New and Notable

Using the Soft Touch on Cell Surfaces

Deborah Leckband
Department of Chemical Engineering, State University of New York at Buffalo, Buffalo, New York 14260 USA

The past several years have seen an explosion in the number of available techniques for the direct quantification of molecular forces and the micromechanical properties of cell membranes. In an astonishingly short period, it has become possible to quantify single molecule properties such as the tensile strengths of receptor-ligand bonds. This has precipitated substantial breakthroughs in our understanding of biological adhesion. In principle, the goal of such measurements is to establish the in vivo function/behavior of biomolecules, but the sample requirements, sensitivity limitations, or the limited ranges of measurable forces often have restricted measurements to purified preparations or to model systems. The latter approach obviously decouples measurements from both the in vivo microenvironment of, for example, the cell surface and from adhesion (or compression)-induced biological responses. Because of experimental limitations, however, direct measurements of the full range of forces exerted by molecules at the surfaces of living cells have been tantalizing but difficult goals. In this issue of *Biophysical Journal*, through an elegant modification of the micropipette aspiration (MPA) technique, Evans et al. have achieved a tremendous advance in the ability to conduct measurements of single molecules at the surfaces of living cells and soft materials.

The micropipette aspiration technique (MPA) has been used extensively to probe the interactions between such soft materials as red cells or model lipid membranes. The adhesion between and mechanical properties of the membranes are determined from micromechanical analyses of the global deformations of two interacting membrane capsules that are held in contact by suction at the tips of opposed micropipettes. In this case, the force transducers are soft membrane capsules rather than mechanical springs, and the membrane tension controls the transducer stiffness. The measurable forces range from 0.01 pN — nearly 1000 times more sensitive than are attainable with the softest cantilever spring of an atomic force microscope (AFM) — to ~1000 pN, which is fivefold greater than the maximum force exerted by optical tweezers on living cells. Thus, MPA can probe the entire repertory of relevant biological forces that operate at the single molecule level. Moreover, the ability to tune easily the membrane tension by aspirating increasing fractions of the capsules into the pipettes is a distinct advantage over AFM for which a given cantilever spring constant is fixed.

Despite these advantages, previous MPA measurements were limited primarily to the interactions between lipid membranes. In the current article, however, Evans et al. have expanded tremendously the versatility of their technique by biochemically coupling a 2- to 3-μm-diameter microsphere (test bead) to an aspirated vesicle. This enabled them to probe directly the forces between ligand-coated microspheres and the surfaces of cells on an opposed glass slide. The AFM community recently began attaching microspheres to cantilever tips as more versatile probes of the forces between a variety of different materials (Ducker et al, 1991). This method also avoids the stringent sample requirements of, for example, surface force (SFA) measurements (Marra, 1985). Consequently, with this modification the biological systems amenable to these highly sensitive force measurements are now limited only by the available microspheres, the materials with which they can be modified, and materials that can be coated on the glass slide.

A second limitation of the earlier MPA design was the inability to determine the submicroscopic distances between the interacting materials. The distance-measuring capabilities of SFA, AFM, or TIRM (total internal reflection microscopy) made possible the direct measurements of both the molecular forces acting between two materials and the local material deformations in response to local stresses. For example, discrete jumps in the unbinding trajectories of AFM cantilever tips corresponding to single receptor-ligand bond rupture and local discontinuities in the optical interference patterns in SFA measurements signaling the onset of membrane bilayer fusion have both been measured directly (Florin et al., 1994; Helm et al., 1989). With the MPA technique, similar information could be only indirectly inferred from global deformations in the capsule transducer. Thus, extracting the local contributions to membrane interactions was not possible.

The second major advance in the current work, therefore, is the implementation of reflectance interference contrast (RIC) microscopy in conjunction with MPA to quantify the submicroscopic distances between the test bead and the coated glass substrate. This has enabled Evans et al. to determine directly both the shapes of the contact regions and the distances between the bead and glass surface from the patterns resulting from the interference between light reflected from both the glass slide and the test bead surfaces. The reported resolution is ~5 nm. Although much higher resolution (0.1 nm) can be achieved with the optical techniques of AFM and the SFA, the difficulty in measuring soft, rough surfaces with the AFM and the sample requirements and detection limits of the SFA limit their usefulness in live cell measurements. Because the roughness of membrane interfaces is ~10 nm, the modified MPA technique is quite adequate for measurements at the surfaces of intact cells and soft materials. Consequently, Evans et al. now have at their disposal a highly sensitive and tremendously

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versatile tool for direct measurements of local membrane compliances and of forces exerted by single molecules on live cells.

