

GENERAL PHYSICS

I. MOLECULE MICROSCOPY

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We have been developing instruments called molecule microscopes based on the simple idea that neutral molecules travel in straight lines between collisions. We are interested in producing magnified images (micrographs) of samples that emit neutral molecules as a result of various stimuli.

The contrast in a molecule micrograph arises from spatial variations in properties of the sample such as permeability, diffusion, binding of neutral molecules, and spatial distribution of enzymes. These properties cannot be observed directly in other forms of microscopy.

Although molecule microscopy can be used to study problems in various fields, we think that the most significant and valuable applications, especially while the spatial resolution is of the order of 1 μm , will be to problems in the life sciences. Because there are many kinds of sample, sample preparation or treatment, sample stimulation, and molecular emissions, molecule microscopy is rich and complex and no single apparatus is the Molecule Microscope. For instance, the sample can be dead or alive, in vacuo, in vitro, or in vivo, denatured or surviving. Stimuli include heat, light, charged particles, currents, toxins, hormones, and so forth. The observed molecules may have come through the sample, been part of it, been previously placed on it as a stain, or may have been generated by it in such processes as specific enzyme catalyzed reactions or metabolism.

During recent years, we have constructed and tested a lot of apparatus and performed experiments relevant to understanding the various mechanisms involved. We have studied, more or less thoroughly, the desorption of water and ethanol from model biological surfaces by both thermal desorption and electron-stimulated desorption techniques, the interaction of CO with silicon surfaces, variations of permeability and metabolism of dead and surviving tissue (frog skin, toad bladder, and vascular smooth muscle), and immobilized and solubilized enzyme catalyzed reactions.

Now we are beginning to use our instruments and techniques in their preliminary form to study what we hope will prove to be important scientific problems. Our

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collaborators are interested in such diverse systems as the mechanism of thrombus formation on materials, cell surface properties and active transport in tissue, enzymology, and toxicology.

1. SCANNING PINHOLE MOLECULE MICROSCOPE (SPMM)

The apparatus is being modified to allow the study of living tissue in vitro, thereby eliminating the problem of vacuum dehydration. The modified apparatus will be used to study, with spatial resolution, the active and passive transport properties of frog's bladder. This epithelium (a single layer of epithelial cells, $\sim 15 \mu\text{m}$ on a side, on a thin, $\sim 20 \mu\text{m}$, basement membrane), as well as toad's bladder and frog's skin, has long been studied without spatial resolution by other researchers as a model of the human kidney. We intend to observe the epithelial cells in vitro by differential interference light microscopy simultaneously with SPMM to correlate transport properties with tissue morphology at the cellular level. This work is being done by Joseph A. Jarrell in collaboration with Dr. Alvin Essig and Dr. Michael Lang of the Boston University Medical Center.

2. DESORPTION EXPERIMENTS RELATED TO THE SCANNING DESORPTION MOLECULE MICROSCOPE (SDMM)

Bruce R. Silver has performed some experiments relating to scanning desorption molecule microscopy (SDMM) with emphasis on neutral molecule staining for biological surface studies. With this technique a scanning electron beam is used to desorb water or other small molecules as a means of mapping weak chemical properties of the surface monolayer. We are interested in direct electron stimulated desorption (ESD) and also in desorption by local electron beam heating.

The apparatus comprises a scanning electron beam, neutral molecule staining beam, quadrupole mass spectrometer, and sample holder. It has been used to measure the ESD cross sections of D_2O and ethanol stains from platinum and three model biological surfaces as a function of electron energy. The three biological surfaces included a protein (bovine serum albumin), a carbohydrate (glycogen), and a nonpolar lipid (tristearin). We compared the cross sections with binding energy data determined by flash desorption in the same apparatus.

The ethanol is desorbed with a total cross section, Q , of approximately $1 \times 10^{-15} \text{ cm}^2$ at 1 kV, falling to $2 \times 10^{-16} \text{ cm}^2$ at 4 kV, which is nearly the same for all four surfaces. The flash desorption data show similar binding energies and simple first-order kinetics for all four surfaces.

The D_2O data on tristearin and BSA surfaces show ESD cross sections of $1 \times 10^{-15} \text{ cm}^2$ at 1 kV, falling to $1 \times 10^{-16} \text{ cm}^2$ at 4 kV, while on platinum and glycogen surfaces Q is $3 \times 10^{-16} \text{ cm}^2$ at 1.5 kV, falling to $5 \times 10^{-17} \text{ cm}^2$ at 4 kV. The flash

desorption data indicate kinetics of order less than one, and can be interpreted in terms of condensed and mobile phases of the adsorbed D_2O . In this model, which is examined through the peak shift with coverage, peak width, and initial desorption rate, the binding energy of the mobile phase is found to be 3 kcal/mole for BSA and tristearin and 5 kcal/mole for platinum and glycogen, correlating with the ESD cross sections.

