Nanostructures

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Formation of Polydopamine Nanofibers with the Aid of Folic Acid**

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Abstract: *Polydopamine (PDA) generated by the oxidative self-polymerization of dopamine shows great potential for surface modification. Observed PDA nanostructures are nanoparticles and thin films. The formation mechanism of PDA is still unclear; thus, the manipulation of PDA nanostructures is a big challenge. In this study, we first demonstrated that folic acid shows a dramatic effect on the PDA nanostructure: New aggregated nanostructures of PDA, nanobelts and nanofibers, were generated in a dopamine/folic acid system. We hypothesized that folic acid may be involved in the stacking of protomolecules of PDA by π–π interactions and hydrogen bonding. Herein we describe the first experimental strategy to manipulate the aggregation of PDA by using small molecules. This study not only provides a new method for generating PDA nanofibers, which are proposed bioorganic electronic materials, but also a possible way to understand the formation mechanism of PDA and its analogues in nature, melanins.*

Eumelanins, a class of biomacromolecules present in humans and animals, are thought to be extended heteropolymers of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid. They demonstrate multiple critical functions in humans and animals, such as photoprotection and free-radical scavenging, but their phototoxicity is also implicated in melanoma skin cancer.[1] Researchers believe that there is a crucial link between the fundamental molecular structure of melanins and their observable macroscopic behavior.[2] Meredith et al. proposed that the functional characteristics of eumelanin are related to extreme chemical and structural disorder at the secondary level.[2c] Although the debate about the structure of melanins—a polymer or a supramolecular aggregate—is ongoing, the research interest in melanins extends to physics and materials science, far beyond the traditional areas of melanin research in biology, chemistry, and medical science. More and more researchers have found that melanins are unexplored biooptoelectronic materials.[3] Oetzel et al. proposed self-assembled one-dimensional π-conjugated stacks of guanine- and melanin-based molecules as promising candidates for applications in organic electronics.[3d] Natural eumelanins are spherical granules, which are composed of aggregates of protomolecules that stack by strong π–π interactions, hydrogen bonding, or other kinds of supramolecular interactions.[4] Natural eumelanin fibrils are unusual and hardly observed. Only a few researchers have reported the observation of eumelanin filaments in special drying processes.[5] It has been proposed that the study of eumelanin fibrillation may help us to understand the origin of neurological diseases.[5c] On the other hand, with the aim of developing organic optoelectronic devices, researchers have a great desire to synthesize different melanin nanostructures, particularly one-dimensional nanofibers.[3d]

The self-polymerization of 3,4-dihydroxyphenylalanine (DOPA) and coordination with metal ions in mussel foot protein have been confirmed to be correlated to the hardness and high extensibility of the cuticle of mussel byssal threads and thus mussel adhesion, and proposed to endow self-healing properties.[6] Inspired by mussel natural "glue", researchers have given much attention to polydopamine, the sticky product of the oxidation and self-polymerization of dopamine, because of great applications in the surface modification of various materials.[7] Polydopamine is also recognized as an analogue of natural eumelanin.[8] Surface modification with PDA has broad application in tissue engineering and other biomedical areas. In studies aiming to modify the functions of PDA coatings, different functional molecules have been immobilized in PDA through copolymerization with dopamine in a basic environment or the postmodification of PDA.[7d,e,9] These immobilized target molecules have a wide range of size and functionality. However, no influence on the supramolecular nanostructure of PDA was observed. The controllable construction of new nanostructures of PDA is still a big challenge and to our knowledge has not yet been reported.

