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Improved electrical conductance through self-assembly of bioinspired peptides into nanoscale fibers

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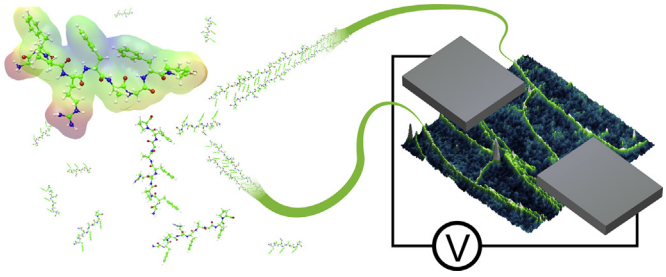
HIGHLIGHTS

- We designed a novel peptide sequence from natural amino acids which forms nanofibers in water.
- The nanofibers were investigated by AFM, fluorescence and CD spectroscopy.
- A film of self-assembled peptide shows conductivity in air and vacuum.
- We propose that stacking of phenylalanine between peptides leads to conductivity.

GRAPHICAL ABSTRACT



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ABSTRACT

We investigated the electrical conductance of films consisting of bio-inspired peptide molecules and of their extended form, self-assembled nanoscale fibers. Here, the entirely natural and novel peptide sequence, GFPRFAGFP, was designed based on naturally occurring fibrous proteins. To attain electrical conductance, we implemented phenylalanine residues in the sequence such that the aromatic rings are present along face of the molecule. We confirmed self-assembly of nanoscale fibers in pure water after incubating the peptides at 37 °C by AFM. The morphology and conformation of the incubated peptide fibers were studied using AFM, fluorescence spectroscopy and circular dichroism spectroscopy. It was shown that very thin fibers with a single-molecule-level diameter form. The helical feature of the peptide backbone and enhanced stacking of aromatic residues were also investigated. This aromatic stacking is important to our electrical measurements as, even in vacuum environment, films of non-incubated GFPRFAGFP sometimes show apparent conductance while those containing self-assembled nanoscale fibers show stable and improved conductance. We propose that this effect may be due to extended stacking of aromatic residues providing $\pi - \pi$ conjugation along the fiber.

1. Introduction

In the last decade, increasing needs for electrically conductive nanomaterials have been emerging for use in electronic devices [1], medical [2,3], and sensing [4,5] applications, among others. In particular, one dimensional conductive nanostructures are essential for the construction of nanodevices [5–7]. One method of

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construction at the nanoscale is utilizing self-assembly. Much self-assembly research has focused on mimicking nature's building blocks [8–10]; however, without modification these organic materials are intrinsically insulating, or conduct via relatively slow mechanisms [11]. Some notable exceptions exist, such as the bacteria *Geobacter Sulfurreducens*, capable of producing nanowires with metal-like conductivity [5,12,13]. Also, recent studies have shown that electron conductivity through natural peptides is theoretically plausible [12,14]. The suggested manner of conduction is via delocalized electron states and enhanced molecular orbital states from self-assembly of aromatic and charged residues. However, thus far, the potential of this emergent functionality has not been utilized.

In this work, we present a novel short peptide formed entirely from naturally occurring amino acids which dynamically self-assembles into conductive fibers. This peptide, containing residues of glycine (G), phenylalanine (F), proline (P), arginine (R), and alanine (A) in the sequence GFPRFAGFP, was shown to self-assemble under aqueous conditions, forming long nanofibers. These nanofibers form from left-handed helical backbone conformations of the monomer peptides, allowing aromatic group stacking. It was shown that a film made from these nanofibers is conductive, highlighting the potential for electronic functionality to emerge from the assembly of bioinspired materials. This and similar peptides offer controlled functionality and interesting properties suitable for a variety of nanoelectronic and bio-nanotechnological applications.

2. Materials and experimental methods

2.1. Peptide

De Novo design of peptide was inspired by the fibrous proteins found in the extracellular matrix of mammalian tissues, such as elastin and collagen [15,16]. Utilizing similar sequences, the peptide backbone comprising of GFPRFAGFP was prepared for this study for the following two reasons: First, GFPRFAGFP was expected to adopt a helical structure [15,17], allowing for one-dimensional assembly. Second, periodically inserted F was expected to promote intermolecular coupling [18–22], and increase conductance of the assembled construct owing to $\pi - \pi$ conjugation [23.].

The chemical structure and the ball-and-stick model of a GFPRFAGFP molecule are shown in Fig. 1(a) and (b), respectively.

