Resonance Reflectivity from Langmuir Monolayers of Fatty Acids Incorporating a Covalently Bound Resonant Species

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Introduction

Resonant x-ray reflectivity can simultaneously determine the overall electron density distribution of a monolayer and the precise location of resonant atoms within that layer, with sub-angstrom resolution [1]. This makes it a valuable tool for studying proteins whose function can often depend both on the presence of a single metal atom bound to a specific site within the macromolecule and on their incorporation into a membrane [2]. The role of Langmuir monolayers as reasonable model membrane systems makes it desirable to adapt resonant x-ray reflectivity to the air-water interface [3]. The technique makes use of the change in the scattering factor of atoms in the vicinity of their absorption edge — $f(E) = f'(E) + if''(E)$ — and compares reflectivity data collected at the absorption edge and data collected at energies above and below the edge. It requires a liquid surface spectrometer and constant exit-height monochromator so that the alignment of the spectrometer is maintained as the energy is changed. As our test case, we chose to work with a bromine-labeled fatty acid in order to have a system with known stoichiometry and molecular geometry.

Methods and Materials

The fatty acid $CH_3(CH_2)_{16}COOH$, called stearic acid and abbreviated SA, and the fatty acid $CH_3(CH_2)$15CHBrCOOH, called 2-bromostearic acid and abbreviated 2BrSA, were purchased from Sigma (St. Louis, MO), dissolved in high-pressure liquid chomatograph (HPLC)-grade chloroform at 1 mg/mL, spread onto a pure MilliQ water subphase maintained at 5°C, compressed to 5 mN/m, and maintained at that surface pressure during the reflectivity measurements. The third harmonic of the undulator was used to obtain photon energies of K = 13,474 eV, calibrated by the absorption from KBr powder. Reflectivity was recorded at energies of K, K + Δ, K – Δ, and K for SA and at energies of K, K + Δ, K – Δ, K, K + Δ, K – Δ, and K for 2BrSA, where Δ = 200 eV.

Results

Measuring the reflectivity from water, which is well understood, allowed us to normalize our data to account for small systematic energy-dependent effects. After energy- and Fresnel-normalization, reflectivity data collected from the SA monolayer showed no change with energy (Fig. 1), while data from the 2BrSA monolayer showed definite and reproducible energy-dependent effects (Fig. 2). The model-independent box refinement algorithm [4] applied to the data collected at E = K – Δ (where f″ is ~0) determined the nonresonant electron density distribution of the monolayers, $\rho_{nonres}(z)$. We then modeled the data at E = K, K + Δ as arising from $\rho_{nonres}(z)$ plus a Gaussian of amplitude $\Delta f'(E) + i\Delta\ f''(E)$ and used a simultaneous nonlinear fitting to locate the Gaussian representing the Br atom at z_{res} = with a width of σ. Since Br is the densest atom present and is adjacent to the electron-dense carboxyl headgroup of the fatty acid, its location at the peak in the electron density distribution is as expected.

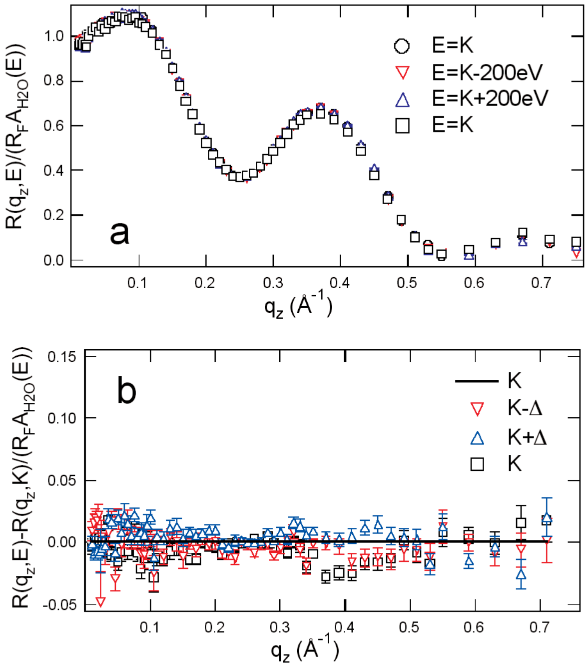

FIG. 1. Top graph a: Fresnel- and energy-normalized reflectivity data collected from an SA monolayer at various energies. Bottom graph b: Differences between the first data set at E = K in top graph a and the other data sets reveal no energy dependence in the reflectivity data.

