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Locating Calcium in Membranes with X-ray Standing Waves

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Calcium plays a pivotal role in the life of the cell. Unfortunately, calcium is not particularly easy to monitor directly in intact biological or even simple model systems. In what follows, we show that synchrotron-radiation-based x-ray standing waves can be used to monitor directly and with atomic resolution the location of calcium in the profile structure of a model membrane under ambient conditions. This new capability has far-reaching implications for biological structure−function studies involving calcium at surfaces and interfaces and in thin films.

While the biochemical functions ascribed to calcium are many and varied, investigations of same are often limited by a paucity of techniques available for its direct measurement in natural and model systems. Thus, indirect studies concerned with calcium typically resort to replacing it with a spectroscopically active ion such as a lanthanide or to complexing it with a bulky fluorophor. To date, X-ray standing wave (XSW) measurements in the biomaterials area[1,2] have relied on the use of such heavy atoms as As, Br, Cd, Mn, Se, and Zn with atomic numbers *Z* of 25 and above. Access to the lighter, biologically important elements such as calcium under physiologically relevant conditions, i.e., without the need for an ultrahigh-vacuum environment,[3,4] was considered unrealistic due to an intrinsically weak and low-energy characteristic X-ray fluorescence. As a result, air attenuation presents a major obstacle to calcium detection as does interference from the intense fluorescence of argon (dry air is ca. 1 mol % argon). These factors, coupled with a small surface coverage ($<10^{14}$ atoms/cm^2), conspire to make XSW measurements of calcium in biologically relevant systems problematic. The results described herein show that XSWs can now be used to locate calcium in membranes and at surfaces with angström resolution under ambient conditions when the sample is housed in a helium-filled enclosure.

Variable period XSWs are generated when X-ray plane waves striking a reflecting mirror interfere with the corresponding reflected plane waves.[5−7] The positions of the nodes (minima) and antinodes (maxima) of such an XSW *E*-field can be manipulated in a controlled and predictable way, most simply, by changing the angle of incidence θ. The position of a layer of heavy atoms suspended above the mirror surface in an organic thin film or liquid can be determined by recording the X-ray fluorescence from the heavy atom layer as θ is adjusted in the range $0 < \theta < \theta_c$, where θ_c is the critical angle for the reflecting surface. The fluorescence yield is minimized and maximized, respectively, when the nodes and antinodes of the XSW are brought into coincidence with the heavy atom layer during a scan in θ.

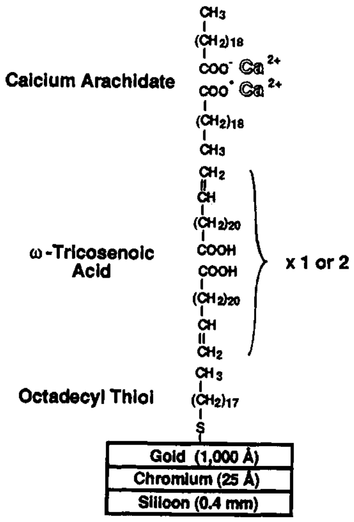

Figure 1. Schematic representation of the gold mirror, substrate, and deposited films. The hydrophobic gold mirror and the ω-tricosenoic acid film were prepared as described previously.[7,8] The Langmuir−Blodgett film of calcium arachidate was deposited by using arachidic acid spread on a $CaCO_3$ subphase (0.1 mM) at 30 mN/m.