Also, the calculated polar surface area of the molecule is shown in Fig. 1(b), where the blue and the red areas highlight polar and apolar residues, respectively. Amphiphiles such as this molecule have been extensively exploited for controllable aqueous self-assembly [8,24–26]. Fig. 1(c) – 1(e) show the CPK model of a single peptide down different axes of view, expressing dimensions of approximately 15.8 × 8.9 × 28.6 Å. The peptide model with PPII backbone [27] was initially built using VegaZZ (v3) [28]. After inserting a water layer of 10 molecules thickness, molecular conformation of the model was further optimized through MM2 force field method [29].

All water used in this study is 0.22 μm filtered ultrapure MilliQ water (>18.2 MΩ cm). The designed peptide molecule, NH_2-GFPRFAGFP-COOH, was synthesized and purified by Eurofins Operon (Operon Bio-Technology Co., Ltd., Japan). 1 mg lyophilized aliquots were prepared in water to a concentration of 20 mg/mL as stock solution. Prior to further dilution as described, the stock solution was sonicated in an ice bath for 30 s.

2.2. Atomic force microscope (AFM) observations

GFPRFAGFP solution of 10 mg/mL in water was prepared and incubated at 37 °C either overnight or for 7 days. The incubated peptide solution was further suspended to a concentration of 1 mg/mL in water, and 5 μL was placed onto a freshly cleaved mica surface and incubated for 15 min. Prior to AFM observations, the sample was again rinsed in water and dried under pure Argon gas to remove excess peptide from the surface. For comparison, peptide samples which were prepared immediately after dilution (non-incubated) were used as a control. Images were acquired in AC tapping mode on an Asylum MFP3D SPM (Asylum Research, USA) using Olympus OMCL-AC240TS probes (nominal values: k = 1.8 N/m, f = 75 kHz, radius > 10 nm) in air at RT. A free air amplitude of 500 mV was used (20–50 nm) with minimum engage setpoint (<10% of free air amplitude). The phase was monitored to ensure stable imaging conditions. A phase increase was observed on engaging with the surface, indicating that imaging was in the repulsive regime.

2.3. Optical spectroscopy measurements

GFPRFAGFP solution of 10 mg/mL was used for the optical spectroscopy measurements of both incubated and non-incubated peptide. Samples were diluted to 0.1–0.3 mg/mL (measured by UV absorbance at 257 nm) in water immediately prior to the following measurements.

2.3.1. Fluorescence spectroscopy

Fluorescence spectroscopy measurements were carried out at room temperature on a Hitachi F-7000 (Japan) fluorescence spectrophotometer with a small volume cuvette (pathlength 0.1 cm) with excitation wavelength of 265 nm, emission wavelength in the range of 275–500 nm, and a scan speed of 240 nm/min. Emission data was normalized against the fluorescence peak of phenylalanine (~281 nm) in order to average results of repeated measurements on separately prepared samples.

2.3.2. Circular dichroism (CD) spectroscopy

CD spectroscopy was performed on a J-725 spectrophotometer (Jasco Co. Ltd., Japan) using a small-volume cuvette (pathlength 0.1 cm). CD spectra were acquired in the range of 185–500 nm with a step of 0.2 nm, at a scan rate 100 nm/min. Dichroic angle was measured with a resolution of 100 mdeg under an N_2 gas flow rate of ~25 L/min. To reduce noise, we averaged over 3 scans per sample. We acquired CD spectra at temperatures of 4, 21, and 60 °C.

2.4. Electrical measurements

Electrical conductances were investigated for the films of incubated and non-incubated peptide, biotin, and insulin, using a Keithley 4200 semiconductor parameter analyzer equipped with a 4200-preamplifier (Keithley Instruments Inc., USA) which enabled the highest relative resolution of 100×10^{-18} A in current measurement. To prepare the films, the method was similar to that for AFM observations: 5 μL droplets of incubated and non-incubated peptide, biotin and insulin (Sigma–Aldrich) solutions (10 mg/mL in water) were cast onto freshly cleaved mica substrates and dried under Argon gas. Then, two molybdenum plates with a size of 3 mm × 6 mm were placed with a spatial gap of 1 mm, directly onto the sample films (Fig. S1): Effectively, conductances of the films with a width of 3 mm and a length of 1 mm were measured by flowing current in the direction of the length. The current–voltage characteristics (I–V curves) were collected by sweeping the voltage of one electrode from −2 to +2 V (and vice versa) with steps of 0.01 V while the other electrode was grounded. Measurements were performed in ambient conditions or under vacuum conditions.