Arrangement of Ferritin Molecules on a Gold Disk Array Fabricated on Highly Ordered Anodic Porous Alumina Substrate

Hideki Masuda,*[†,††] Hiromi Hogi,[†] Kazuyuki Nishio,[†,††] and Futoshi Matsumoto[††]
[†]*Department of Applied Chemistry, Tokyo Metropolitan University, 1-1 Minamiosawa, Hachioji, Tokyo 192-0397*
[††]*Kanagawa Academy of Science and Technology, 5-4-30 Nishi-Hashimoto, Sagamihara 229-1131*

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An ordered array of ferritin, in which the arrangement of ferritin coincided with the arrangement of Au disks, was prepared on Au nanodisk arrays fabricated on a highly ordered anodic porous alumina substrate. Selective adsorption of ferritin onto the Au disk by controlling the modification time and ferritin concentration in the modification solution was confirmed by scanning electron microscopy.

The arrangement of functional biological molecules such as proteins, DNA, and antibodies into biomolecular arrays has attracted considerable interest because of the potential application of such arrays to useful tools for estimating specific signals in living cells and molecular electronics.[1–3] To date, many studies on the arrangement of biological molecules have been limited to submicron-level patterning within two dimensions using conventional photoresist lithography, microcontact printing, and a self-assembled monolayer.[4–6] In order for the biological arrays to become widely used, arrays with smaller disk interval and diameter, on which biological molecules are patterned on the nanometer level, need to be developed. In addition, in order to confirm the arrangement of biological molecules on the nanometer level on 2-D substrates and detect accurately and rapidly signals from each pattern, the patterns of biological molecules should be highly ordered with nanometer-sized intervals. In this study, we show for the first time the highly ordered arrangement of biological molecules on the nanometer scale using Au nanodisk arrays (Figure 1) fabricated on a highly ordered anodic porous alumina substrate, which has a nanometer-scale porous structure with hexagonally arranged close-packed cells over a large area,[7,8] by filling the pores with Au. The selective adsorption of biological molecules onto the Au disk surface, that is, the specific interaction between biological molecules and the Au disk surface, leads to the simple formation of a highly ordered biomolecular array with a high density. The fabrication of an array of ferritin molecules, which has a ferric oxide core and a spherical shape and whose molecular size is 13 nm,[9,10] as a model for nanometer-sized biological molecule array, was performed on the Au nanodisk array with a disk period (center-to-center distance between the disks) of 63 nm and a disk diameter of 46 nm.[11]

A Au disk array with a disk period of 63 nm and a disk diameter of 46 nm was prepared on an anodic porous alumina substrate having the same structure. Aluminum (99.99% purity) was anodized using the Al plate as a counter electrode for 4 h in 0.3 M sulfuric acid solution under a constant voltage of 25 V at 16 °C with stirring, after electrochemical polishing using a mixture of perchloric acid and ethanol under a constant current of 100 mA·cm^{-2} for 4 min at 0 °C. The substrate was dipped into a 5% (by weight) phosphoric acid solution for 10 min at 30 °C to widen the pore diameter. Then, Au was deposited in the pores of the porous alumina by AC electrolysis at 11 V until the deposited Au overflowed the pores using a Pt plate as a counter electrode in an aqueous solution containing 2.5 mM $HAuCl_4$, which was adjusted to pH 1.2 with H_2SO_4.[12] The over-flowed Au was then etched using Ar ion milling to planarize the surface. Ferritin from an equine spleen (90% purity) was obtained from Sigma and used without further purification. Ferritin patterns on the Au disk array were observed using a scanning electron microscope (SEM, JSM-6700F, JEOL).

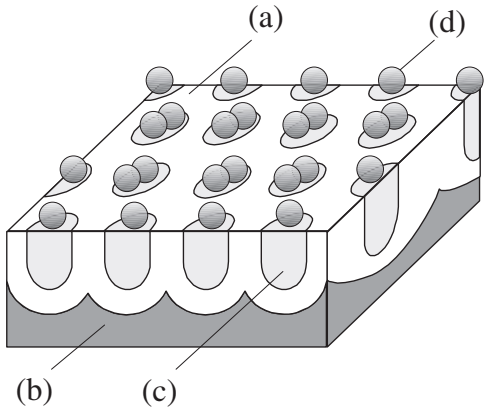

Figure 1. Schematic of highly ordered ferritin molecules obtained using a highly ordered Au disk array fabricated with anodic porous alumina. (a) porous alumina, (b) aluminum, (c) Au, and (d) biological molecule.

Figure 2 shows typical SEM images of the ordered Au disk array fabricated using highly ordered anodic porous alumina with a pore diameter of 46 nm and a pore period of 63 nm. The Au disks are observed as bright spots having uniform diameters,

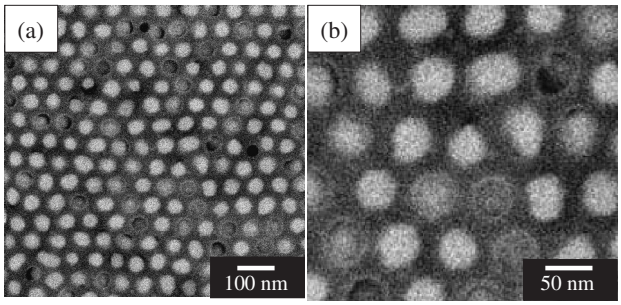

Figure 2. Typical SEM images of an ordered Au disk array with a disk diameter of 46 nm and disk period of 63 nm. (a) lower magnification and (b) higher magnification.

