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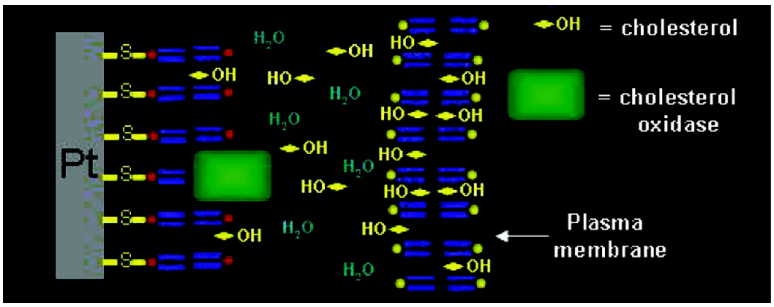

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Steady-State Detection of Cholesterol Contained in the Plasma Membrane of a Single Cell Using Lipid Bilayer-Modified Microelectrodes Incorporating Cholesterol Oxidase

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Cholesterol is a major structural component of the mammalian cell plasma membrane that regulates fluidity and permeability.[1] Cholesterol is also a primary constituent of protein and lipid assemblies in the plasma membrane (i.e., rafts) that are involved in signal transduction among cells, immune response, cell infection, and cell surface polarity. The ability to evaluate the dynamics of intracellular cholesterol trafficking to and from the plasma membrane would allow characterization of pathways governing cholesterol homeostasis and, in particular, of the initial steps in atherogenesis.[1] An attractive approach to track, in real time, changes in the cholesterol content of the cell plasma membrane involves the use of a microelectrode to study the behavior of a single cell. This communication reports electrochemical detection of cholesterol contained in the plasma membrane of a single cell using platinum microelectrodes modified with a lipid bilayer membrane containing cholesterol oxidase. The steady-state electrode response appears to correlate with the cholesterol content of the cell plasma membrane.

The idealized structural model of the thiolipid/lipid bilayer membrane with cholesterol oxidase partially inserted in the outer lipid leaflet is taken from the literature describing binding of cholesterol oxidase to cell plasma membranes.[2] These studies indicate that association of the enzyme with the cell plasma membrane allows cholesterol contained in the membrane to move directly into the enzyme active pocket without interaction with the aqueous phase. Therefore, in the work described here, cholesterol is believed to partition into the electrode-supported lipid bilayer membrane prior to enzymatic oxidation.[3]

Lipid bilayer vesicles have been used as solution-phase acceptors of cholesterol in cellular efflux studies.[4] This literature provides the basis for using microelectrodes modified with a lipid bilayer membrane containing cholesterol oxidase to extract and detect plasma membrane cholesterol. Cholesterol molecules contained in the plasma membrane move across an aqueous (membrane solvation) layer and partition into the electrode-supported lipid bilayer membrane. Detection is achieved through electrochemical oxidation of hydrogen peroxide generated upon enzyme-catalyzed oxidation of cholesterol by molecular oxygen.

Experiments for detection of cholesterol contained in a single oocyte (*Xenopus* frog) are conducted by initially positioning the electrode about 5 μm from the cell surface (Figure 1A) for acquisition of baseline data (no cholesterol detection). Repositioning the electrode and contacting the cell plasma membrane (Figure 1B) results in a steady-state current response. Contact is defined as the electrode position that corresponds to maximum electrode response. As shown in Figure 2, positioning the electrode directly adjacent to the cell (within ca. 1 μm or partially touching) produces an intermediate response, while contacting the cell with some force yields maximum current. The response observed adjacent to the cell may reflect detection of cholesterol efflux (i.e., solution-phase

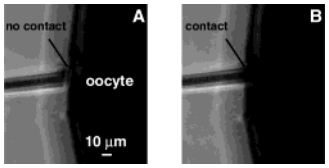

Figure 1. Photographs showing the electrode: (A) positioned about 5 μm from the plasma membrane and (B) contacting the plasma membrane.

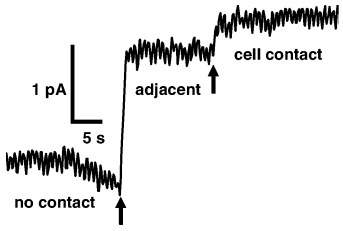

Figure 2. Amperometric data for detection of cellular cholesterol at a microelectrode (11.5 μm diameter) modified with a lipid bilayer membrane containing cholesterol oxidase. No contact (Figure 1): baseline data; no cholesterol detection. Adjacent: data for positioning the electrode within about 1 μm of (or partially touching) the plasma membrane. Cell contact: data for contacting the oocyte plasma membrane. Arrows approximate the times of changing electrode position. The buffer is 0.1 M sodium phosphate, pH 6.5. The electrode potential is 800 mV vs NHE.

cholesterol). Control experiments for contacting cells with lipid bilayer-modified electrodes containing no enzyme show no response. Additional control experiments using bare platinum electrodes also show no response. These experiments, in conjunction with data suggesting that electrode response correlates with the cholesterol content of the cell plasma membrane (vide infra), imply that the anodic current observed at the enzyme-modified electrodes reflects detection of cellular cholesterol. Cholesterol oxidase does have activity for oxidation of other sterols. However, cholesterol is the only sterol reported to be a constituent of the cell plasma membrane.[5] Additionally, the microelectrodes have been used to detect cholesterol within the lipid bilayer membrane of giant vesicles[6] (Supporting Information).