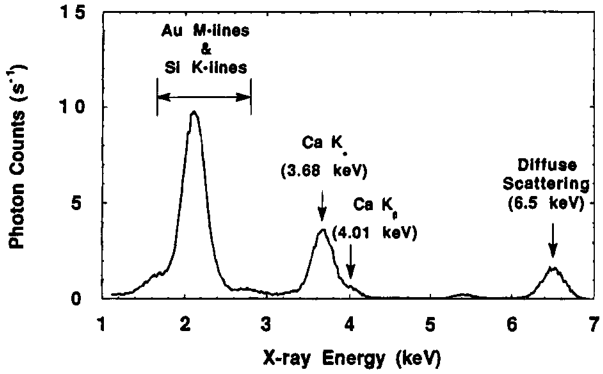

Figure 2. X-ray fluorescence spectrum from sample 1 at an incident angle of 5.0 mrad. The fluorescence measurement was made using monochromatic light (6.5 keV) on the X-15A line at the National Synchrotron Light Source, Brookhaven National Laboratory, with a helium-filled sample chamber. The experimental arrangement is described in the text and in refs 7 and 8. The data in this figure were collected in 260 s to ensure satisfactory counting statistics. In the spectrum, the gold M- and possibly silicon K-fluorescence lines ranging from 1.5 to 3 keV dominate the low-energy region. These data suggest therefore that if XSW measurements are to be made on systems with a lower calcium concentration or on elements lighter than calcium and which have fluorescence lines at even lower energies, the gold mirror must be replaced by a different material. Copper is a likely candidate.

The strategy used to address the usefulness of an XSW as a long-distance surface probe for light elements such as calcium follows (see Figure 1). A hydrophobic gold mirror with one inverted bilayer of ω-TA (sample 1) and an inverted bilayer of calcium arachidate was prepared which posits the calcium layer ca. 100 Å above the mirror surface. By way of contrast, a second sample (sample 2) was prepared incorporating two ω-TA inverted bilayers placing the calcium layer some 200 Å above the gold surface.

The K-fluorescence from calcium has a low characteristic energy ($E_{K\alpha} = 3.68$ keV, $E_{K\beta} = 4.01$ keV) in the soft X-ray region. Calcium X-ray fluorescence attenuation in air can be sufficiently great to prevent its detection. Additionally, fluorescence at 2.96 keV from argon in the air is close enough in energy to the Kα line of calcium and is of sufficient intensity to interfere with the calcium fluorescence measurement. These problems are obviated simply by enclosing the sample in a helium-filled chamber fitted with X-ray transparent windows.

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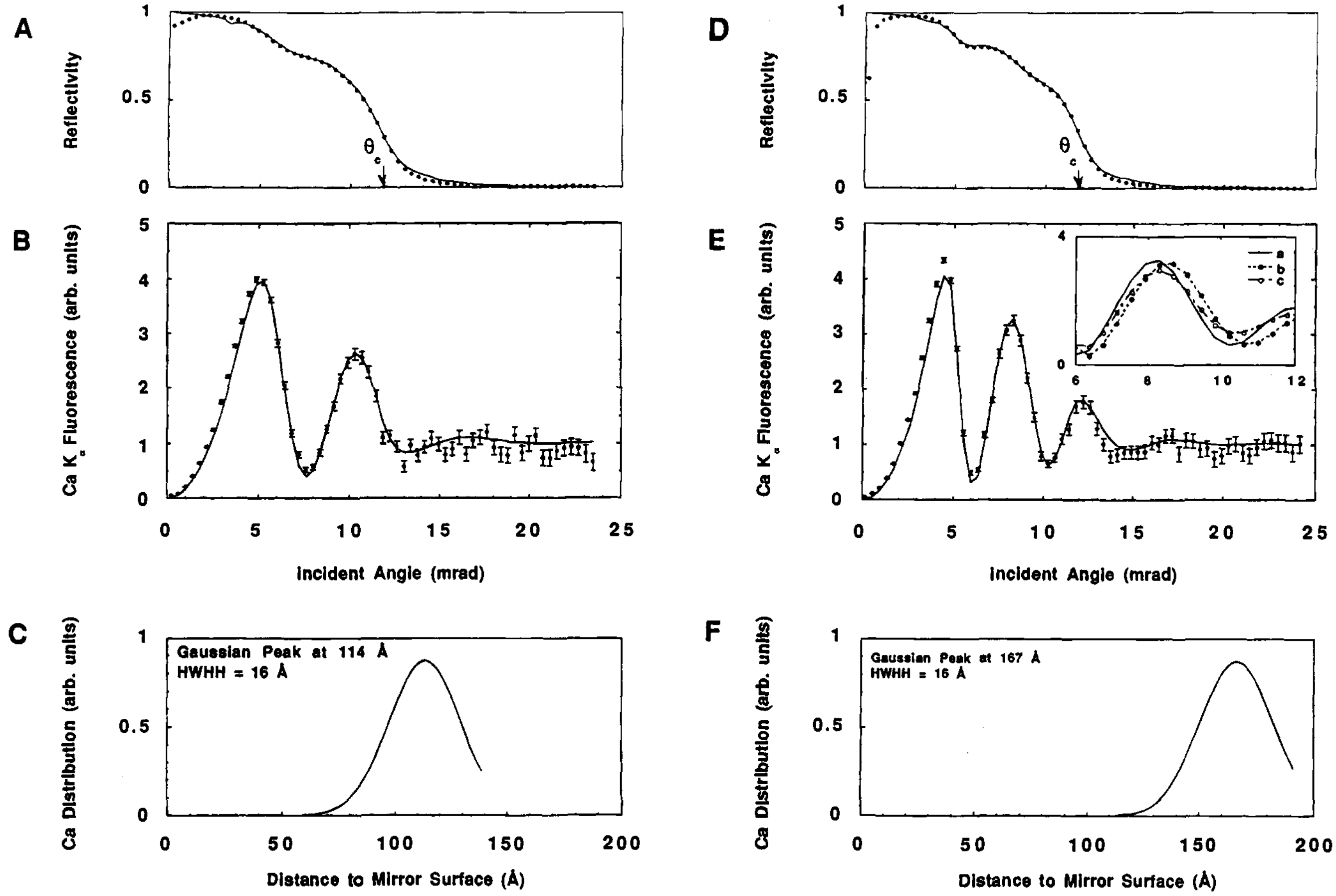