and can be seen to be arranged uniformly over the sample except for a few holes in which the Au metal was not fully deposited. The average diameter of the Au disks as estimated from the SEM observation was 46.2 nm. The high-resolution image (b) of the Au disk surface confirms that it is clean and uniform. On the other hand, in the image of the Au disk array on which the ferritin molecules adsorbed (Figure 3), the ferritin molecules are observed as brighter spots because of the ferric oxide core.[9,10] The figure shows that many ferritin molecules are adsorbed selectively on the Au disks to form a clearly defined array of ferritin with a Au disk arrangement, while very few ferritin molecules are adsorbed onto the surrounding alumina. The selective adsorption of ferritin onto the Au disk is thus confirmed to occur. From the SEM image of Figure 3, three to five bright spots, due to the ferric core of the ferritin molecules, can be observed on each Au disk. The SEM images in Figure 4 show the dependence of the amount of adsorbed ferritin on the concentration of ferritin in the modification solution. The extent of adsorption depends strongly on the concentration of ferritin. At a ferritin concentration of 0.125 $mg \cdot mL^{-1}$, a few ferritin molecules adsorb onto the Au disk. The Au disk onto which ferritin molecules do not adsorb can also be observed. On the other hand, at a ferritin concentration of more than 0.5 $mg \cdot mL^{-1}$, ferritin adsorbs not only onto the Au disk but also onto the surrounding alumina. A number of ferritin molecules are strongly attracted by ferritin adsorbed onto the Au disk surface with van der Waals attraction between molecules. Ferritin layers larger than the Au disks are formed on the Au disk array. It is difficult to form a ferritin array with the size coincided with one of the Au disk at a ferritin concentration of more than 0.5 $mg \cdot mL^{-1}$. The trend in the surface coverage with modification time is similar. At a modification time of more than 30 min, ferritin molecules start to adsorb onto the alumina. A modification time of 30 min and a ferritin concentration of 0.25 $mg \cdot mL^{-1}$ are therefore considered most suitable for achieving a well-separated arrangement of ferritin. Moreover, the amount of adsorbed ferritin on the Au disks was sensitive to the change in pH of the modification solutions. Selective adsorption of ferritin onto the Au disk could not be observed in the acidic solution with a pH of less than 6.5 and an alkaline solution with a pH of more than 9. The adsorption behavior of ferritin onto the Au disk surface may be attributed to the surface charge of the ferritin molecule and the porous alumina substrate. Therefore, the control of the concentration of ferritin, immersion time in the modification solution, and solution pH are important in the fabrication of a biomolecular array of regular size and arrangement.

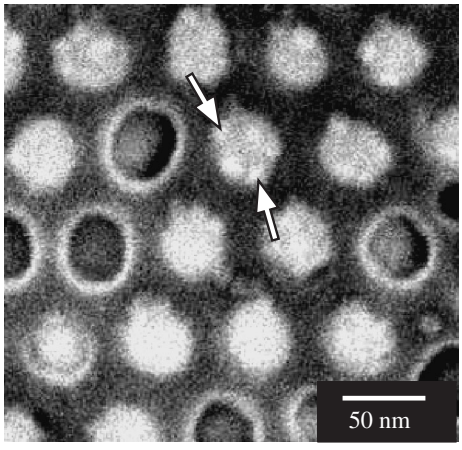

Figure 3. Typical SEM image of ferritin molecules adsorbed onto the Au disk array with a disk diameter of 46 nm and disk period of 63 nm. Modification with ferritin was performed by immersion in 0.25 $mg \cdot mL^{-1}$ ferritin phosphate buffer solutions (pH 8.0) for 30 min.

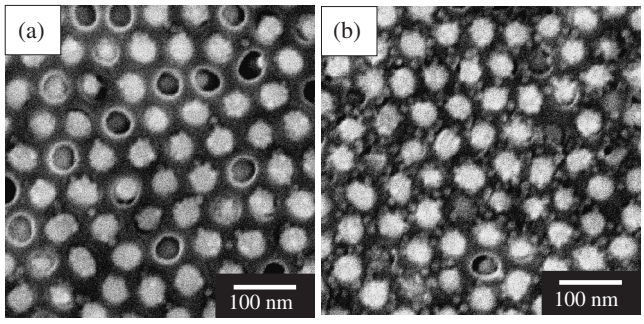

Figure 4. Typical SEM images of ferritin molecules adsorbed onto the Au disk array with a disk diameter of 46 nm and disk period of 63 nm. Modification with ferritin was performed by immersion in (a) 0.125 and (b) 0.5 $mg \cdot mL^{-1}$ ferritin phosphate buffer solutions (pH 8.0) for 30 min.

In conclusion, an ordered array of ferritin molecule using a Au nanodisk array, which has a disk period and diameter of nanometer sizes, was fabricated using a highly ordered anodic porous alumina substrate. Ferritin was confirmed by scanning electron microscopy to adsorb selectively onto the Au disk by controlling the modification time and ferritin concentration in the modification solution. The process used here has the advantage that the size and shape of the Au disk array is highly controllable, and thus it allows for the preparation of biomolecular arrays having various shapes, sizes, and spot spacing. Further reductions in disk area and spacing could lead to high-density, highly ordered isolated biomolecular arrays for the analysis of signal transduction and molecular electronics.

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