Controlled self-assembly and templated metallization of fibrinogen nanofibrils†

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Fibrinogen (Fg) nanofibrils were prepared by the ethanol-induced self-assembly of Fg molecules, which can be used as fibrous scaffolds to organize gold nanoparticles (NPs).

It is a feasible strategy to fabricate many composite materials by the self-assembly of NPs on biomolecular templates.[1] These organic templates, such as proteins,[2] viruses,[3] and DNA strands[4] have been used to organize NPs on solid surfaces. With the development of one dimensional (1-D) nanomaterials for nanoelectronics and nanobiotechnology, the method to prepare 1-D nanostructures by absorbing and assembling NPs on natural and artificial 1-D biomolecules templates has been investigated recently.[5–7] Among these artificial templates, protein fibers are very important in the fields of biomaterials science,[8] tissue engineering,[9] and biomedicine.[10–12] Although the formation and deposition of amyloid-like protein fibers in the human body are not desired,[11,12] some of the properties of protein fibers are useful for nanotechnology. These protein fibers are characterized not only by their suitable physical size in nanoscale, high degree of stiffness and great stability, but also by their extraordinary functionalities which are helpful for the binding to the electronic circuitry in the construction of nanoscale devices. Many strategies are known to prepare amyloid-like fibers by aggregating proteins and peptides, such as elevating the system temperature,[11,13] changing the pH of protein solution higher or lower than the isoelectronic point of proteins,[11,12,14–16] introducing reagents that can denature the proteins,[17,18] self-assembling the engineered peptides,[19–21] and so on.[22] For the applications in nanotechnology, a variety of protein fibers have been used to organize metallic NPs and quantum dots.[10,23,24]

In this work, we report that one of the important plasma proteins, *i.e.* human plasma fibrinogen (Fg), can be denatured to form amyloid-like nanofibrils by the stimulation of ethanol, and the self-assembled protein nanofibrils can be used as flexible templates to prepare 1D inorganic–organic composite.

Fg plays crucial roles in mediating clot formation, cellular and matrix interactions, fibrinolysis and wound healing. Fg is a 340 kDa glycoprotein that contains two identical subunits, and the native structure of Fg contains a central E and two outer D globular domains connected by an α-helix. The two C-terminals depart from the D domain and fold back to form a single globular domain located closely to the E domain. The length of the Fg molecule is 47.5 nm.[25] The structure of Fg and the atomic force microscope (AFM) image of a single extended Fg molecule on new cleaved mica are shown in Fig. S1 (ESI†). AFM data showed that the height of the two D domains and length of single Fg molecule on mica are 0.58 ± 0.12 and 50.98 ± 3.57 nm, respectively.

The introduction of ethanol into the Fg aqueous solution caused the formation of linear protein fibrils. Different protein fibril structures were formed with different concentrations of Fg at the same ethanol concentration. Fig. 1 shows the AFM height images of protein fibrils formed when Fg solutions with different concentrations were mixed with ethanol (1 : 1, v/v) and incubated at 37 °C for 1 h. When the concentration of the initial Fg solution was 5 ng μL^{-1}, thin and straight fibrils with 4–5 μm length were formed, as shown in Fig. 1(a). When the concentration of Fg was increased to 20 ng μL^{-1}, the formed protein nanofibrils became shorter (1.92 ± 0.22 μm), and were accompanied by numerous globular protein aggregates. When the concentration was 50 ng μL^{-1}, protein fibrils with branches were formed, and the length of the fibrils was about 0.59 ± 0.17 μm. At the same time, globular protein aggregates were also formed (Fig. 1(c)). Higher Fg concentration

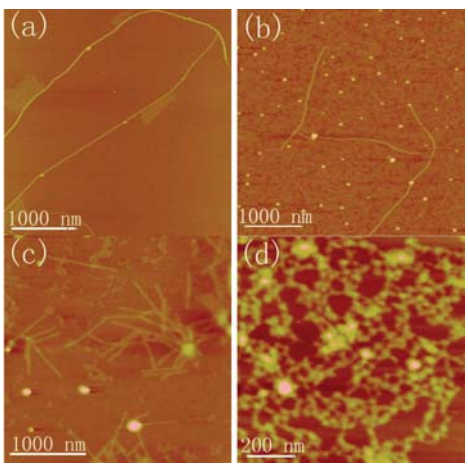

Fig. 1 Typical AFM images of Fg nanofibrils formed when the ratio (v/v) of initial Fg solutions with different concentrations to ethanol (95%) was 1 : 1. The concentrations of Fg were: (a) 5 ng μL^{-1}, (b) 20 ng μL^{-1}, (c) 50 ng μL^{-1} and (d) 200 ng μL^{-1}, respectively. The Z-range scale is 20 nm for all these images.

