Resonant Reflectivity from Langmuir Monolayers of a Synthetic Peptide with Metalloporphyrins Bound at Designed Sites

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Introduction

Synthetic peptides based on a di-α-helical design motif provide a robust protein scaffold whose design can be easily manipulated to incorporate features, such as binding sites for metalloporphyrin cofactors, that allow them to mimic properties of naturally occurring proteins, such as their redox behavior, but with much simpler structures.[1] Such peptides, with their surface activity enhanced by the addition of a C_{16} hydrocarbon chain to the N-terminus of each helix, can form Langmuir monolayers at the vapor/water interface that at high surface pressure (π) are vectorially oriented with the helical axes nearly normal to the plane of the interface, as demonstrated by x-ray reflectivity measurements of the electron density distribution of the monolayer, ρ(z).[2,3] These monolayers therefore could be developed as tools for studying intramolecular electron transfer in proteins, especially considering that they can be transferred without loss of orientation to solid supports[4] that may incorporate electrodes. Theoretical calculations of electron transfer in proteins requires detailed knowledge of the separation between redox centers, moieties that can change their oxidation state and so provide stable sites for electrons along the transfer path. In one class of redox centers, metalloporphyrins, a single transition metal atom (e.g., Fe, Zn) is bound within an organic macrocycle. In the vicinity of their K absorption edges, the structure factor f(q) of these metals possesses complex resonant (or "anomalous") components f′ and f″: $f(q)=f_0(q) + f'(q,E) + if''(q,E)$. We sought to exploit this property to localize the discrete metal sites within ρ(z) by comparing reflectivity data collected at the K edge of the metal and at energies slightly off-resonance (K±Δ, Δ = 50, 100 eV). This information can be joined with independent optical measurements of the orientation of the macrocycle to give a detailed picture of the monolayer's structure.[5] The constant-exit-height monochromator of beamline 9-ID and the liquid surface spectrometer (LSS) available at CMC CAT make it feasible to attempt to measure resonant reflectivity from peptide Langmuir monolayers.

Materials and Methods

We used a palmitoylated synthetic peptide, BBC16,[2,3] prepared by automated solid phase synthesis and comprising 31-mer mostly α-helical units with amino acid sequence
$CH_3(CH2)_{14}$CO-CGGGE IWKLH EEFLK KFEEL LKLHE ERLKK L
that are dimerized via a disulfide bond between the N-terminal cysteine residues and form two bis-His binding sites for metalloporphyrins between the H10,H10′ and H24, H24′ residues of one such dihelix. Hemo (zinc) BBC16 has a stoichiometric amount of Fe- (Zn-) protoporphyrin IX titrated in from DMSO stock solution while the apo peptide has no redox centers added to it. The monolayer spreading conditions are described in Ref. 3.

Results and Conclusions

As the resonant effect is small (for Fe at the K edge, $f' = -8e^-$, $f'' = 4e^-$ in a molecule weighing approx. 8 kD), its contribution to the total reflectivity signal is expected to amount to about 1%. This puts stringent requirements on the stability of the monolayer and the instrument. Using the unfocused undulator beam, we looked at apo and hemo BBC16 monolayers (we expect to see resonant effects in the latter but not the former) at and near the K absorption edge (7112 ± 50 eV), and saw variations between scans of about 5-10% in the reflectivity from both samples. Moreover, the difference signal between reflectivities collected from the hemo monolayer did not follow the behavior that we expected from our model calculations. More work will be needed to isolate the cause of the changes we observed and to try and mediate them. It may be better to concentrate future efforts on monolayers on solid multilayer supports, where resonant diffraction has already been demonstrated and correlated electrochemical measurements may also be developed.

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