evolve into more efficient chemical and biological catalysts.

With our surfactant-like peptide system we can also incorporate particular amino acid residues, such as a cysteine, at any location of the peptide. Since the side chain of cysteine can covalently couple onto gold or other metal nanocrystals and surfaces, the peptide nanotubes and nanovesicles provide a new class of molecular scaffolds for organizing metal and inorganic nanocrystals to fabricate devices at the nanometer scale. Moreover, some designed molecular recognition ligands may also be incorporate into the nanostructures for the delivery of a wide range of substances into cells and other targets.

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