

19. Molecule Microscopy

Academic and Research Staff

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19.1 Research Objective

Our principal research objective for the last decade has been to develop a new microscopy that can show the location and binding energy of molecules or atoms on or near the surface of a sample. We believe that molecule microscopy (MM) will be of unique importance in explaining diverse phenomena that take place on surfaces of samples of interest in biological and materials science without requiring samples that are orderly or flat as are required with diffraction methods or the newly developed scanning tunnelling microscope (STM). The surface-specific chemical contrast mechanism and potentially high spatial resolution of the MM will make it a valuable extension of the various forms of electron microscopy and microanalysis now in use.

19.2 Applications of Molecule Microscopy

What samples should we examine and what information should we seek initially? We chose to examine the distribution of water on biological materials, arguing that the presence or absence of water would give information about the surface beneath, in particular the electric charge and dipole distributions on the surface which must play important roles in the specific interaction of tissue with ions, macromolecules, antibodies, viruses, etc.

In previous studies we have shown that water binds differently to representative frozen lipid, carbohydrate, and protein samples, so that logical initial samples would be frozen artificial lipid mono- and multilayers with and without protein. Later we will examine natural material including cells, cell ghosts, and freeze-fracture samples. In each case we have identified local collaborators to supply samples and help with sample handling and interpretation of observations.

Some freeze-fracture observations of cells, in which water was found to crystallize preferentially or "decorate" certain sites forming ice crystals large enough to be seen by electron microscopy, suggest that our approach is valid.

We have also considered in the past various applications of the MM to materials science. These are now being explored in detail, and we expect to propose studies to be carried out, with appropriate collaboration, of hydrogen transport in metals, and of the interaction of foreign atoms

with metal surfaces. The results of such studies would be useful in furthering understanding of the mechanisms of hydrogen embrittlement, corrosion and stress-corrosion failure.

19.3 Kinds of Molecule Microscope

MM works by detecting the spatial variations of the emission of neutral molecules from the sample. Various methods of detection are possible, spatial resolution can be achieved in different ways and molecules (of many species, parts of the sample, coming through the sample, or placed on it as a stain) may be emitted from the sample as a result of various stimuli. The earliest MM used a field ionizing point and electron multiplier as a detector, a pinhole to achieve spatial resolution, and a sample which emitted molecules continuously ("self-luminous"). The MM under development for the biological and metallurgical work mentioned above (called the Nanometer Resolution Scanning Desorption Molecule Microscope) will use a relatively large (1 μm) field ionizing tip which oscillates from above the sample, where it collects neutral molecules emitted by the sample, to the entrance of a time-of-flight mass spectrometer where the collected molecules are field desorbed and field ionized. A pulsed beam of focused electrons from an appropriate electron gun causes the desorption of neutral molecules from the sample surface.

This system has the following advantages. The cross-sections for ESD of neutral molecules from samples of interest are large. The ESD is produced by a focused beam of electrons which can be of a size comparable to that achieved in EM thus affording high spatial resolution. The relatively large field ionizing tip close to the sample gives a large solid angle for collecting neutral molecules. The sticking coefficient of neutral molecules on the tip is near unity. The field desorption and field ionization processes make the detection of the neutral molecules highly efficient.

In the MM described above the sample must be in ultra high vacuum, which is permissible for frozen biological material at 77 K, or metallurgical samples. For live tissue, or some corrosion studies, the sample must be bathed in an appropriate fluid. We have developed a MM in which the sample is scanned or probed with a micropipette connected to an ionizer and mass spectrometer for identifying and detecting molecules emitted by the sample and picked up by the micropipette. Unique information can thus be obtained concerning fluxes of small volatile molecules in tissue. This instrument is called the Scanning Micropipette MM (SMMM).

19.4 Nanometer Resolution Scanning Desorption Molecule Microscopy

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Progress

We have undertaken the detailed design of this instrument. We have also continued the development of various subsystems, principally the high voltage pulse generators and oscillating needle driver systems. We have also made a direct measurement of the cross-section for ESD of neutral water molecules confirming the indirect results of previous work.

