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Soft Bio-materials in Solid State Devices

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1. Introduction

Biomolecules are members of soft-materials and their characters can be summarized as their abilities of specific, or selective, recognition of other molecules. The combinations of 20 amino acids make up various polypeptides (=proteins) with the ability to distinguish particular target molecules from others. What's more, developments in molecular evolutionary systems have enabled us to create artificial peptides (= peptide aptamers) that specifically bind to selected target molecules assigned by researchers [1]. The system was originally applied for creating peptides that would bind to biomolecular targets such as receptors and antibodies. Over the years, the targets have been extended to solid-state molecules [2, 3], and peptide aptamers have been created that recognize a variety of materials [4]. By using these solid material-binding peptides, we can endow the surfaces of soft or hard materials with the specific binding ability of these peptides. Using our titanium (Ti) binding peptide, TBP-1, as a model, I will introduce how such soft bio-materials can be used for the field of solid state devices.

2. Titanium binding peptide, TBP-1

TBP-1 is a 12 amino acids peptide that was selected for its capacity to bind Ti particles through a bio-panning process using a peptide phage system [5]. Further analyses have revealed that the N-terminal hexapeptide (RKLPDA), which we named "minTBP-1," was sufficient for Ti binding, and that that the arginine (R1), proline (P4) and aspartate (D5) residues are essential for the binding. Among these three essential residues, proline is known to introduce a kink into the main chain of peptides, and the side chains of arginine and asparate act, respectively, as a base and acid at neutral pH. Considering the fact that the surface of Ti in water is covered with an oxidized film displaying both acidic ($-O^-$) and basic ($-OH_2^+$) hydroxyl groups, we proposed a model in which minTBP-1 binds electrostatically to Ti (Figure 1) [5].

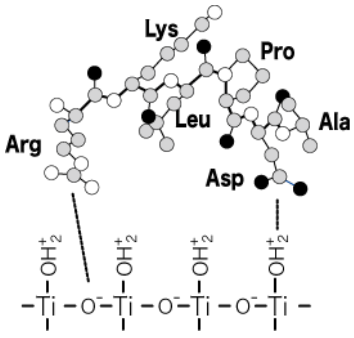

Fig. 1 Titanium binding peptide, TBP-1 and its binding mode.

We also evaluated the binding capacities of TBP-1 to various solid-state materials, including Si, Ag, Au, Cr, Pt, Sn, Zn, Cu and Fe, and found that, in addition to Ti, TBP-1 also binds Si and Ag, but not Au, Cr, Pt, Sn, Zn, Cu or Fe [6]. Quantitative analyses using atomic force microscopy indicated that the adhesion force between minTBP-1-displaying ferritin and the surface of Si was weaker than the force observed between the particle and Ti [7]. We therefore conclude that TBP-1 is a strong binder of Ti and Ag and a moderate binder of Si.

3. minTBP-1-displaying ferritin

Ferritin is a nanometric scale spherical protein composed of 24 subunits, whose diameter is approximately 12 nm. Ferritin has a cavity that is 7-8 nm in diameter, within which ferrihydrite is stored *in vivo* and it has been shown that various inorganic materials, including CdSe, Co_3O_4 and ZnSe, among others, can be deposited into the inner space *in vitro* [8]. We considered the possibility of endowing the exterior surface of ferritin with the ability to specifically bind Ti by grafting minTBP-1 to ferritin and then selectively positioning nano-dot-loaded ferritin in a nanometric Ti pattern lithographed onto a base material. We inserted a DNA cassette encoding minTBP-1 at the 5' region of the subunit gene so that minTBP-1 and a ferritin subunit would be translated as a fusion polypeptide. The resultant minTBP-1-displaying ferritin was endowed with Ti-binding ability [9] and has been used to selectively positioning ferrihydrite- or Co_3O_4-loaded minT1-LF on lithographically prepared Ti nanopatterns [10].

4. Multifunctionality of TBP-1

Recent studies have revealed that peptide aptamers against solid-state materials have often mineralization activity as well as binding ability. For instance, silicification from prehydrolyzed tetramethoxysilane (TMOS) was markedly accelerated in the presence of TBP-1. Likewise, when we incubated the peptide with $AgNO_3$ at room temperature, Ag nanoparticles were formed that ranged from 300 to 400 nm in size with good crystallinity [6]. Thus, TBP-1 is a bifunctional peptide: it functions as both a binding protein and as a mediator of mineralization.

5. BioLBL

BioLBL is a novel method for fabricating multilayered nanostructures [11]. The first step starts with the specific binding of a nanomaterial displaying TBP-1, minT1-LF for instance, to a substrate. Within minTi-LF, 24 minTBP-1 molecules are nearly symmetrically distributed on the surface of each ferritin molecule. Some of them are engaged in binding to the Ti plate, but the rest are free to access chemical compounds in solution. Consequently, the addition of prehydrolyzed TMOS resulted in the deposition of a thin silica film on the layer of ferritins by virtue of the mineralization mediated by minT1-LF. If we used Ti(IV) bis(ammonium lactato)-dihydroxide (TiBALDH) instead of TMOS, a thin titania film was formed on the minT1-LF layer [12]. The newly formed mineral film was then the binding target of minT1-LF, and a second minT1-LF monolayer was formed on the silica (or titania) layer. Because the first and second layering of ferritin can be performed in separate reaction vessels, ferritin containing different metal nanodots can be layered in a stepwise manner.

This second layer of minT1-LF could then serve as a catalyst for a second deposition of mineral film, which in turn could served as the target for a third minT1-LF layer. This cycle of minT1-LF binding and mineralization can be repeated until the desired number of layers is reached. The resultant structure, comprised of alternating layers organic molecules (loaded metal nanodots) and mineral deposits. In addition, the intervening mineral layers prevented interlayer diffusion of minT1-LF, so that each ferritin layer was well separated from all others, even in the vicinity to the base substrate, which is difficult to achieve using conventional layer-by-layer methods. The excellent ability of BioLBL to segregate the contents one layer from the others enables us to fabricate heterogeneous multilayer structures by using different metal-containing ferritins.

6. *in aqua* structuralization of 3-D nanostructures using DP-BioLBL

In DP-BioLBL [12], we first lithograph Ti nano-patterns on a thin Pt film using the conventional top-down method. Then layers of minT1-LF are selectively positions on the Ti patterns. This X-Y positioning reflects the ability of minTBP-1 to distinguish between Ti and Pt. Within these constrained minT1 LF regions, multiple layers of titania and minT1-LF are further stacked by alternately applying the binding and mineralization capabilities of minTBP-1. Because the Pt regions are not a binding target of minT1-LF, nor do they mediate mineralization of titania, layers are only formed in the patterned Ti region.

7. Conclusions

TBP-1 is a peptidic Ti, Ag and Si binder, and has the ability to enhance the formation of titania and silica as well as nanoparticles of Ag. TBP-1 is thus a bifunctional soft-material: a binder that also acts as a mediator of mineralization. When the surface of ferritin was ornamented with minTBP-1, the resultant modified ferritin acquired the ability to bind Ti and mediate mineralization. By alternately applying the binding and mineralization activities of the minTBP-1-modified nano-cage, we were able to construct multilayer structures composed of titania (or silica) and nano-cages. By coupling BioLBL with a conventional top-down lithographic method, in aqua structuralization of a three-dimensional (3D) configuration of nanomolecules was realized.

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