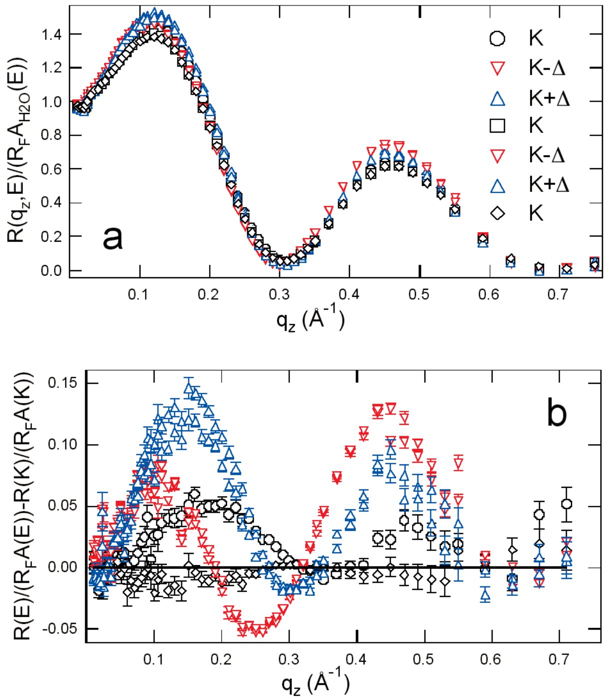

FIG. 2. Top graph a: Fresnel- and energy-normalized reflectivity data collected from a 2BrSA monolayer at various energies. Bottom graph b: Differences between the second data set at E = K in top graph a and the data at E = K ± Δ reproduce well, and energy-dependent resonant effects exist in the data at E = K ± Δ.

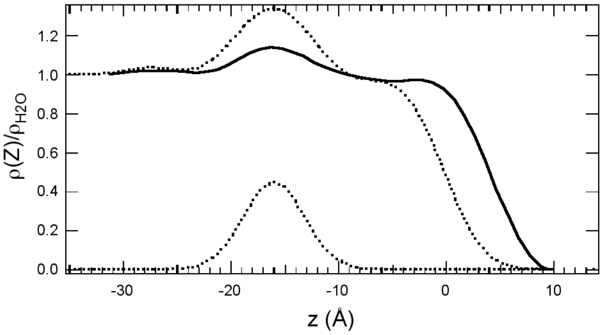

FIG. 3. The electron density distributions derived via box refinement for the SA monolayer (top solid curves) and 2BrSA monolayer (top dotted curve). The contribution to the monolayer from Br as determined from the resonance data is shown as the dotted Gaussian (bottom dotted) curve.

Discussion

The measurement demonstrates the first application of resonance reflectivity to locate the position of an atom covalently bound to an organic monolayer at the air-water interface. For a molecule as small as SA, labeling with Br does not preserve the isomorphism of the monolayer, but for larger molecules such as proteins, substitution of Br for H should not be such a significant perturbation of the structure. Coupled with solid-phase peptide synthesis, which allows the labeling of individual residues in the sequence, x-ray resonance reflectivity should prove quite useful for studying peptide monolayers.

Acknowledgments

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References

[1] J.M. Pachence, R.F. Fischetti, and J.K. Blasie, Biophys. J. **56**, 327-337 (1989).
[2] J.K. Blasie and J. Stamatoff, Annu. Rev. Biophys. Bioeng. **10**, 451-458 (1981).
[3] D. Vaknin, P. Krüger, and M. Lösche, Phys. Rev. Lett. **90**,178102 (2003).
[4] J.K. Blasie, S. Zheng, and J. Strzalka, Phys. Rev. B **67**, 224201 (2003).