The apparent steady-state responses suggest that cholesterol oxidation is not limited by lateral diffusion of cholesterol in the plasma membrane to the electrode contact site (i.e., no current decay for depletion is observed). The steady-state responses could reflect a slow rate of cholesterol mass transfer from the plasma membrane to the electrode-supported lipid bilayer membrane compared to the rate of replenishment to the contact site though lateral diffusion. However, this possibility is not consistent with data indicating that response magnitude is dependent on electrode pretreatment conditions. Electrode response depends on the time allowed for immobilization of enzyme on the electrode with longer incubation (in enzyme solution) leading presumably to more immobilized enzyme and larger responses (Supporting Information). These data

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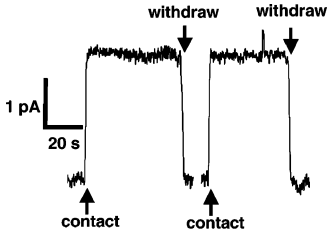

Figure 3. Replicate experiments showing amperometric responses for contacting an oocyte with a lipid bilayer-modified microelectrode containing cholesterol oxidase. Other conditions as in Figure 2.

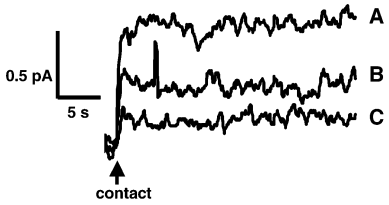

Figure 4. Amperometric responses for contacting an oocyte (A) prior to cholesterol depletion, (B) after partial cholesterol depletion, and (C) after near complete cholesterol depletion. All data collected at the same oocyte using the same electrode. Other conditions as in Figure 2.

suggest that response magnitude is dictated by the rate of enzyme catalysis for the cholesterol concentration reached in the electrode-supported lipid bilayer membrane under steady-state turnover. It is noted that the cholesterol content of the plasma membrane (0.5 cholesterol/phospholipid ratio[7]) is more than sufficient to produce the observed responses. For example, the cholesterol contained in the region of the plasma membrane defined by the electrode contact area (ca. 100 amol cholesterol) could produce 20 pC.[8] The total amount of cholesterol contained in the oocyte plasma membrane is estimated at 4−6 pmol (oocyte diameter is ca. 800−1000 μm).

It is not yet known if cholesterol contained in the inner leaflet of the plasma membrane significantly contributes to electrode response. The reported $t_{1/2}$ values for cholesterol transbilayer movement (flip-flop between the leaflets of the cell plasma membrane) range from <1 s to several hours.[9] This uncertainty extends to the possibility that cholesterol diffuses to the electrode contact site from membranes inside the cell. Intracellular cholesterol trafficking between the endoplasmic reticulum, Golgi, and plasma membrane is believed to involve vesicular transport and energy consumption.[9] Lateral diffusion of cholesterol in the plasma membrane to the electrode contact site is likely the primary source that feeds cholesterol oxidation. The steady-state nature of the response is consistent with the notion that cholestenone (oxidized cholesterol) does not significantly accumulate in the electrode-supported lipid bilayer membrane and affect the rate of enzyme catalysis.

As shown in Figure 3, replicate experiments for contacting a cell using the same electrode show reproducible responses. This result is significant because it allows an electrode to be used for evaluating changes in the cholesterol content of the cell plasma membrane. Such behavior is demonstrated by measuring electrode response at cells that have been partially depleted of plasma membrane cholesterol through incubation in cyclodextrin solution[9] (1 mM). Even electrodes yielding a relatively small response (e.g., 0.8 pA) show a discernible decrease when cholesterol has been removed from the plasma membrane (Figure 4). Exposing the cell to cyclodextrin solution for 5 min results in a decrease in response of about 50% (compare traces A and B). Exposing the cell to cyclodextrin solution for an additional 20 min results in a further decrease in response (trace C). Although cyclodextrin selectively removes cholesterol from the cell plasma membrane,[9] the possibility that other changes in the plasma membrane (as a result of cyclodextrin exposure) cause the decrease in electrode response cannot conclusively be ruled out. It is noted, however, that cholesterol can be delivered to the plasma membrane from a cyclodextrin solution containing cholesterol and electrode response is reestablished (Supporting Information).

Platinum microelectrodes modified with a lipid bilayer membrane incorporating cholesterol oxidase are used for detection of cholesterol contained in the plasma membrane of a single cell. Electrode response appears to correlate with the cholesterol content of the cell plasma membrane. Experiments using giant lipid bilayer vesicles as a model system for the cell plasma membrane are underway to calibrate electrode response and gauge the rate of transbilayer movement of cholesterol.

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Supporting Information Available: Figure S1; vesicle photograph, Figure S2; data collected at vesicles, Figure S3; dependence of response on electrode pretreatment, Figure S4; delivery of cholesterol to cell from cyclodextrin solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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