kayama, Yl Kanaoka, M. Minamino, K. Kangawa, H. Matsuo, M. A. Raftery, R. Hirose, S. Inayama, H. Hayashida, T. Miyata, and S. Numa. 1984. Primary structure of Electrophorus electricus sodium channel deduced from cDNA sequence. *Nature.* 312:121–127.

Noda, M., H. Suzuki, S. Numa, and W. Stühmer. 1989. A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. *FEBS Lett.* 259:213–219.

Terlau, J., S. H. Heinemann, W. Stühmer, M. Pusch, F. Conti, K. Imoto, and S. Numa. 1991. Mapping the site of block ty tetrodotoxin and saxitoxin of sodium channel II. *FEBS Lett.* 293:93–96.

Yang, J., P. T. Ellinor, W. A. Sather, J.-F. Zhang, and R. W. Tsien. 1993. Molecular determinants of Ca^{2+} selectivity and ion permeation in L-type Ca^{2+} channels. *Nature.* 366:158–161.

Want to Exchange Your Virus? Try Microdialysis and Raman

Paul R. Carey
Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106-4935 USA

Three years ago I presented a New and Notable review on the use of a microdialysis cell to observe the time-dependent effects of deuterium-hydrogen exchange on the Raman spectrum of bean pod mottle virus (Carey, 1993). In the source paper, Li et al. (1993) showed that as a solution of virus particles in H_2O is exposed to D_2O, it is possible via the Raman spectrum to follow the replacement of hydrogens by deuterium atoms in protein capsids and separately in the RNA bases, as a function of time. From this observation of exchange dynamics, the authors were able to reach structural information on the RNA-protein contacts, concluding, for example, that the 4CO-3NH-2CO network of uracil and the 6CO-1NH-2CNH_2 network of guanine form extensive and rigid contacts with the protein capsids. These results from Li et al. highlighted a unique advantage of Raman spectroscopy in that the technique is able to follow the details of $H \leftrightarrow D$ exchange in complex assemblies such as virus particles. They used a "homemade" microdialysis flow cell to bring about the exchange, which, at the same time, served as the Raman cell. Although the initial results from the use of this cell were never in question, it is necessary to define quantitatively the cell's isotope exchange characteristics in both spatial and temporal terms and to probe its limitations. These are the goals of Tuma and Thomas's (1996) latest publication, "Theory, design, and characterization of a microdialysis flow cell for Raman spectroscopy."

As any old-time Raman spectroscopist (tell me about mercury lamps, Grandpa!) will confirm, one of the delights of the Raman technique is its experimental flexibility. The laser, the spectrometer, and the photon detector may, of necessity, be "high tech," but the sample arrangement could still almost be back in the era of string and sealing wax science. As long as you can focus the laser beam in or on the sample and can collect the scattered light (not forgetting the direction of the incident **E** vector), there is complete flexibility over the nature of the sample. Thus, the sample arrangements can be fiendishly complex or, as in the case of Thomas's dialysis cell, simple and elegant. The latter is also easy to describe to the nonspecialist: imagine a standard glass capillary tube, 1 mm in internal diameter and 2 cm in length, in the horizontal plane and containing a drop of the macromolecular solution for examination. A laser beam entering the capillary in the vertical direction is focused into the drop, and the scattered light is collected by a lens and analyzed by a spectrometer to provide the Raman spectrum of the drop. Tens of thousands of such experiments have been performed, but Thomas and coworkers realized that if they threaded a narrow ($\cong$200 μm) dialysis tube through the glass capillary and the droplet, and sealed the capillary ends with wax, they could flow D_2O through the dialysis tubeand slowly (on the time scale of a few minutes) exchange D_2O into the H_2O solution in the drop. They could then observe the effect of this exchange on any macromolecules contained in the drop. Thomas's group has now used this approach with considerable effect in studying the exchange characteristics of the P22 virion (Reilly and Thomas, 1994) and the PRD1 virus II (Tuma et al., 1996), as well as the bean pod mottle virus, alluded to above. There are very interesting differences in the hydrogen exchange properties of the constituents in these three systems, but here the emphasis will be on the biophysical/chemical experiments undertaken to characterize the microdialysis cell.

The underlying assumption governing the operation of the microdialysis cell is that diffusion governs the transport of small molecules between the membrane boundary of the microdialysis tubing and the glass capillary containing the macromolecular solution. The applicability of a diffusion model was justified by agreement between calculated and measured efflux rates for a number of solutes. Thus, the efflux of D_2O, calcium ions, and EGTA were measured via the appearance of their characteristic Raman features and found to be consonant with calculated values. Applicability of the microdialysis technique to pH titrations was explored by measuring the kinetics of glutamate protonation in the Raman spectrum of a peptide copolymer [poly-(Glu,Lys,Tyr)]. The results showed that pH-titration rates of up to 3.3 pH units/min can be monitored. The kinetics of deuterium exchanges in single and double stranded nucleic acids and with basic pancreatic trypsin inhibitor were all in agreement with the values obtained by other techniques. The message from all this is that the Raman microdialysis cell is a reliable means of measuring isotope exchange kinetics and that it appears to be free of unpleasant surprises for the unwary investigator. Tuma and Thomas also discuss modifications to the design of the cell to improve the present time resolution of about 2 min; they discuss

Received for publication 10 October 1996 and in final form 10 October 1996.

0006-3495/96/12/2918/02 $2.00

ways to increase the dialysis tube surface area-to-sample volume ratio that could improve time resolution by a factor of 10. However, they point out that the simple diffusion model would no longer apply and data analysis would become complex. Ah well, as the old timers know and the aspiring student will soon find out, in science there is always one more hurdle to jump!

REFERENCES

Carey, P. R. 1993. Virus dynamics lead to structure, by George! *Biophys. J.* 65:1749–1750.

Li, T., J. E. Johnson, and G. J. Thomas, Jr. 1993. Raman dynamic probe of hydrogen exchange in bean pod mottle virus: base-specific retardation of exchange in packaged ssRNA. *Biophys. J.* 65, 1963–1972.

Reilly, K. E., and G. J. Thomas, Jr. 1996. Hydrogen exchange dynamics of the PZZ virion determined by time-resolved Raman spectroscopy. *J. Mol. Biol.* 241, 68–82.

Tuma, R., and G. J. Thomas, Jr. 1996. Theory, design, and characterization of a microdialysis flow cell for Raman spectroscopy. *Biophys. J.* 71:This issue.

Tuma, R., J. H. K. Bamford, D. H. Bamford, and G. J. Thomas, Jr. Structure, interactions and dynamics of PDR1 virus II. Organization of the viral membrane and DNA. *J. Mol. Biol.* 257:102–115.