Dusan G. Lysy investigated the desorption of water on five different model biological surfaces by a thermal desorption technique. In the basic experiment a platinum ribbon was coated with a thin layer of the biological macromolecule under consideration and cooled to liquid nitrogen temperatures in vacuum, the adsorbed water molecules were thermally desorbed by ohmic heating of the sample ribbon, and then the desorbed water molecules were detected with a quadrupole mass spectrometer system. The resulting plot of the desorbed water flux as a function of the sample temperature exhibited a series of peaks corresponding to different binding states of the adsorbate molecules on the sample. The observed peaks divide naturally into three groups on the basis of the sample temperature: the low-temperature peak between $-140^\circ C$ and $-50^\circ C$, the middle-temperature peaks between $-50^\circ C$ and $210^\circ C$, and the high-temperature peaks between $210^\circ C$ and $560^\circ C$.

The samples investigated were a protein (bovine serum albumin), a polar and a non-polar polypeptide (poly-L-lysine hydrobromide and poly-L-alanine), a carbohydrate (glycogen), and a completely nonpolar lipid (tristearin).

The low-temperature peak was present in all samples, as well as in the controls, and no sample specificity was demonstrated. At high coverages this peak can be interpreted as the sublimation of ice. Adsorption into this peak takes place with a sticking coefficient of at least very close to unity for sample temperatures during adsorption, $\leq 125^\circ K$, for coverages between ~ 0.1 and ~ 300 monolayers of water.

The middle peak groups showed very strong sample specific peak patterns with major peak energies from 16.5 to 22.2 kcal/mole, and desorption in the range of energies from 14.5 to 27.5 kcal/mole. The peaks each contained a range of activation energies for desorption, and the peak patterns were consistent with the assumption that the number and types of polar groups present in the sample determine the amount of water adsorption in this peak group and the complexity of the peak patterns, thereby giving the observed sample specificity. This peak group appears to correspond to the water adsorption traditionally measured by water adsorption-desorption isotherms taken near room temperature and often described by the Brunauer-Emmett-Teller (BET) theory of multilayer adsorption, with the important differences that in our experiment kinetic rather than equilibrium parameters were measured and that different adsorption states could easily be differentiated. Lower limits on the sample specific water adsorption in this peak group of $\sim 15\%$ of the BET monolayer values were established, and the observed coverages were shown to be correlated with the trends obtained from the BET monolayer values. The

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sizes of these peaks were shown to be proportional to the sample amount, and the peak locations to be reproducible even when the amount of applied sample was varied.

The high-temperature peak group gave sample specific peak patterns. Evidence was found to support the interpretation of this peak group as representing pyrolysis of the sample.

3. MOLECULE FLUXES IN TISSUE

The application of mass spectrometry methods to the study of fluxes of volatile molecules through biological tissues and membranes continues. This work is being performed by Stanley J. Rosenthal in the laboratory of Dr. Alvin Essig at Boston University Medical School. Simultaneous measurement of O_2 consumption and CO_2 production has been demonstrated, and much time has been devoted to studies of calibration methods, design of appropriate Ussing chambers, and control and stabilization of the flow of Ringer's solution. We are now ready to start a series of investigations of various epithelia. Later, this approach can be extended to nonvolatile species such as lactate, by use of the Volatile Enzyme Product (VEP) method.

4. VOLATILE ENZYME PRODUCT (VEP) TECHNIQUE

Recently, we have been pursuing the development of a technique that interfaces enzyme-catalyzed reactions with a quadrupole mass spectrometer (employed as a sensitive mass-filter detector) by means of a semipermeable membrane.¹ All that is required is that at least one of the reactants of the enzyme-catalyzed reaction be volatile and able to permeate the membrane, so that a large number of enzymes can be considered. In short, the VEP technique offers the potential of high sensitivity, specificity, and speed in the assay of substrates, cofactors, effectors, and enzymes. Although much work needs to be done, the ultimate sensitivity should allow, for example, continuous monitoring of metabolic fluxes (e. g., CO_2 and lactate) from single cells with an associated time constant of approximately 10 seconds. Furthermore, in combination with scanning pinhole molecule microscopy, this method should permit mapping of suitable enzyme distributions in nonstained and nonfixed tissue slices.

Recent work continues to emphasize preliminary exploratory studies² and, most important at the present stage of development, improvement of the technique's capabilities. In order to facilitate understanding of results, all recent work emphasizes steady-state conditions, and transitions between steady states. Specifically, we have used both catalase (EC 1.11.1.6; volatile product is O_2) and urease (EC 3.5.1.5; volatile product is CO_2) to examine effects of volume flow rate, \dot{V} , passive continuous degassing, pH and continuous electronic averaging over the volatile product molecule's mass peak. In addition to using the two enzyme systems, brief trials with suspensions of viable

cells (one species of yeast) have been made.