Herein we report the successful fabrication of PDA nanofibers with the assistance of folic acid. We observed that PDA nanofibers longer than tens of micrometers were generated during the oxidation and self-polymerization of dopamine upon the addition of folic acid. The length of the nanofibers varied depending on the reaction time from several hundred nanometers (essentially nanobelts) to over several tens of micrometers (nanofibers). The characterization of these nanofibers by UV/Vis and FTIR spectroscopy, X-ray photoelectron spectroscopy (XPS), and MALDI-TOF MS showed that these nanofibers are similar to PDA nanoparticles in their chemical properties. We suppose that folic acid, functioning as a structure-directing agent, manipulates the aggregated nanostructure of PDA during self-polymerization. This molecule is the first reported to show an impact on the supramolecular aggregation of PDA. Because the self-polymerized product of dopamine is similar to eumelanin, the successful fabrication of PDA nanofibers not only expands our knowledge of the self-polymerization of dopamine, but also provides us with a good chance to explore their possible

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application as biooptoelectronic devices. It also helps us on the road to the discovery of the structural secrets of melanins.

In a typical experiment, dopamine (0.3 mg mL^{-1}) and folic acid (0.15 mg mL^{-1}) were dissolved in water and stirred for 1 day at 60 °C. Tris buffer (10 mM, pH 8.8) was added to the resulting solution and triggered the self-polymerization of dopamine. In all experiments, we kept the concentration of dopamine at 0.3 mg mL^{-1}. A higher concentration of dopamine usually led to the generation of small folic acid/PDA nanoparticles (see Figure S1 in the Supporting Information). We studied a broad concentration range of folic acid in the self-polymerization of dopamine, from 0.07 to 0.25 mg mL^{-1}. We found that the synthesis proceeded through two stages, which are divided by the addition of Tris buffer (see Figure S2b; see Table S1 for important experimental parameters). There is an optimal temperature window of 45–60 °C for the fabrication of PDA nanofibers. Although nanofibers can be generated at room temperature (ca. 20 °C), the required time is much longer (over 7 days for stage I) than that at elevated temperature, and the yield of nanofibers is too small (see Figure S3). However, the generation of PDA nanoparticles is significantly accelerated at higher temperature (80 °C). It is hard to produce clean PDA nanofibers at 80 °C (see Figure S4). Furthermore, it may be possible to enhance the yield of PDA nanofibers by carrying out the reaction in the dark (see Figure S5). Chedekel et al. showed that UV light induces DOPA to polymerize into melanin.[10] In our synthesis of PDA nanofibers, it is necessary to avoid the generation of normal aggregates of the self-polymerized product of dopamine. On the other hand, folic acid is known to be photolabile.[11] Keeping the reaction mixture in the dark is one important step to protect folic acid. Finally, the time period of stage I needs to be long enough: Stage I takes several days at room temperature and 45 °C, or 1 day at 60 °C (see Figure S6). During shorter time periods, PDA nanofibers are hardly generated at all. In particular, the time period of stage II influences the length and morphology of PDA nanofibers.

Figure 1 presents SEM images of nanostructures obtained at different periods of stage II, at two different concentrations of folic acid (0.15 and 0.25 mg mL^{-1}). We investigated the products obtained after reaction in stage II for 1 (a, d), 3 (b, e), 9 (c), and 30 h (f). Belts were observed as the aggregation product after reaction for 1 h, the length of which varied from several hundred nanometers to 2 μm at folic acid concentrations of 0.15 (Figure 1a) and 0.25 mg mL^{-1} (Figure 1d). Some belts started curling. After 3 h, nanofibers appeared mixed with nanobelts, and the length of nanobelts and nanofibers was over several micrometers (Figure 1b,e). In some cases, PDA nanofibers coiled into circles, which are highlighted by arrows in Figure 1b,e. Further elongation of the reaction time in stage II (9 h) produced longer PDA nanofibers and nanobelts, whose length reached 5–10 μm (Figure 1c). The length of PDA nanofibers was in direct proportion to the reaction time in stage II. However, when the reaction time in stage II was too long, massive hybrid nanostructures of PDA were formed. After 30 h, the obtained nanofibers were covered by thick layers, which may be aggregated PDA nanoparticles (Figure 1f). We observed that