Figure 3. The experimental (circles) and theoretical (solid lines) angular dependence at 6.5 keV of the specular reflectivity (A, D) and the calcium Kα fluorescence yield (B, E) for samples with one (sample 1: A, B) and two (sample 2: D, E) ω-tricosenoic acid bilayers. The fluorescence data were corrected as described previously.[7,8] The calculated calcium distribution above the mirror surface is shown for samples with one (C) and two (F) inverted bilayers of ω-tricosenoic acid (see Figure 1). The inset in E shows the sensitivity of the fluorescence yield profile to the mean position of the calcium layer above the mirror surface and to the HWHH about the mean position. Curve a represents the best theoretical fit and corresponds to the solid line between 6 and 12 mrad in E. Curves b and c were generated by assuming a mean calcium position of 157 Å with a HWHH of 16 Å and a mean calcium position of 167 Å with a HWHH of 26 Å, respectively. Radiation damage to the sample was not monitored in this study. However, similar measurements on a related zinc arachidate system showed no evidence of radiation damage.[8]

Such an arrangement provides a well-resolved calcium peak in the X-ray fluorescence spectrum recorded during the calcium XSW measurement (Figure 2).

The results of the XSW measurements are presented in Figure 3 for the two samples studied. Included in this figure are the experimental reflectivity (Figure 3A,D) and fluorescence yield profiles (Figure 3B,E) along with the calculated calcium distribution above the mirror surface (Figure 3C,F). The theoretical curves were determined by minimizing the χ^2 fit to the data in the region between 5 and 11 mrad. The data show that the calcium-to-gold separation is 114 ± 3 Å and 167 ± 3 Å in samples 1 and 2, respectively, with an associated spread (half-width-at-half-height, HWHH) of 16 ± 3 Å in each sample. These results compare favorably with those obtained with related thin films incorporating the heavy atom zinc.[8] The data also show that XSWs offer extremely good spatial resolution, of order several angströms (see inset, Figure 3E), in determining the distribution of light atoms such as calcium when dispersed in organic thin films at low surface coverage.

With this new capability in hand, all of the advantages that go with the XSW method now extend to calcium. These include the ability to obtain structure information directly with atomic resolution in an element-specific manner on dilute samples that may take the form of an isolated membrane, an organic thin film, or an interface. Further, the method can be used with intrinsically ordered and disordered (liquid) systems and in static as well as in kinetic studies. Since calcium can be imaged directly, the need for potentially perturbing reporter ions or molecules is eliminated.

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