Fibrinogen Nanofibril Growth and Self-Assembly on Au (1,1,1) Surface in the Absence of Thrombin

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Direct Observation of Self-Assembled Fibrinogen Fiber Formation on Au(1,1,1) in the Absence of Thrombin

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Abstract

Fibrinogen (fg) molecules were observed to form very well organized patterns of nanofibrils by self-assembling on Au (1,1,1) surface without any addition of thrombin, growing in two orientations (longitude and transverse). This observation is new and unique for gold surfaces, in contrast with Mica or HOPG surfaces. Based on the experimental results, we proposed an assembly mechanism: Au-S interactions and its activated interactions in the 'αC-domain' are two main causes for the patterned assembly on Au(1,1,1) surface, and 'D: D' and 'γ_{XL}' interactions help the elongation and strengthening of the fibril assembly.

Understanding protein adsorption on gold surface bears increasing importance for many reasons.[1] For example, colloidal gold and gold nanoparticles are finding very useful applications in disease treatment including as therapy for rheumatoid arthritis and Alzheimer's disease and as drug delivery carriers;[2] gold surface is widely used in label-free detection methods such as surface plasmon resonance; and gold materials are also used in the development of biosensors and tissue engineering scaffolds.[3] Due to its paramount role in blood coagulation and its high concentration in the blood, fibrinogen (fg) has been extensively investigated for its absorption on to different surfaces,[4–9] particularly for its surface-induced changes in conformation and bioactivity. However, the absorption of fg on gold surface is least investigated, lagging the advancement of gold nanoparticle studies for biomedical applications. Here we report our finding that fg self-assembly into highly ordered nano-fibrils in two growing orientation on bare Au(1,1,1) surface in the absence of thrombin, and propose a possible mechanism.

We observed large variations of fg assembly patterns on Mica (Figure 1(a)), HOPG (Figure 1(b)), and Au(1,1,1) (Figure 1(c)) surfaces. The samples were prepared and studied under the same conditions (4 μg/mL fg and 50 min incubation, see detailed methods in SI A). As shown in Figure 1(a), most fg molecules on the mica surface maintain its native trinodules structure, which was first proposed by Hall and Slayter.[10] The length of one molecule is about 50~60 nm, which is very close to the reported data.[7] In contrast to the mica surface, the HOPG surface was densely covered by an amorphous protein layer, which indicates that the hydrophobic HOPG surface has stronger adhesion to fg than the hydrophilic mica surface. A similar conclusion was also attained by Agnihotri[4] and Geer.[5] The isoelectric point of gold surface has been reported to be around pH 4.5,[11] indicating that the gold

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Supporting Information Available Sample preparations, Experimental procedures and controls, and additional experiments are provided (5 pages) (PDF).

surface is negatively charged in a neutral buffer. In addition, gold surface has been reported to be hydrophilic,[12] which would not normally favor protein assembly as much as hydrophobic surfaces such as HOPG. However, in our study, more fg molecules were absorbed on the gold surface than on the mica surface and they form highly ordered patterns, as shown in Figure 1(c). The highly ordered patterns on gold surface are in a sharp contrast to the disorganized fg layer on the HOPG surface. Since the UV405 nm data already excluded the possibility of fg coagulation in the solution (SI C, fibrinogen clotting tests), one can conclude that fg absorption and organization on the gold surface is due to special interactions between the gold surface and protein molecules.

To study fg absorption on Au(1,1,1) surface, we performed in-situ imaging, as shown in Figure 2(a)-(f). It is clear that fibrinogen grows into fibril in two orientations (longitude and transverse), step by step, once the first fibrinogen molecules are attached on the surface. The single fibrinogen strand is clearly resolved, with ~5 nm in width, which is very close to the diameter of the nodule of D domain and E domains.[13] The observed assembly process is totally different from the cases with thrombin addition[8] or the denaturation of fg.[14,15] It was proposed that the coupling of 'αC domains' on adjacent molecules could contributes to the formation of fibrinogen fibril.[9,14] However, coupling of 'αC domains' cannot be used to fully explain our observation since the electrostatic interactions between the negatively charged gold surface and positively charged 'αC domains' would make these domains unavailable to engage in lateral intermolecular interactions.[9] One logical hypothesis is that the strong interactions between Au and sulfides could be the driving force of the self-assembly, given that there are many cysteine residues in fg.[16] Specifically, fg crystal structure shows that many cysteine residues are accessible and could be available for Au-S bond formation. As an example, part of the protein structure and cysteine residues is presented in SI E. Although some of the Cys residues are in the of form of disulfide bonds,[17] it is well known that they can be reductively broken with the formation of strong Au-S bonds as the driving force.[18]

Human fg is a dimeric molecule, the disruption of disulfide bonds in the E domain would result in the appearance of half-molecules.[17] We did observed fg patterns with different length in the transverse growing fibrils as shown in Figure 3(a). The length of part A is around 22 nm, which is almost exactly half of the dimeric molecule. Nevertheless, Part B (~41 nm) is consistent with a dimeric fg molecule based on its length. Part C can be one dimeric fg and one half fg bound to each other, giving rise the length of ~65 nm.

