spectrometer design, and laser light rejection. Multielement detectors, initially vidicons and photodiode arrays, and more recently charge coupled devices (CCDs), have provided the hardware necessary to monitor the complete spectral distribution of Raman scattered light with both the spectral resolution and high quantum yield necessary. Moreover, the most recent generation of back-thinned CCD detectors has extended the useful spectral range accessible to Raman spectroscopy well into the near infrared, as experiments with the photosynthetic bacterial reaction center have demonstrated (Palaniappan et al, 1993). Holographic notch filters, which attenuate elastically scattered laser photons effectively without inordinately decreasing the intensity of inelastically scattered Stokes photons, have been coupled with compact, efficient spectrographs to provide high resolution and high throughput. These have eliminated the need for complex, double, or triple monochromators which, because of the necessarily high number of optical components, have poor optical throughput.

Salmaso and co-workers have capitalized on a number of these advances in their continuing development of a confocal Raman microscope (Puppels et al., 1990). In their article in this issue, the Dutch group has applied the technology to the study of the immunologically important enzyme, eosinophil peroxidase (EPO), at the level of a single living cell. EPO is a member of a larger class of heme-containing mammalian peroxidases that includes lactoperoxidase, intestinal peroxidase, and myeloperoxidase (MPO). At least two of these proteins, EPO and MPO, are critical to antiparasitic defenses, because these enzymes catalyze the formation of cytotoxic hypohalous acids from hydrogen peroxide and halide ions after phagocytosis of the invading microbe. EPO has also been implicated in the anti-tumor activity of interleukin 4. The optical and Raman spectroscopic properties of these proteins are unusual, as might be expected from the unorthodox chemistry they catalyze, and they have generated considerable debate in the literature. The origin of the striking red-shift in the optical spectrum of MPO, for example, continues to be debated, despite the fact that a high resolution crystal structure now exists (Zeng and Fenna, 1992). Recent Raman and biochemical data suggest that charged amino acids in the active site play a significant role in altering the electronic and vibrational properties of the heme chromophore in this peroxidase (Floris et al., 1994).

Within this context, the work of Salmaso et al. is of considerable interest. As they demonstrate, Raman microspectroscopy of human eosinophilic granulocytes allows in situ measurements to be made with high spectral resolution. In the current implementation of their microscope techniques, they have developed methods for variable laser wavelength excitation, which provides access to excitation profile characterization, and for depolarization measurements, which is critical in assigning observed vibrations to chromophore normal modes. By using these methods, they provide a detailed interpretation of the vibrational properties of EPO, both in situ and in isolated form. They refine earlier assignments of the Raman spectral characteristics so that a clear picture of the active site in the resting enzyme is now available. As with the well characterized plant peroxidases, such as horseradish peroxidase, the heme chromophore is iron protoporphyrin IX, which assumes a six-coordinate, high-spin configuration in the ferric resting enzyme, both in granulocytes and in its isolated form. Similar to the situation in MPO, local protein effects appear to modulate the properties of the chromophore significantly. Salmaso et al. localize these interactions to the protoheme vinyl groups, which can relate critically to activity through redox and conformational mechanisms. Their low frequency data suggest that active site tailoring through the peripheral substituents is a general mechanism in the mammalian peroxidases for implementing cytotoxic product generation.

The present work systematizes the interpretation of the vibrational data available on resting mammalian peroxidases. Extension of the Raman approach to outstanding questions is now feasible. Key among these are the following: 1) Can axial ligand assignments in the resting enzyme be confirmed by isotope substitution?; 2) Is the unusually high iron-histidine vibrational frequency in reduced plant peroxidases also a characteristic of the mammalian peroxidases?; 3) What is the detailed nature of the local protein/heme peripheral substituent interaction and how does it relate to activity; 4) Can the microspectroscopic technique be extended to resolving mechanistic issues in real time on single cells?; 5) How do the mammalian peroxidases protect themselves from the corrosive products of their catalysis? Raman methods will undoubtedly continue to play a large role in addressing these issues.