19.5 Scanning Micropipette Molecule Microscopy (SMMM)

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Joseph A. Jarrell, John G. King

The major goal of this research is the study of water transport at the cellular level across hormonally responsive tissues that model the distal tubules of the mammalian kidney, specifically amphibian urinary bladder. The elucidation of these processes is basic to an understanding of the cellular and molecular mechanisms by which the kidney maintains salt and water balance in man.

During the past year, we have developed a new flow chamber for the SMMM (boundary layers in this chamber can be as thin as 10 μm) and a technique for casting composite cellulose acetate membranes to provide support for our experimental samples. These membranes are 25–30 μm thick except for a central region, 1 mm diameter, which may be as thin as 3 μm .

We have also done preliminary experiments to examine the effects of quin2 (a fluorescent calcium chelator that has been introduced into some biological cells nondestructively) on the hydro-osmotic vasopressin response in amphibian urinary bladder. If, as has been hypothesized by others, a change in intracellular calcium activity is an important link in the chain of biochemical events that are initiated by vasopressin binding, then the presence of quin2 would delay or blunt this response. Our results so far are inconclusive. We plan to repeat these experiments with radioactively labelled quin2. This will enable us to make well-defined measurements of the cellular uptake of the chelator.

Publications

Jarrell, J.A. and C.A. Rabito. "Development of Gap Junctions Analyzed by Metabolic Cooperation between Ouabain-Resistant and Wild-Type LLC-PK1 Cells," submitted for the 1985 FASEB meetings.

19.6 Scanning Micropipette Molecule Microscopy - Boston University School of Medicine Collaboration

National Institutes of Health (Grant AM-25535)

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A SMMM has recently been made operational and applied to tissue transport studies with the following initial results. sampling tracer concentrations above a transporting Necturus gallbladder. Experiments were conducted with a probe of drawn quartz with tip dimensions 4.5 μm I.D., 11 μm O.D., plugged with DMS in a thickness less than 0.5 μm and protruding beyond the tip. This probe provided an initial sensitivity of 7×10^{-9} amps/mole/cc, which degraded to 2×10^{-9} amps/mole/cc after one week of use. Necturus gallbladder was dissected, rinsed, and quickly mounted with moderate stretching in a chamber exposing a tissue area of 0.22 cm^2 . The mucosal bath contained a standard Ringer solution with 2.5 mM HCO_3 and either 100 mM sucrose or 10^{-3} M EGTA. The small volume of serosal Ringer required was made using 50% H_2O^{18} .

The epithelium and probe were easily observed simultaneously under a Leitz microscope. Cell borders were discernible at 400X magnification. The positioning of the probe at a border or cell center was reliable as was the ability to lower the probe, touch the cell (as indicated by a cell shape change), and raise the probe 2 μm above the surface.

The existence of unstirred surface layers was made tangible as the tracer signal dramatically increased when a probe was within 300 μm of the surface. Quantitative studies of the concentration profile in this region are in progress.

Four tissue were mounted and explored for localized sites of tracer water flux. The first mounting had too much tissue movement for reliable probe positioning due to inadequate stretching of the bladder. In one tissue the serosal compartment was closed and sucrose added to the mucosal bath to encourage a directional water flux, but hydrostatic pressure was poorly controlled so that net flux was uncertain. Under these conditions the concentration difference between junctional and cell-centered sites was insignificant, 0.03 ± 0.09 mM/cc (N = 15 that is, 15 observations). We added EGTA to remove Ca^{++} , which has been reported to loosen junctions. An opening in the cell continuum appeared and was scanned with the probe. A 0.5 mM/cc concentration increase was observed above that site, demonstrating that the probe function had not been compromised in the above series of measurements.

