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SCANNING ELECTROCHEMICAL MICROSCOPY

Stephen W. Carmichael[1], Mayo Clinic

The atomic force microscope (AFM) has been shown to be very versatile. Fu-Ren Fan and Allen Bard have extended this versatility by imaging biologic molecules based on electrochemical reactions, referred to as scanning electrochemical microscopy (SECM). Imaging in this mode required the specimen to be covered with a thin film of water. An electrical potential of about 3 volts was maintained between the tungsten tip and an electrode near the specimen, so that electrochemical reactions that occurred within the aqueous medium resulted in a detectable current (a few picoamps).

As you can appreciate, the film of water was critical. Fan and Bard accomplished this by having the specimens on a mica chip in a environment with high relative humidity. The mica was coated with gold on one side to create the reference/counter electrode. When the SECM was operated on a dry mica chip, very small currents were measured. Then the relative humidity was increased to 93% within the specimen chamber, the current increased at least 5 orders of magnitude, and the current increased about 2 more orders of magnitude when a physiologic buffer was added. The current at the tip was attributed to oxidation or reduction of species within the liquid layer. Most of the images of the biologic molecules were made with the relative humidity between 65% and 85%. Higher humidity caused the feedback system to be too unstable for good imaging.

The liquid layer was measured to be about half a nanometer. This had important consequences on the resolution since the current anywhere within the radius of curvature from the tip contributed to the image. The thin layer limited this radius, giving a resolution on the order of 1 nanometer.

For imaging biologic molecules, Fan and Bard maintained a constant current, as is usual for the scanning tunneling microscope (the "grand daddy" of all scanning probe microscopes). A feedback mechanism was used to move the tip up and down to maintain contact with the thin liquid layer. Thereby, the z-position of the tip provided the surface topology information.

Fan and Bard chose to image 4 molecules whose structures are well characterized: DNA, mouse monoclonal IgG, glucose oxidase, and keyhole limpet hemocyanin. As you would expect, the image of the DNA showed an irregular, supercoiled geometry. There was a tendency to lose the image of the DNA after repeated scans over the same area, presumably because the electroactive species near the tip were depleted. Interestingly, the signal recovered if a few minutes were allow to pass between images, or a different area was scanned, suggesting that the electroactive species are transient. The proteins were prepared at sufficiently dilute concentrations to minimize aggregation, yet concentrated enough to be easily located in the microscope. The IgG molecule was seen to have a C shape, with two "arms" and a "body," perhaps corresponding to the two Fab and one Fc fragments. The shape was similar to that seen by other imaging methods, but the dimensions were somewhat larger by SECM, suggesting that realm of electrochemical activity exceeds the dimensions of the molecule. The oxidase molecule could be visualized as a dimer, with the monomeric unit being a compact spheroid. Again, the size measured by SECM was larger than the accepted size. The hemocyanin is a fairly large molecule that appeared as a cylinder with a suggestion of 9 parallel sections. The ability to image this molecule indicated that a specimen thickness of up to 20 nm may not pose serious limitations to the SECM imaging technique, as long as the water film can provide an ion conductive path to the tip.

The overall shape of the specimens were well produced by SECM, and are in agreement with other more established techniques. Fan and Bard pointed out that whereas more information is needed about the physical and chemical processes within the water film, the potential of this technique is very attractive. ■

1. The author gratefully acknowledges Drs. Allen Bard and Fu-Ren Fan for reviewing this article.

2. Fan, F-R and A.J. Bard, Imaging of biological macromolecules on mica in humid air by scanning electrochemical microscopy, *Proceed. Nat. Acad. Sci.* (USA) 96:14222-14227, 1999. See also Commentary by Helen Hansma in the same issue, pages 14678-14680.

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For registration information contact Matthew Hulbert, email: matthew_hulbert@hotmail.com

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Suggestions from some SPM literature
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