Our current objectives include a continuing attack on limiting sources of noise (fluctuations in \dot{V} , residual partial pressures, electronics drift, and a nonoptimal value of counting efficiency), and exploratory studies of other enzymes.² Also, in addition to the continuous assays now being studied, the assay of small discrete samples (e. g., homogenized brain tissue) will be pursued, and application of the VEP technique to environmental measurements such as sensitive detection of pesticides will be explored.

References

1. J. C. Weaver, M. K. Mason, J. A. Jarrell, and J. W. Peterson, *Biochim. Biophys. Acta* **438**, 296-303 (1976).
2. J. H. Abrams, J. C. Weaver, F. Villars, and A. J-Y. Chong, RLE Progress Report No. 118, Research Laboratory of Electronics, M. I. T., July 1976, pp. 3-8.

5. THERMAL ENZYME PROBE

The thermal enzyme probe (TEP)^{1, 2} is a tantalizingly attractive biochemical transducer because of its potential universality and basic simplicity (two thermistors, one coated with immobilized enzyme, the other without). The steady-state response, which can be reached within a few seconds, is a temperature difference, ΔT , which is converted into an electrical signal. Almost any enzyme can be used, since nonzero enthalpy changes occur with almost every reaction. A major difficulty, however, is that the TEP is not a particularly sensitive device; both simple theory and experiments already performed show that small ΔT 's, of the order of 10^{-4} °C for 10^{-3} molar substrate concentrations, are expected. For this reason, the primary technical problems involve the physics of differential thermometry in flowing aqueous solutions.

All recent work (primarily by Scott P. Fulton) has been directed toward understanding and reducing various sources of noise. It is still not clear what the fundamental limits of ΔT measurements are in a flowing aqueous solution. Based on the work of others,³ as well as on our own,⁴ a goal of $\Delta T = 10^{-6}$ °C seems feasible. Under the assumption of an associated typical enthalpy change of 10 kcal/mole⁻¹, this leads us to estimate the minimum detectable substrate concentration to be ~10 micromolar. We have achieved an rms noise level of approximately 10^{-5} °C and plan soon to use a recently improved apparatus with hexokinase (EC 2. 7. 1. 1) or urease (EC 3. 5. 1. 5) to study the TEP performance under a variety of conditions.

In the longer term, we believe that the use of state-of-the-art electronics, including microprocessors, might allow the TEP to provide the basis for a relatively simple and inexpensive biochemical "multimeter" that would be useful in a wide variety of research and clinical applications.

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References

1. C. L. Cooney, J. C. Weaver, S. R. Tannenbaum, D. V. Faller, A. Shields, and M. Jahnke, in E. K. Pye and L. Wingard (Eds.), Enzyme Engineering, Vol. 2 (Plenum Press, New York, 1974).
2. J. C. Weaver, C. L. Cooney, S. P. Fulton, P. Schuler, and S. R. Tannenbaum, Biochim. Biophys. Acta 452, 285-291 (1976).
3. L. D. Bowers and P. W. Carr, Thermochim. Acta 10, 129-142 (1974).
4. S. P. Fulton, S.B. Thesis, Department of Physics and Department of Nutrition and Food Science, M.I.T., June 1975.

6. LIQUID HELIUM RESEARCH

We are interested in studying droplets of liquid helium so small that their dissimilarity from bulk properties is significant. From a theoretical standpoint these droplets are similar to atomic nuclei, but have several interesting features. First, the interaction between pairs of helium atoms is well known, while the details of the nuclear force are not. Bulk liquid helium is available for measurement of such properties as equation of state, surface tension, and excitation spectrum, whereas nuclear matter can be explored only in the limit of large finite nuclei. We also have a choice of statistics between Bose ^4He and Fermi ^3He .

Several sources and detectors of helium clusters in the 1-100 atoms range have been proposed; we are now developing a supersonic nozzle beam source. When cold helium gas expands adiabatically through a nozzle into vacuum, much of the random thermal energy becomes directed along the streamlines; in a co-moving frame the gas cools, becomes supersaturated, and condenses into droplets. This process has been studied extensively, principally with other gases, by several investigators.

Peter W. Stephens has constructed a vacuum system with a supersonic helium beam capable of operating at 4.2°K, up to 760 T stagnation pressure, with an approximately 10 μm pinhole as a nozzle. The beam is detected by a mass spectrometer in a separately pumped chamber. Measurements of the size distribution of clusters are now being made.

Future work may include measuring the spin of ^3He drops by molecular beam resonance on the nuclear magnetic moment, and measurement of vibrational and rotational excitations by light scattering.