The need for careful control of hydrostatic pressure became obvious and a manometer and calibrated capillary tube were added. The pressure gradient could now be measured to within 1/2 millibar and flow rates to within a few microliters per hour. A tissue exposed to a 5 mbar (serosal above musocal) hydrostatic pressure gradient responded with a 130 $\mu\text{l/hr-cm}^2$ flow (s \rightarrow m). Again junctional sites showed no significant concentration difference to within

0.3 mM/cc. The appearance of hemispherical blisters 50 μm to 300 μm in radius was noted. These areas of tightly stretched cells showed no difference in junctional permeability. But the blisters were easily damaged when poked too hard by the probe. A defect thus produced showed a high water flow rate, as indicated by a high concentration (1.7 ± 0.4 mM/cc (N = 4)). As a further demonstration of feasibility, we increased the pressure gradient to 14 mbar ($s > m$) and the flow rate went up to 400 $\mu\text{l/hr-cm}^2$. Sampling above three different junctional sites showed a significant increase in tracer concentration as compared with that over cell-centered sites, although the variability was high. The mean difference was 2.2 ± 2.1 mM/cc (N = 6).

In a final tissue we were able to control blisters (which might distort the unstirred layer) by keeping the pressure below 1 mbar ($s > m$) and the flow rate less than 47 ml/hr-cm²; however an overheating wire in the vacuum caused spurious noise and forced termination of this run. These tests show the feasibility of the approach and that quantitative results can be obtained from careful data accumulation.

19.7 Desorption Studies

Whitaker Health Sciences Fund

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We are continuing the experiments begun by D. Lysy and B. Silver on the thermal and electron stimulated desorption of water and other small molecules from biological materials for four reasons. (1) To understand the contrast mechanism in molecule microscopy; (2) to measure the total water content of various samples; (3) to measure the binding energy of this water; and (4) to determine the order of the kinetics of the desorption process. The importance of these experiments comes from the central role of water in biological processes at the molecular level.

Progress

Most of 1984 was spent in apparatus improvement and training new students in an effort to establish a routine to permit 100 desorptions per day instead of 10 or 1.

19.8 Electrical Neutrality of Molecules

International Business Machines, Inc.

Anthony P. French, John G. King

The present experimental upper limit on any departure from neutrality of molecules is approximately 10^{-21} of one elementary charge. This can be interpreted as a limit on the proton-electron charge difference, or on the charge on the neutron, or on some combination of these. The occurrence of innumerable transformations involving both leptons and baryons, and

our long-standing belief in the conservation of electric charge as a fundamental law of nature, certainly support the assumption of exact equality of charges of either sign on all elementary particles. Nevertheless, the possibility that there might be some minute difference between electron and proton charges is something that cannot be ruled out on the basis of any existing experimental evidence or theoretical scheme, and its existence would have profound implications.

The fundamental interest of this question has led to a number of experiments by various investigators. These experiments have involved three different approaches: (1) direct tests for any net charge in a volume of gas released from a container; (2) tests for a charge on an isolated body (analogous to the Millikan oil-drop experiment); (3) molecular-beam deflection experiments.

In 1973 King and Dylla¹ pioneered a new technique based on the fact that an electric field, periodic in time, applied to a homogeneous medium will excite acoustic waves at the same frequency if the molecules of the medium are not completely neutral. (This is separable from any induced polarization effects, which will occur at doubled frequency).

Progress

The apparatus described in RLE Progress Report No. 126 has been assembled and tested. The first runs in air show that there is a fundamental frequency signal present that is ca. 10^3 larger than the minimum detectable signal. We attribute this to forces arising from the interaction of the electric field and frozen-in charges in the mylar rings used to separate the copper equipotential rings. Other arrangements to avoid this effect are being studied. We have begun development of high voltage audio-frequency sources with exceptionally low harmonic content as needed to exploit the intrinsic sensitivity of this technique.

References

1. H. Dylla and J. King, Phys. Rev. A 7, 1224 (1973).