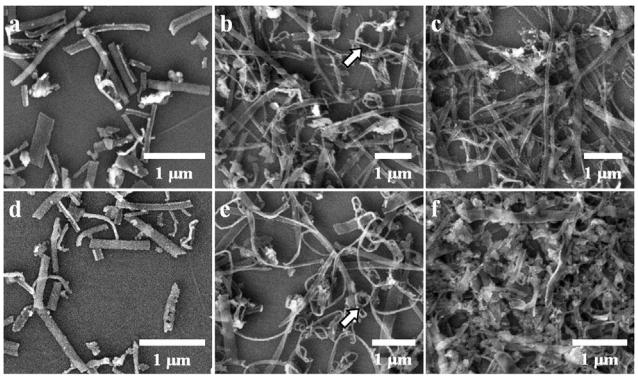


Figure 1. SEM images of PDA nanofibers obtained at 60 °C with varying reaction periods in stage II and a folic acid concentration of a–c) 0.15 and d–f) 0.25 mg mL^{-1}. The concentration of dopamine was 0.3 mg mL^{-1}, and the mixing period (stage I) for folic acid and dopamine in water was 1 day. The reaction period for stage II was 1 (a, d), 3 (b, e), 9 (c), and 30 h (f). Arrows in (b,e) indicate circles of PDA nanofibers.

when the reaction time was over 12 h at 60 °C, the increase in the amount of PDA nanoparticles in the obtained self-polymerization products of dopamine was significant (see Figure S7), and it may become dominant when the reaction time is over 1 day. Furthermore, we found that PDA nanobelts and nanofibers could be successfully generated when Tris buffer was replaced with aqueous NaOH (0.1M, pH 8.3; see Figure S8). However, the yield and quality of the obtained PDA nanofibers were lower than with Tris buffer (see Figure S9).

The above morphology characterization demonstrated that the self-polymerization products of dopamine in a basic environment were nanobelts and nanofibers in the presence of folic acid. One of the most attractive points is that these PDA nanofibers are probably formed by curling from nanobelts or nanosheets. We further characterized the nanostructures by TEM and AFM. Figure 2a–c shows TEM images of these nanofibers and nanobelts. One significant feature of the nanobelts is that they have different contrasts within one belt: One half appears lighter than the other half (Figure 2b), supposedly because these nanobelts are curled. AFM presented detailed morphological features of the curled nanobelts. Figure 2d,e shows two curled nanobelts, which have higher heights at the edges of the belts (50–60 nm) and a lower height in the middle of the belts (32–35 nm). Figure 2f presents a nanobelt which curled from only one side; we observed a plateau in its height profile on the other side. By analysis of the AFM height profile of this nanobelt, we can estimate its thickness to be approximately 31 nm, which is consistent with the lower height of nanobelts curled from two sides.

We further investigated the chemical nature of these nanofibers by UV, FTIR, and XPS spectroscopic analysis (see Figures S10 and S11; see Figure S12 for MALDI-TOF characterization of the PDA nanofibers). We found that nanofibers have monotonous absorbance similar to that reported for PDA nanoparticles,[8a] PDA thin films,[12] and melanins[2a] in the UV spectrum. XPS provided information