This promises to be an extremely powerful tool for probing molecular interactions at cell surfaces. Although the limitations in the lateral spatial resolution and in the distance resolution normal to the interface do not permit molecular scale definition of the cell surface, the ability 1) to quantify both the static and dynamic properties of single cell surface receptors in their native environments and 2) to characterize local membrane deformations in response to focal attachments is currently unmatched. It is interesting to speculate on the potential information that will be gained from future studies with this method. Perhaps one of the more intriguing questions in cell adhesion research is how the rate of increasing the force on a bond impacts both the measured bond strength and the detachment rate (Dembo et al., 1988; Kaplanski et al., 1993). In addition, what influence does the microenvironment have on cell surface receptor function? Preliminary results suggest that it will also be possible to detect dynamic responses to the formation of receptor-ligand bonds, and this opens up exciting possibilities for elucidating the molecular mechanisms that couple receptor-ligand bond formation to a number of ligand-induced cell responses.

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Burst Busters: Uncovering a New Mechanism in Pancreatic β-Cells

Joel Keizer

Institute of Theoretical Dynamics, University of California, Davis, California 95616 USA

In this issue of *Biophysical Journal*, Bertram et al. present evidence that a nonspecific cation conductance that is activated by calcium depletion of internal stores plays an important role in bursting electrical activity and insulin secretion in pancreatic β-cells — as if the mechanisms underlying secretion in β-cells were not complex enough already! Located in the pancreatic islets of Langerhans, β-cells are responsible for the synthesis, storage, and exocytosis of insulin granules. The primary endogenous secretagogue for insulin is glucose, and therein lies the complexity. Rather than using a receptor, glucose stimulates secretion only after being taken up through specific glucose transporters (GLUT2) and then metabolized via the glycolytic pathway, the Krebs cycle, and oxidative phosphorylation.

According to the traditional paradigm, increases in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) are responsible for triggering exocytosis. So how does glucose metabolism accomplish this? Two classes of mechanisms are at work here: those that mediate Ca^{2+} influx through the plasma membrane and those involving Ca^{2+} uptake and release from internal compartments. Plasma membrane influx of Ca^{2+} is complicated by the fact that β-cells are electrically excitable. In an islet perifused at low glucose concentrations (≤5 mM), microelectrodes register stable membrane potentials of about −65 mV. This resting potential is determined primarily by large numbers of ATP-sensitive potassium channels (KATP) that are active at resting concentrations of ATP (Satin and Smolen, 1994). At higher concentrations of glucose (10–16 mM), elevated ATP concentrations due to metabolism inhibit these channels, depolarizing the β-cell to such a degree that voltage-activated Ca^{2+} channels now become active, further depolarizing the cell. This self-regenerating depolarization, however, is counteracted by delayed-rectifier K^+ channels that partially repolarize the membrane. This occurs on a time scale of several hundred milliseconds and produces action potential-like spikes of electrical activity that, because the KATP channels remain inactive, repeat over and over again. During this so-called "active phase," each action potential spike (~20 mV in amplitude) brings a small increment of Ca^{2+} into the cell while at the same time the plasma membrane gradually repolarizes over the course of ~15 s.

Although the origins of this slow hyperpolarization have been attributed variously to slow voltage inactivation of Ca^{2+} channels, Ca^{2+}-activated K^+ channels (KCa), and the indirect activation of KATP channels by Ca^{2+} uptake into the mitochondria, the ultimate effect is a sudden drop of membrane potential and a "silent" phase lasting ~10 s. During this period, Ca^{2+} influx is minimal, and simultaneous measurements using fluorescent Ca^{2+} dyes reveal a slow decrease in $[Ca^{2+}]_i$. At the same time, the membrane slowly depolarizes until another volley of action potential spikes occur. These repetitive bursts of electrical activity occur in phase with both an elevated plateau in $[Ca^{2+}]_i$ and increases in the rate of insulin secretion. At even higher concentrations of glucose (20 mM), the silent phase disappears and only continuous spiking is observed. Using extensive data collected by electrophysiologists, a number of realistic kinetic models of bursting electrical activity have been developed (Satin and Smolen, 1994).

What makes β-cell Ca^{2+} handling really complicated is that plasma membrane influx is strongly coupled to uptake and release by internal stores,

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