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Cross-Talk Between Membranes

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In this issue of *Biophysical Journal*, Lückhoff and Clapham (1994) describe

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a surface membrane Ca channel that is influenced by depletion of intracellular Ca stores. In 1986, when Putney proposed the "capacitative model . . . for receptor-regulated calcium entry," few electrophysiologists must have predicted its impact. The capacitative model was a generalization and extension of earlier proposals of van Breemen (1977), Casteels and Droogmans (1981), and Brading et al. (1980) for smooth muscle: that the sarcoplasmic reticulum (SR) forms a "superficial buffer-barrier" against the movement of extracellular calcium to regions deep within the cell, and that the filling of the SR occurs by a special access pathway to the extracellular medium. Thus, the SR and surface membrane were hypothesized to act as a coordinated system controlling Ca penetration. Putney posed that in cells that are regulated through surface membrane receptors, emptying the endoplasmic reticulum (ER) would increase Ca permeability of the surface membrane. In other words, he emphasized that the *degree of filling* of the ER was the important parameter regulating the movement of Ca into the cell through this special access pathway, hence the name "capacitative model."

Occupation of surface membrane receptors regulates intracellular Ca stores through the diffusible intracellular second messenger, D-myo-inositol 1,4,5-trisphosphate (IP_3). A salient feature of the Putney hypothesis is that IP_3 or other metabolites of the inositol lipid system do not act directly on specialized Ca channels of the surface membrane; rather, they act only indirectly by emptying the intracellular stores.

Signaling from surface membranes to intracellular membranes has been firmly established: for example, via IP_3 or direct coupling between membranes. The capacitative model requires that information be transferred in precisely the opposite direction, i.e., from intracellular to surface membranes. Evidence has recently begun to support this idea in a number of cellular systems. Notably, in T-lymphocytes (Zweifach and Lewis, 1993) and in mast cells (Hoth and Penner, 1993), a Ca current can be elicited by a number of maneuvers that deplete ER stores. In both cells, the Ca pathway appears to have a very low single-channel conductance. In lymphocytes, the single-channel conductance has been estimated by noise analysis to be ~24 fS in 110 mM $CaCl_2$, too low for detection in conventional single-channel records.

Furthermore, a diffusible messenger that increases the surface membrane permeability has been found and partly characterized in human tumor lymphocytes (Randriamampita and Tsien, 1993). The soluble mediator has a molecular weight under 500, is inactivated by alkaline phosphatase but not by heat or protease, and is released into the cytoplasm (and into the extracellular medium) upon depletion of the intracellular Ca stores.

The article by Lückhoff and Clapham describes a Ca channel in an epidermal cell line (A431) that is transiently activated by experimental conditions expected to reduce the Ca content of the ER. The slope conductance, although small (2 pS in 200 mM $CaCl_2$), was larger than the 24-fS channel of lymphocytes and large enough to be observed as single-channel openings. Their report represents the first single-channel records of a Ca depletion current, and it demonstrates that all Ca depletion channels cannot be identical because theirs has different conductance and selectivity properties than the 24-fS channel already described.

Now that a Ca depletion channel has been found with conductance large enough for single-channel recordings, more techniques can be applied to the problem. In addition to having the availability of single-channel analysis, one can envision a time when such channels (and perhaps accessory molecules) will be incorporated into artificial membranes, and purified chemical messengers will be added to regulate the channel openings.

A large number of unknowns remain. How many types of Ca channels are coupled to the depletion of internal stores? What are the coupling mechanisms? Might physical, as well as chemical, links be utilized? What is the nature of the chemical messenger that has already been partly identified (Randriamampita and Tsien, 1993), and are there more than one? Finally, can this pattern of functional communication from intracellular stores to the surface membrane be generalized? Are there comparable signals between other intracellular membranes; for example, does the ER talk to the mitochondria?

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Reining in Calcium Release

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In striated muscles, skeletal or cardiac, contraction is triggered by a sudden increase in the concentration of free Ca^{2+} near the myofilaments. The relaxation that follows contraction is brought about by the return of $[Ca^{2+}]$ to resting levels. A fast contraction-relaxation cycle depends on the mechanisms that ensure a fast decay of the elevated $[Ca^{2+}]$ as much as on those that determine its rapid increase.

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