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† Electronic supplementary information (ESI) available: Preparation of Fg fibrils/Au NPs composites, AFM image of Fg molecules, TEM image of Fg fibrils, and EDX spectra of Au NPs. See DOI: 10.1039/b806316h

(200 ng μL^{-1}) prevented the formation of protein fibrils in this system, as shown in Fig. 1(d). Larger protein aggregates appeared and these spherical aggregates formed a network-like structure by connecting to each other. It should be noted that these results (Fig. 1(a)–(c)) differ from other previous studies, where higher protein concentrations (about 10 mg mL^{-1}) were used to prepare lysozyme fibers.[12,14,17] In our experiment, Fg concentrations between 5 and 50 ng μL^{-1} were suitable to form Fg fibrils.

In a control experiment, the effect of different ratios of Fg (20 ng μL^{-1}) with ethanol (95%) on the morphology of formed fibrils was investigated. At a ratio of 4 : 1 (v/v) of Fg to ethanol, Fg molecules revealed amorphous aggregates, as shown in Fig. 2(a). In this situation, no fibrils were formed in the solution, which indicated that it is difficult to form Fg nanofibrils at low concentration of ethanol. By increasing the ratio of Fg to ethanol, we were able to generate fibrils of different shape and length (Fig. 2(b)–(d)). At an Fg to ethanol ratio of 2 : 1, protein fibrils with lengths of 1–3 μm were formed. A typical AFM image of the products is shown in Fig. 2(b). It was found that both protein aggregates and fibrils existed in the solution, and the yield for the fibrils was very low. When the ratio of Fg to ethanol was 1 : 2, shorter protein fibrils with high yields were generated, as shown in Fig. 2(c). The histograms show that the height of the fibrils was 1.15 ± 0.12 nm and the length was 0.81 ± 0.28 μm (Fig. S2, ESI†). The structure of formed fibrils was also characterized by transmission electron microscope (TEM). Negative staining of the self-assembled Fg nanofibrils with phosphotungstic acid (PTA) was done according to the method described in ESI†. The TEM image of the Fg fibrils shows the width of the formed fibrils to be about 10 nm (Fig. S3, ESI†). Fig. 2(d) indicates a typical AFM image of fibrils that formed when the ratio of Fg to ethanol was 1 : 4. In this situation, long and thick fibrils with branched nanostructures were produced. These results concerning the ethanol induced uniform aggregation of protein molecules agree with the results reported by Goda *et al*., who prepared the amyloid protofilament of hen egg lysozyme in highly concentrated ethanol solution (>80%).[17]

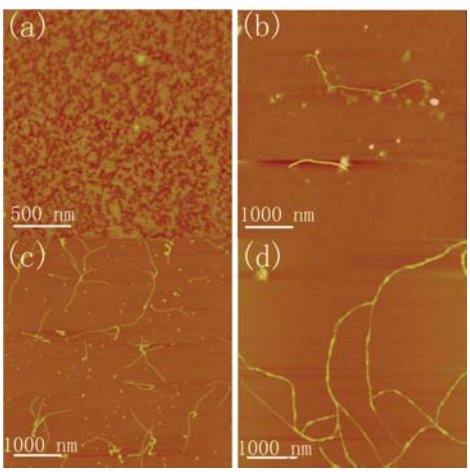

Fig. 2 Typical AFM images of Fg nanofibrils formed when the ratios (v/v) of Fg (20 ng μL^{-1}) to ethanol (95%) were: (a) 4 : 1, (b) 2 : 1, (c) 1 : 2 and (d) 1 : 4. The Z-range scale is 10 nm for (a), (b), and (c), 20 nm for (d).

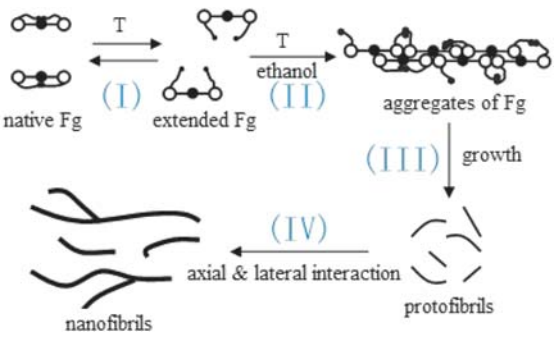

Fig. 3 Possible mechanism for the formation of Fg nanofibrils in highly concentrated ethanol solution.

It should be noted that the speed to form fibrils in the present study is much more rapid than that reported by others.[11–14] In our experiments, one hour was enough to generate a large amount of fibrils in highly concentrated ethanol solution (about 80%). A possible reason is that highly concentrated ethanol has stronger ability to change the conformation of Fg molecules than acids. We suggest that these branched and network-like structures were determined by the molecular structure of Fg. The two arms (αC terminals) in the Fg molecules contain complementary sites which can bind intramolecularly or intermolecularly to form superstructures.[26,27]