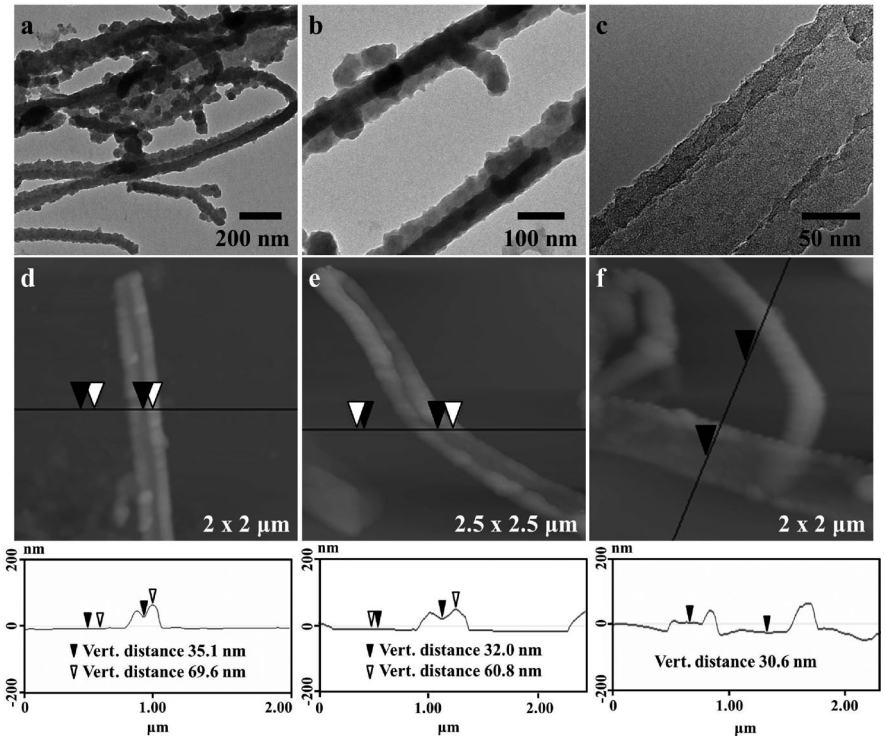


Figure 2. Morphological characterization of PDA nanofibers. a,b) TEM images. c) High-resolution TEM image of a PDA nanofiber. d–f) AFM analysis of several nanofibers, showing that nanobelts rolled up to form nanofibers. The vertical distance in (f) demonstrates that the thickness of nanobelts is about 30 nm, which corresponds to the vertical distance of the cavity part of nanofibers in (d,e) of approximately 35 and 32 nm, respectively.

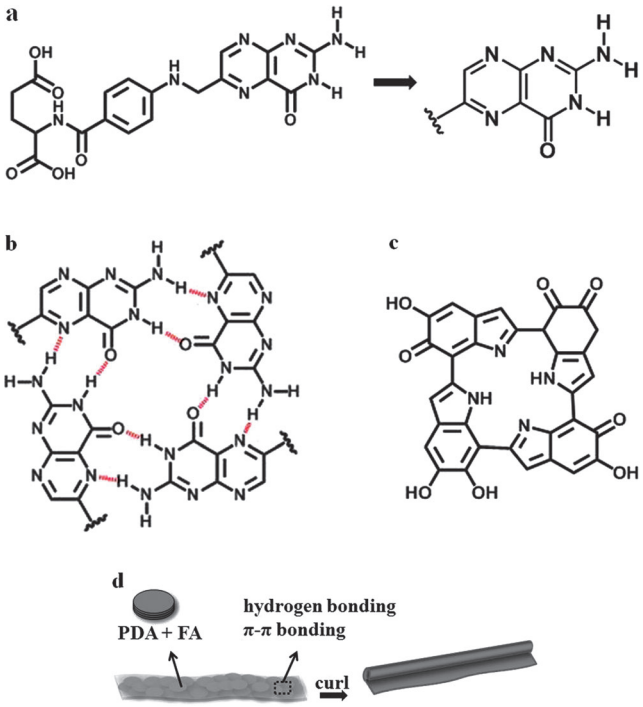
about the chemical composition of the PDA nanofibers (see Figure S11 and Table S2). The value of N/C is often used to monitor the growth of PDA layers and ranges from 0.08 to 0.17 in different cases.[7c,13] In our case, the N/C value is 0.115. The best fit for the C 1s spectrum was obtained with four components corresponding to the carbon atoms (see Table S2): C–C (284.7 eV), C–N/C–OH (286.1 eV), C=O (287.9 eV), and a π–π* transition (291.3 eV). π–π* transitions were observed in autooxidized DHI melanin free acid, DHI methyl ester,[13b] and PDA thin films.[14] In our PDA nanofibers, the ratio of the π–π* transition (5.2%) was slightly higher than those reported previously (2.6 and 4%). The N 1s region was fit with three peaks assigned to primary ($R-NH_2$), secondary (R^1-NH-R^2), and tertiary/aromatic (=N–R) amine functionalities. The content of tertiary/aromatic amines (8.9%) was lower in PDA nanofibers as compared with reported values (13.6[12] and 11%[14]). FTIR spectroscopic analysis also provided information about the functional groups in the PDA nanofibers. They were similar to those in PDA nanoparticles (see Figure S10). A broad and strong peak at 3400–3300 cm^{-1} was assigned to ν(N–H) and ν(O–H) stretching, a peak at 2922 cm^{-1} is due to ν(C–H) stretching modes, a weak peak at 1749 cm^{-1} is due to ν(C=O), a peak at 1618 cm^{-1} was attributed to aromatic C=C bonds of indole, and a peak at 1385 cm^{-1} was assigned to C=N–C stretching modes.[15] The peak at 1749 cm^{-1} was not present in PDA nanoparticles, but was observed for PDA nanofibers. We think that its existence in the spectrum of the PDA nanofibers may be due to the C=O bond in folic acid. MALDI-TOF MS showed ionic species of low m/z ranging from 490 to 1200 Da (see Figure S12), in agreement with previous MALDI-TOF MS analysis of natural melanins.[16] The similarity of the MALDI-TOF mass spectrum of the PDA nanofibers generated in the presence of folic acid to that of pure PDA nanoparticles confirms the similarity of the chemical nature of these nanostructures. We also conducted experiments to investigate the chemical reactivity of these PDA nanofibers. The reduction ability of PDA has been used to generate metal/PDA hybrids.[3b] In particular, the growth of gold nanoparticles on a PDA thin film and PDA nanoparticles was reported previously.[17] We attempted similar experiments and found that gold nanoparticles were also loaded on PDA nanobelts and nanofibers (see Figure S13) without any additional reductive agents. This result further supports the proposed chemical nature of the PDA nanofibers.

