

Protein adsorption to lipid membranes through metal-ion chelation studied by X-ray and neutron reflection and GIXD

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The association of proteins to lipid membranes plays a critical role in many biochemical processes within living systems such as membrane signaling and recognition. Targeting the adsorption of proteins to synthetic surfaces is key to many biotechnologies. Often it is required that a protein be adsorbed in a specific orientation and with the native conformation. From a fundamental point of view, a better understanding of a number of specific issues is needed: protein orientation and surface density, monolayer versus multilayer adsorption, the role of and competition between specific and non-specific interactions, and lateral order and conformational changes upon adsorption including denaturation of protein.

In this work neutron and X-ray reflection (NR and XR) are combined with grazing incidence X-ray diffraction (GIXD) to study the interactions of myoglobin with Langmuir monolayers of synthetic lipids [1]. NR and XR are used to determine the adsorbed amount and to obtain information regarding the orientation and conformation of the adsorbed protein. GIXD is used to study the response of the lipid layer that occurs upon protein binding.

The lipid monolayer is composed of the synthetic lipid distearyl imino-diacetate (DSIDA) that contains receptors for divalent metal ions such as Cu^{2+} and Ni^{2+} . Adsorption is due to interaction between the divalent metal ions and histidine moieties at the outer surface of the protein. The use of metal ion coordination to target the adsorption of proteins to lipid membranes has been studied previously [2,3]. Metal ion affinity is a versatile method for targeted adsorption of proteins. This strategy is widely used in proteomic studies [4-6], and for purification and concentration of His-tagged fusion proteins as well as many native and recombinant proteins [3]. It also has been explored for 2-D crystallization of proteins [7-9] and for orienting proteins at engineered interfaces for biofunctionalization [10-12]. A more thorough understanding of the adsorption process would benefit many of these applications.

A Langmuir monolayer of 100 % DSIDA was spread onto the surface of buffered D_2O or H_2O (Fig.1) and then compressed to a surface pressure of ≈ 40 mN/m where it forms a solid condensed phase layer (see GIXD data below.) A dilute solution of CuCl_2 or NiCl_2 was injected into the Langmuir trough and circulated underneath the condensed DSIDA monolayer. The divalent metal ion ions chelate at the imino-diacetate site as indicated in Fig. 1. Neutron reflectivity data for the condensed lipid layer with added Cu^{2+} ions are shown in Fig. 2 for both the H_2O and D_2O buffered subphases. Myoglobin was then injected into the subphase and neutron reflectivity data were collected repeatedly to monitor the time dependence of protein adsorption. The adsorbed protein layers became time-independent at ≈ 14 h after adding myoglobin into the solution. Only final sets of reflectivity data with adsorbed protein (for both H_2O and D_2O subphases) are shown in Fig. 2. The very large change in the reflectivity after protein injection indicates a significant adsorption of protein under the lipid layer. XR data for the same conditions (H_2O subphase) are shown in Fig. 3. Comparison of Figures 2 and 3 demonstrates that the best contrast is obtained with NR using an H_2O subphase. XR data are essential to obtain data over a large q range, where details of the lipid monolayer can be obtained. Solid lines in Fig. 2 and Fig. 3 are best fits from which the

segment concentration profiles of protein and the lipid film can be obtained. The normalized electron density profile from the fit to the XR data is shown in Figure 3c. Simultaneous fits to the XR and NR data for H₂O subphase show that the dimension of the adsorbed myoglobin layer is 43 Å +/- 5 Å, and that the amino acid volume fraction is 0.55 +/- 0.05. The dimensions of myoglobin from its crystal structure are 44 Å x 44 Å x 25 Å. Thus, the protein appears to be bound to the lipid layer in its native state in an end-on configuration.

If no CuCl₂ is added to the subphase, then no change in the neutron reflectivity signal is observed upon injection of myoglobin. Alternatively, if a strong Cu scavenging agent (EDTA) is injected into the subphase after protein adsorption, the reflectivity reverts back to that of the pure condensed lipid layer. This clearly shows that there is very specific binding of the protein to the chelated Cu²⁺ sites on DSIDA. Other data reported elsewhere have shown that the adsorbed layer structure at low coverage is significantly different when Ni²⁺ is loaded into the DSIDA headgroups instead of Cu²⁺[1].