Fig. 3 shows a possible mechanism for the self-assembly of Fg molecules observed in the present study. In step I, when the temperature is being increased from room temperature to 37 °C, the native Fg molecules show a structural transformation to an extended conformation (AFM image of Fg in Fig. S1), and this process is reversible. At this temperature, the addition of highly concentrated ethanol causes the conformation change of Fg and destroys its secondary and tertiary structures.[17] Subsequently the self-assembly of molecules begins by the intermolecular interactions between two α C termini, as well as between two D domains and one E domain of Fg molecules,[27,28] as shown in step II (Fig. 3). Finally, the protofibrils are being formed (step III). Many small fibrils with a length of about 300 nm can be seen in Fig. 2(c). The special aggregation mechanism between Fg molecules causes the two ends and some points of the protofibrils to be active, therefore some of protofibrils could further form long nanofibrils by axial and lateral interaction (step IV). The experimental results shown in Fig. 1(a)–(c) and 2(b)–(d) support this proposed mechanism.

It is well known that uniform protein nanostructures are very suitable precursors to create different metallic nanowires. Templated metallization of Fg nanofibrils was demonstrated in the current study by two different strategies,[29] and the detailed methods are described in ESI†. In the first strategy, Fg fibrils/$AuCl_4^-$ composites were directly reduced in the mixed solution by adding $N_2H_4{\cdot}H_2O$; in the second strategy, Fg fibrils/$AuCl_4^-$ composites were first dropped onto the carbon-coated copper grid and then reduced with $NaBH_4$ to prepare Au NPs on the Fg fibrils templates.

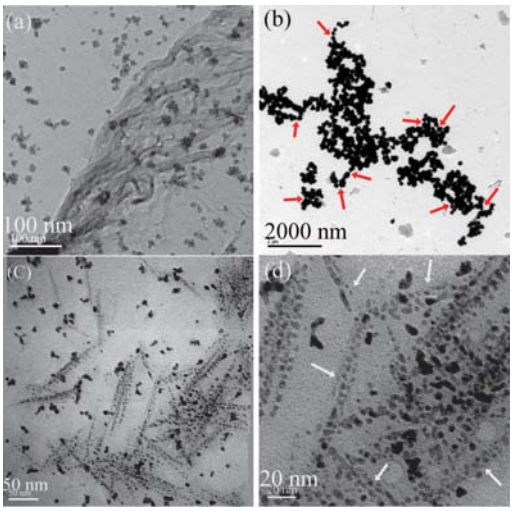

Fig. 4 TEM images of Fg nanofibrils/$AuCl_4^-$ composites before (a) and after reducing by $N_2H_4{\cdot}H_2O$ (b) and by $NaBH_4$ (c and d). (d) A magnified image of (c).

In this experiment, Fg nanofibrils prepared with a ratio of 1 : 2 (Fg : ethanol, v/v, in Fig. 2(c)) were used. Fig. 4(a) presents a TEM image of Fg fibrils/$AuCl_4^-$ composites before reduction. It is clear that the adsorption of $AuCl_4^-$ ions on the Fg fibrils did not change the original structure of the fibrils. Fig. 4(b) shows a TEM image of Fg fibrils/Au NPs composites prepared by the first method. Au NPs with a diameter about 50–100 nm were formed and the Fg fibrils can also be seen in these composites (indicated by arrows), and the formed Au NPs reveal an aggregated status. It is challenging to prepare linear Fg nanofibrils/Au NPs composites by the solution-phase synthesis, because in solution the prepared Au NPs readily connect to each other to form aggregates in the absence of capping reagents, as shown in Fig. 4(b). In the second strategy, the possibility to destroy the templates or cause aggregation was decreased. Au NPs were deposited on the templates of Fg fibrils, as shown in Fig. 4(c). The prepared fibrils/Au NPs composites kept the shape of Fg nanofibrils after coating with Au NPs. The magnified image indicated the formed Au NPs were discrete and uniform, and the mean diameter was 4.5 ± 0.3 nm (Fig. 4(d)). The linear Au NPs assemblies can be seen clearly, as indicated by arrows, and their width was about 13 nm. Furthermore, Au NPs were identified by TEM-energy-dispersive X-ray (EDX) spectroscopy (Fig. S4, ESI†).[29a] Obviously, the second strategy is more feasible to prepare uniform Fg fibrils/Au NPs composites. The stability of Fg nanofibrils/Au NPs is high, and the possible driving force between Au NPs and Fg nanofibrils is the electrostatic interaction (positively charged Fg nanofibrils and negatively charged Au NPs).

In conclusion, for the first time we report the preparation of amyloid-like Fg fibrils by ethanol-induced self-assembly of Fg molecules in the absence of thrombin, and furthermore we produced Au NPs on these fibrous templates. Because the protein fibrils can provide great stability in some special conditions, such as elevated temperature and in strong acid or alkali which are necessary for industrial metallization, the formation of Au NPs on the Fg fibrils shows potential application in nanoelectronics. In the future, attention should be focused on how to achieve the anisotropic assembly of spherical metallic NPs on single Fg nanofibrils, and how to prepare continuous metallic nanowires for practical applications, such as the basic measurement of its Ohmic conductivity.

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