The above analysis and experiments showed that the obtained nanofibers and nanobelts are similar to PDA nanoparticles and thin films in their chemical nature, although they may be hybrid materials and composed of polydopamine, folic acid, and even Tris moieties, as indicated in a previous study.[18] Because folic acid itself cannot form a solid aggregated nanostructure in Tris buffer without dopamine, we propose that the obtained nanofiber is a new kind of aggregate structure of dopamine. The study of melanins shows that melanin is composed of aggregated oligomeric species (tetramers/hexamers), which stack through aromatic π interactions to form planar sheets.[19] In 2001, Clancy and Simon proposed a stacked structure in which the protomolecule is a planar oligomer consisting of approximately five indole units and assembled through π-stacking and side-on interactions.[4d] Such a two-dimensional sheet model is now widely accepted on the basis of different experimental studies.[20] The observation of nanofibers curled from nanobelts also indicates that the two-dimensional planar sheet is formed in the self-polymerization of dopamine in the presence of folic acid; however, the size of these planar units, their width and length, is much larger than we expected, between several hundred nanometers and several micrometers. The enlargement of the planar sheet in the X,Y direction may be due to the influence of folic acid.

The ability of folic acid to manipulate the aggregated structure of self-polymerized dopamine is an unforeseen result. By analyzing the chemical structure of folic acid, we found some important structural features that direct strong supramolecular interactions. Folic acid is composed of pterin

(PT), *p*-aminobenzoic acid, and glutamic acid moieties (see Figure S2a). The self-assembly of pterin rings through hydrogen bonding is well-documented, in contrast to their self-assembly through π–π interactions.[21] The pterin ring of folic acid has the potential to show two hydrogen-bonded self-assembly patterns: ribbonlike and disklike.[21] Gottarelli and co-workers reported that folic acid self-assembles into a tetramer, which forms columnar structures in aqueous potassium salt solution.[21a] The self-assembled folate tetramers can function as structure-directing agents or templates for the formation of mesoporous silica.[21f,g] The pterin moiety of folic acid shares great similarity with guanine, whose supramolecular polymer structure is well-known: Columns are composed of stacked arrays of tetramers, each formed by four Hoogsteen-bonded guanosine residues.[21b–d] However, the PT ring is a planar N heterocycle, which can act as a π acceptor to interact with strong π donors, such as polycyclic aromatic hydrocarbons (PAHs)[22] and polythiophene derivatives.[23] Derivatives of guanosine and pterin assemble into supramolecular polymers in water. The combination of π–π interactions and hydrogen bonding enables the formation of highly ordered supramolecular polymers in a wide range of solvents, which are not limited to apolar solvents.[24] On the other hand, for the generation of eumelanins, π–π interactions show more significant effects.[25] Recently, Mezzenga and co-workers reported that the self-assembly of *N*-(9-fluorenylmethyloxycarbonyl)-3,4-dihydroxy-L-phenylalanine (Fmoc-L-DOPA) led to the formation of twisted fibers at pH 2 through π–π stacking.[26] Because π–π interactions are recognized supramolecular interactions involved in the stacking of protomolecules of melanin, the ability of folic acid to change the nanostructure of PDA may partly originate from its role as a π acceptor.