Figure 3(b) presents another case of assembly: longitude growing fibrils. We also performed surface plasma resonance (SPR) experiments, which are described in detail in SI D, to test the adhesion phenomenon. The data clearly indicated that the adsorption process was not pure physical process and there were strong interactions between the protein and gold surface which are consistent with Au-S bond formation.

Why fg molecules align themselves orderly on Au(1,1,1) surface in their self-assembly? Herein, we propose a mechanism based on our experimental observations. There are three possible non-covalent interactions participating in fg assembly and cross-linking, namely 'αC-domain' in the α chain of the D domain,[19] 'γ_{XL}' and 'D:D' in the γ chain of the D domain.[20,21] Among these, the 'αC-domain' is an important factor in lateral fibril association and extensive network assembly. As discussed previously, the negative charges of gold surface could be impedance to activating the 'αC-domain'. However, the strong Au-S interaction between gold surface and the E domain would overcome the hindrance to separate the 'αC-domain' from the E domain since the interactions between the *C*-terminal of the α-chains and the E domain highly depend on the formation of disulfide bonds.[22] The 'αC-domain' interactions were thought to be a vital factor in the formation of fibril on the

surface.[9,14] For the later two interactions, they are self-association processes, so they can happen when the binding sites are available. Certainly, the very important Au-S interactions could not be ignored.

Patch B in Figure 3(a) could be the simplest case. The released 'αC-domain' by Au-S interaction could be the main reason for the patterned structure, as shown in Figure 3(c). For patch A in Figure 3(a), the half molecule assembly could be possibly attributed to the same reason but with different connectivities as illustrated in Figure 3(d). While the length was getting longer, such as patch C in Figure 3(a), the possible interactions in the assembly could be more complicated; however, 'αC-domain' interactions still could be the main reason. Besides, 'D:D' interactions would be helpful for the elongation, as shown in Figure 3(e). Figure 3(f) illustrated the most complicated case, the longitude growing fibril, as shown in Figure 3(b). All the three interactions could possibly contribute to the formation of the longitude assembly.

In summary, nanofibril structures of fg molecules are self-assembled on Au(1,1,1) surface without any addition of thrombin, growing in two orientations (longitude and transverse). This observation is unique for gold surface, in contrast with Mica or HOPG surfaces. According to the experimental results, we proposed the assembly mechanism: Au-S interactions and its activated interactions in the 'αC-domain' are two main causes for the patterned assembly. Besides, 'D: D' and 'γ_{XL}' interactions do help the elongation and strengthening of the fibril assembly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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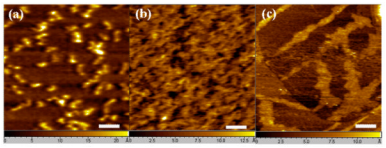

Figure 1.
The topographic images of fg on three different surfaces: (a) Mica; (b) HOPG; and (c) Au(1,1,1) (Scale Bar: 100 nm)

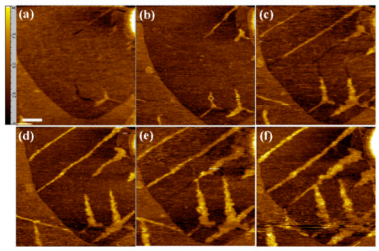

Figure 2.
In-situ imaging of fg self-assembly on Au(1,1,1) (Scale Bar: 100 nm). The time interval between consecutive images is 8 minutes.

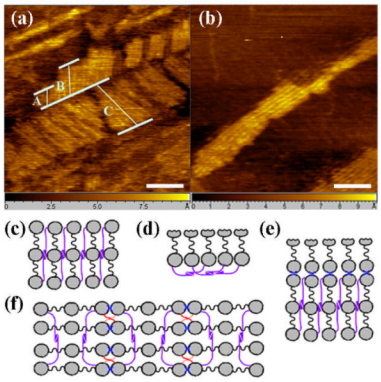

Figure 3.

High resolution images of Fb transverse assembly (a) and longitude assembly (b). (scale bar: 50 nm) The schematic representation of the possible mechanism for the assembly: (c) for patch B in transverse case; (d) for patch A in transverse case; (e) for patch C in transverse case; (f) for the longitude case. (curve with arrow: 'αC-domain' interaction; blue fragment: 'D:D' interaction; red curve: 'γ_{XL}' interaction).