Important insight has been revealed in complementary GIXD data from the DSIDA system. Fig. 4a shows the surface diffraction peak from the tails of a condensed DSIDA monolayer at an in-plane wavevector $Q_{xy} = 1.50 \text{ \AA}^{-1}$. This corresponds to a hexagonal packed lipid layer with a nearest neighbor spacing of 4.21 Å. Insertion of Cu²⁺ ions into the layer does not seem to affect the 2-D crystalline order of the lipid layer except for a slight change in lattice spacing.

When myoglobin is added to the subphase while maintaining a fixed area (after initial compression to 40 mN/m), no change in the surface diffraction peak is observed. However, when myoglobin is added to the subphase while maintaining the pressure constant at 40 mN/m and allowing the area to adjust, the diffraction peak arising from the hexagonal packing of the DSIDA tails (at $Q_{xy} = 1.50 \text{ \AA}^{-1}$) is drastically reduced at short times (Fig. 4b). This indicates a large perturbation in the 2-D crystallinity of the lipid layer upon interaction with the protein. The surface diffraction peak, however, reappears after a few hours (Fig. 4b). The peak is slightly shifted to a higher value of Q_{xy} , and the distribution of intensity normal to the surface (not shown) indicates a substantially increased tilt of the lipid tails. Comparing the time scales for assembly of the adsorbed layer from XR and NR with that observed in Fig. 4, we find that reappearance of the diffraction peak corresponds with assembly of a dense layer of adsorbed protein. Surprisingly, very little adsorbed protein is detected at the time when the diffraction peak first disappears. We hypothesize that the protein initially interacts with the lipid monolayer by transient reversible interactions. These interactions are sufficient to disrupt the packing of the lipid tails. We note that no change in the diffraction peak is observed when protein is circulated underneath a DSIDA monolayer that has not been loaded with metal ions. We further hypothesize that at a later stage, protein adsorption becomes irreversible as a dense layer begins to assemble. The dense irreversibly adsorbed layer of protein facilitates the recrystallization of the lipids tails.

In summary, a combination of neutron reflectivity and GIXD has proven to be a very powerful way to study both the protein adsorption to lipid monolayers as well as structural changes that the lipid layer itself undergoes as the protein adsorbs. This work shows the complementarity of NR, XR, and GIXD. NR and XR profiles are very sensitive to the adsorption of the protein, while GIXD is ideally suited for examining in detail any changes in the 2-D ordered structure of the lipid layer itself. These methods open up a rather large field to examine other important lipid-protein interactions such as the association of biological toxins with lipid layers.

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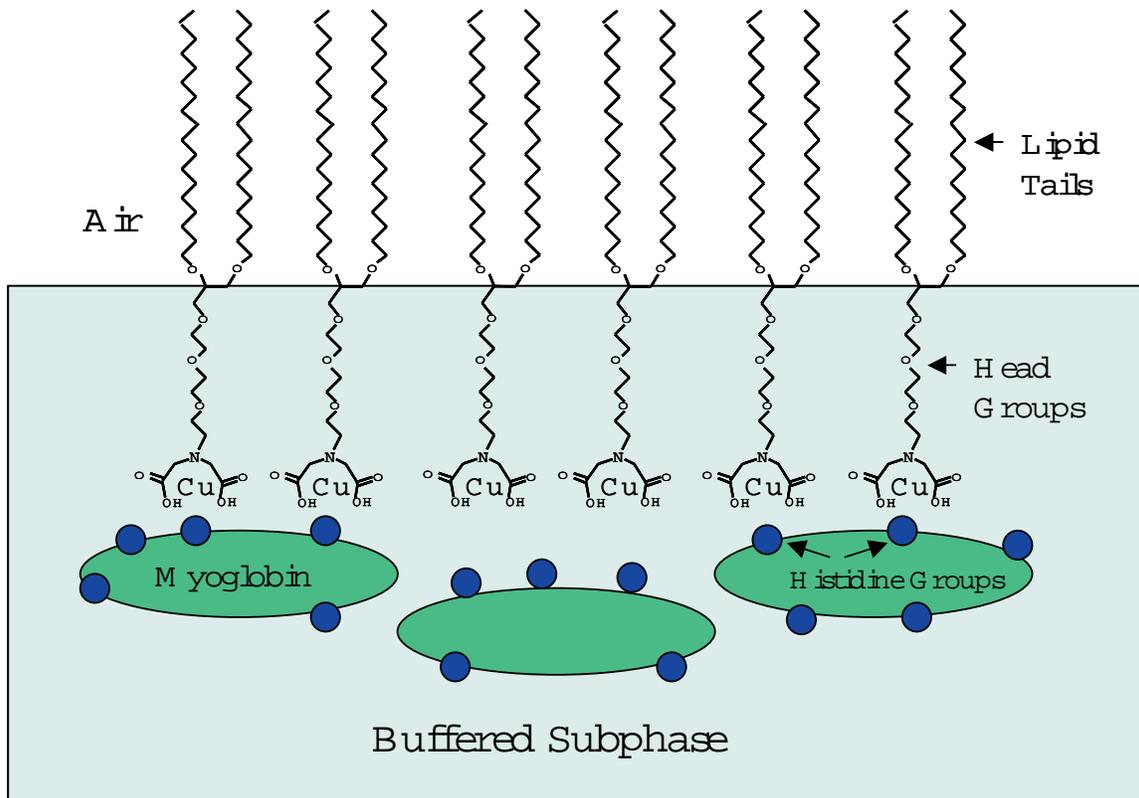