In particular, Kaxiras and Meng have proposed a porphyrin-like protomolecule for eumelanin.[27] Chen et al. reported that the self-assembly of tetramers of DHI can explain the physical properties of eumelanins on the basis of experimental results and density functional theory calculations.[25c] We were surprised to notice that the proposed tetramers of melanins have a similar structure to that of the tetramers formed by the pterin groups of folic acid (Scheme 1b,c).[21a,25c,27] The similarity in structure of these two intermediate products may somehow explain why folic acid plays such a significant role in manipulating the supramolecular structures of PDA. We suppose that the stacking of the pterin part of folic acid through π–π interactions (at 60 °C) may change the supramolecular stacking of protomolecules of PDA. At the same time, the involvement of folic acid in PDA, through noncovalent or covalent interactions, also brings multiple hydrogen bonding to these stacks; as a result, stacks are promoted to aggregate in a side-on style, thus leading to a large extension in the *X* and *Y* directions (Scheme 1d). The curling tendency of PDA nanobelts with elongation of the reaction time may be a result of the increased hydrophobicity of longer nanobelts: The surface tension of aggregates in water can be lowered by curling. A further theoretical study may give a clear picture of the interaction between folic acid and protomolecules of PDA. Investigation of the transport properties of PDA nanofibers generated with the assistance of folic acid may give us experimental data on these 1D organic electronic materials composed of biomolecules.



Scheme 1. a) Chemical structure of folic acid. b) Tetramer composed of pterin rings of folic acid.[21a] c) Proposed intermediate protomolecule in the formation of melanins.[27b] d) Proposed formation mechanism of PDA nanofibers.

Prior to this study, we knew that folic acid and melanin have a strong relationship in terms of their biological functions in humans. Melanins, as a natural pigment in human skin, protect against the UV-light-induced breakdown of folic acid,[28] and folate regulates melanin production as well.[29] Our experiments show that the strong supramolecular interaction between protomolecules of PDA and folic acid may lead to the formation of PDA nanofibers; we wonder whether a similar supramolecular interaction between melanin and folic acid may occur in the organism. There are several open questions about the structure of eumelanins,[2a] and the formation mechanism of eumelanin nanostructures from protomolecules of melanins is a key point in understanding the supramolecular nanostructures and physical properties. We suppose that the knowledge we obtained from the fabrication of PDA nanofibers will help us to reveal functional structures of eumelanin and produce novel materials.

In conclusion, we have reported the first successful fabrication of PDA nanofibers. With the assistance of folic acid, dopamine molecules formed nanofibers through oxidative self-polymerization. Morphological analysis indicates that these nanofibers are curled from nanobelts whose thickness is approximately 31 nm. Characterization indicated that they are chemically similar to the other two well-known PDA nanostructures: nanoparticles and thin films. We proposed that supramolecular interactions between folic acid and protomolecules of PDA, such as π–π interactions

and hydrogen bonding, contribute to the formation of nanobelts and nanofibers. This study reveals the formation mechanism of polydopamine and also sheds light on the mystery of the supramolecular structure of natural melanins. Further study of the physical properties of PDA nanofibers will be useful for developing new bioorganic optoelectronic materials.

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