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FERRITIN DEPOSITION ON FIELD-EMITTER TIPS

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A simple aqueous deposition procedure is described which can produce partial monolayer coverages of ferritin on field-emitter tips. By employing the same deposition procedure with other biomolecules, it may now be possible to prepare field-emitter tips with known molecular coverages – a prerequisite for any attempt to image biomolecules with field-electron, field-ion, or field-desorption techniques.

1. Introduction

Since 1950, several attempts have been made to deposit isolated molecules on a field-emitter tip in order to use the field-electron or field-ion microscope to image their contours [1–5]. The imaging experiments were not particularly successful, and as a result there is no conclusive evidence which indicates that the molecular deposition procedures had been effective. In order to develop an effective procedure, the transmission electron microscope (TEM) was recently used to study the deposition of thick protein layers on tungsten field-emitter tips [6]. The layers were formed by the antigen-antibody reaction, and were deposited from aqueous solution using a simple procedure designed to minimize surface tension forces during drying. It was found that a tungsten tip, when viewed in profile in the TEM, is an excellent substrate on which to observe the results of the immunologic reaction. The entire tip appears opaque in an electron micrograph because tungsten scatters electrons very effectively. Although a single layer of protein is barely detectable as a contrast variation along the tip contour, double and triple layers produced by the immunologic reaction are clearly visible. By correlating changes in the thickness and morphology of these layers with corresponding changes in a dosing parameter, the importance of that parameter could be evaluated.

In this paper we present an extension of the thick layer dosing and observation technique to include single molecules and molecular clusters of ferritin, an electron opaque protein. The present study was initiated as part of a continuing effort to develop a low-field, point-projection microscopy for biomolecules [7,8].

2. Imaging considerations

In order to observe an isolated molecule in the TEM, it must be distinguished from the substrate on which it is deposited. To enhance image contrast, electron scattering from the substrate must be minimized. This can be accomplished by using very thin films of materials which have a low atomic number [9–12]. Since biological molecules are almost com-

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pletely composed of atoms which have a low atomic number, they are inherently weak electron scatters and are difficult to observe. One notable exception is ferritin. Ferritin is a nearly spherical molecule, approximately 11 nm in diameter. It consists of a protein shell (apoferritin) with a molecular weight of about 460,000 surrounding a core of ferric hydroxide micelles. These are arranged in a roughly tetrahedral structure measuring some 6 nm in extent. Because the core contains about 5000 iron atoms, it scatters electrons very well, and is easily seen in the TEM [13]. The visibility of ferritin in the TEM makes it an ideal candidate for evaluating the deposition of single-molecules on a field-emitter tip. Since one can look over the edge of a tip in the TEM, there is no substrate between a deposited ferritin molecule and the fluorescent screen of the microscope to reduce image contrast. As a result, even its protein shell can often be observed. Furthermore, a unique view of the ferritin molecule is obtained because a cross-section *perpendicular* to the emitter tip surface is observed. Various portions of the distribution of ferritin on the tip surface can be sampled by rotating the tip about its axis. In principle, a three-dimensional reconstruction of a single ferritin molecule could be obtained by photographing an isolated molecule on the tip apex at various tip rotations.

3. Experimental technique and results

An emitter tip is prepared by electrochemical etching, followed by thermal annealing in ultra-high vacuum to produce a smooth, thermally faceted tip apex 120–250 nm in radius. It has been found that tips with radii much smaller than 100 nm cannot be deposited with biomolecules in a reproducible fashion, probably because of surface tension effects associated with aqueous deposition. The actual choice of tip material does not seem to be particularly important (tungsten is typically used for convenience). However, the entire tip structure must be made from the same material in order to prevent electrochemical effects from occurring during the tip's total immersion in saline, during the deposition process. These considerations are discussed in greater detail, elsewhere [6].

Fig. 1 is a schematic drawing of the dosing procedure which has evolved from the ferritin study. Several tips can be dosed at one time by placing them in a teflon holder which is immersed into physiological (0.154M) saline solution. A dilute solution of the molecule is added in order to obtain the desired dosing concentration (typically 0.5–50 μg/ml). The tips are not immersed directly into a dilute solution of the molecule in order to prevent the adsorption of a full monolayer when the liquid–air interface is traversed. This phenomenon, studied by Blodgett and Langmuir [14] for soaps and fatty acids, can make submonolayer coverages of molecules on the emitter tip difficult to obtain. The coverage appears to be diffusion limited, depending only on the concentration of the solution and the time of immersion. These parameters can be determined prior to deposition [6] by using a simple technique which allows the adsorption of submonolayer quantities of protein to be determined by the unaided eye [15]. It should be noted that the molecules in solution will tend to deposit on all exposed surfaces until a monolayer-like coverage is achieved. For ferritin and most other proteins, this corresponds to a coverage of $\approx 1\ \mu g/cm^2$. Therefore, the concentration of a dilute solution of protein will continuously change as a function of

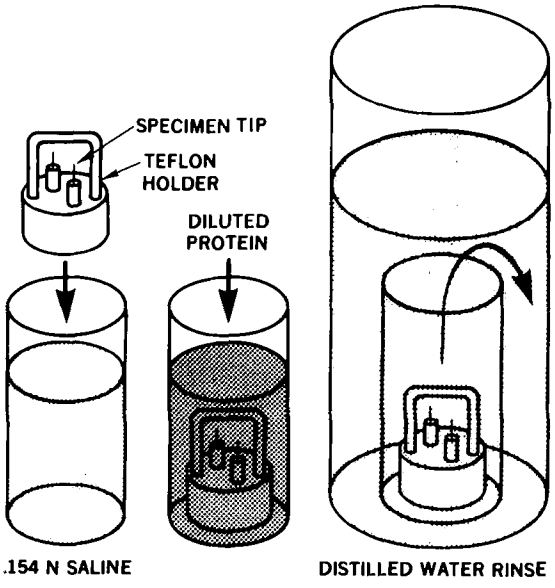

Fig. 1. A schematic drawing of the aqueous dosing procedure developed to place biomolecules on large radius field-emitter tips.

time. This usually limits the maximum tip dosing time to less than 5 min.

Following deposition, the tips are rinsed by immersing the beaker which contains the tips and dosing solution in a volume of distilled water containing no measurable amount of organic contaminant. As shown in fig. 1, the rinse water must completely cover the dosing beaker so that the tip holder can be moved into the rinse water without traversing the liquid–air interface. At this point the dosing solution has been diluted by the ratio of the beaker volumes, typically 400 : 1. Usually, the rinsing procedure is repeated twice with fresh water, without allowing the tips to traverse the interface. This ensures a total dilution of at least 10^5 : 1 before the tips are finally removed through a biomolecule-free interface and allowed to dry. As a result, no additional coverage of biomolecules will be expected to occur.

If large radii tips are used, and the dosing solution concentration and time are adjusted in order to obtain a partial monolayer coverage of ferritin, TEM images such as shown in fig. 2A can be obtained. If small radii tips are dipped directly into a dilute solution of ferritin and then withdrawn, multilayer buildup may be observed. Presumably, the multilayer (which is shown in fig. 2 and is difficult to reproduce) results from two traversals through the liquid–air interface, by the Blodgett–Langmuir effect.

Other dosing procedures (including freeze-drying) have also been evaluated because of their use in the

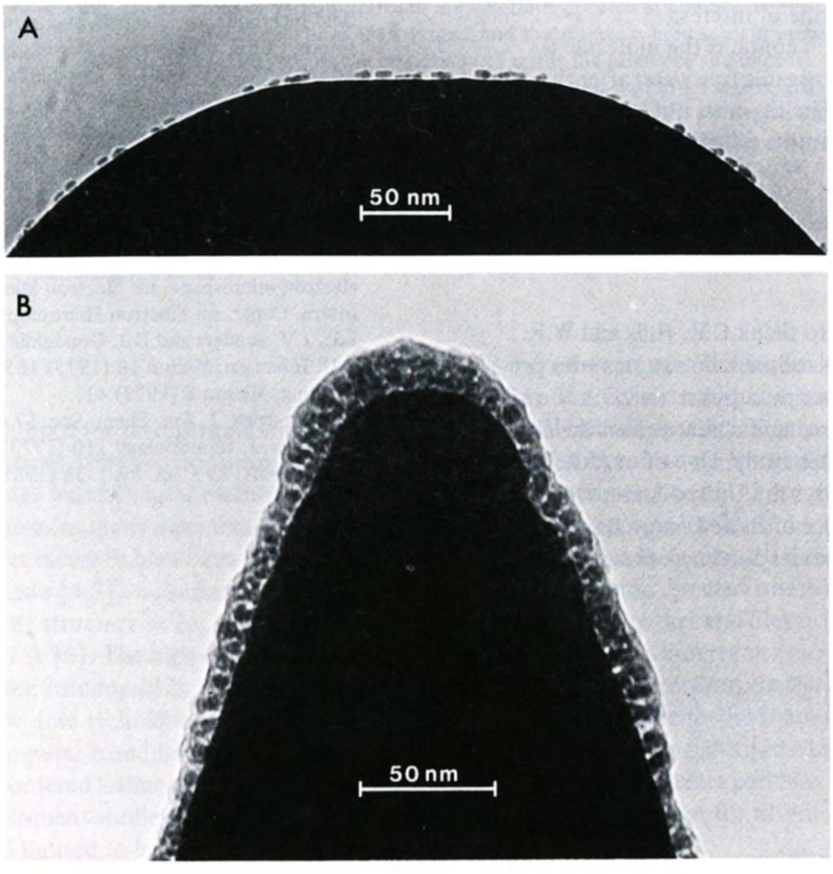

Fig. 2. (A) A large radius (thermally annealed) tungsten field-emitter tip dosed with ferritin (Miles Laboratories, Elkhart, Indiana, 6× crystallized, Cat. No. 96-027). Dosing parameters were adjusted to achieve a partial monolayer coverage of ferritin. JEOL (JSEM-100) micrograph No. 4278. (B) A small radius (field-evaporated) tungsten field-emitter tip dosed with ferritin. The deposition procedures described in the text was not used. The coverage and layer buildup is not reproducible. JEOL (JSEM-100) micrographs No. 1472).

past [4–5,16]. We were unable to obtain reproducible coverages of ferritin with these techniques.

4. Conclusions

A simple aqueous procedure for depositing ferritin onto field-emitter tips has been developed with the aid of transmission electron-microscopy. Reproducible deposition of other biomolecules may also be possible provided:
(a) A field-emitter tip of large, smooth, apex radius (120–250 nm) is used.
(b) The emitter tip and any common supporting structure is made of the same material.
(c) The field-emitter tip is not placed directly into a solution of the molecule of interest.
(d) The solution which contains the molecule is thoroughly diluted in organic free water after the deposition is completed. The tip must not traverse the liquid–air interface until a dilution of at least 10^5 : 1 is achieved.

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