Fig. 1. Schematic of histidine groups on myoglobin adsorbing to distearyl imino-diacetate (DSIDA) molecules at chelated Cu^{++} ion sites.

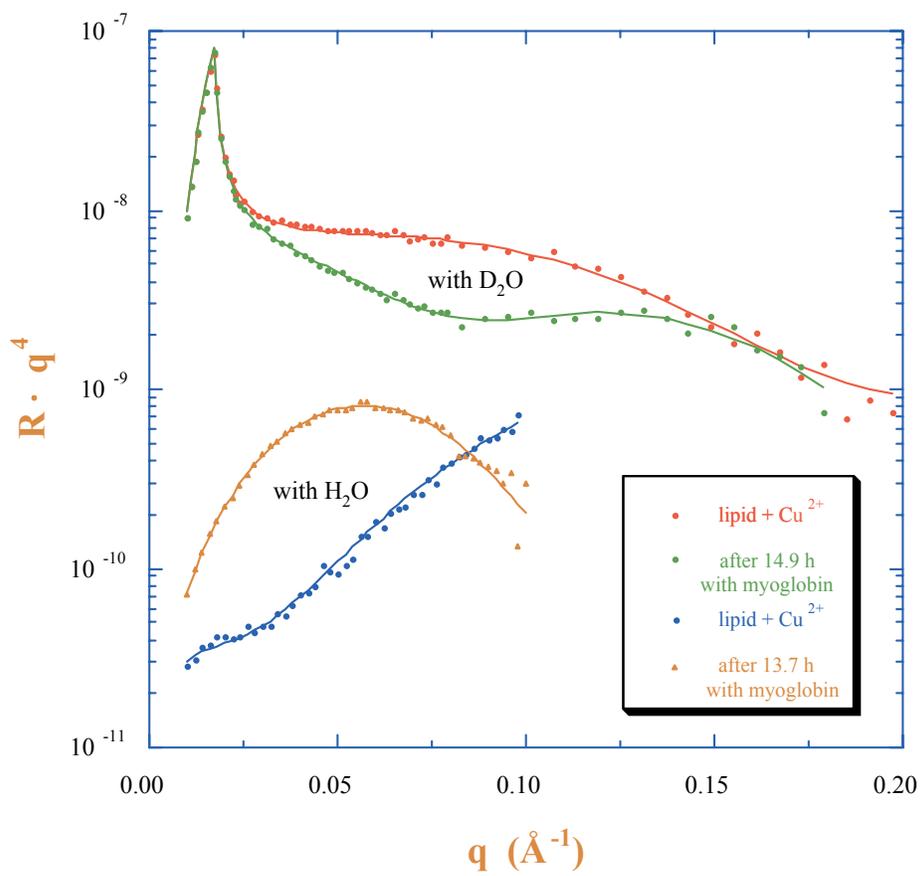


Fig. 2. Neutron reflectivity for 100 % DSIDA on buffered D₂O and H₂O subphases with and without myoglobin in the subphase. Solid lines are fits to the data.

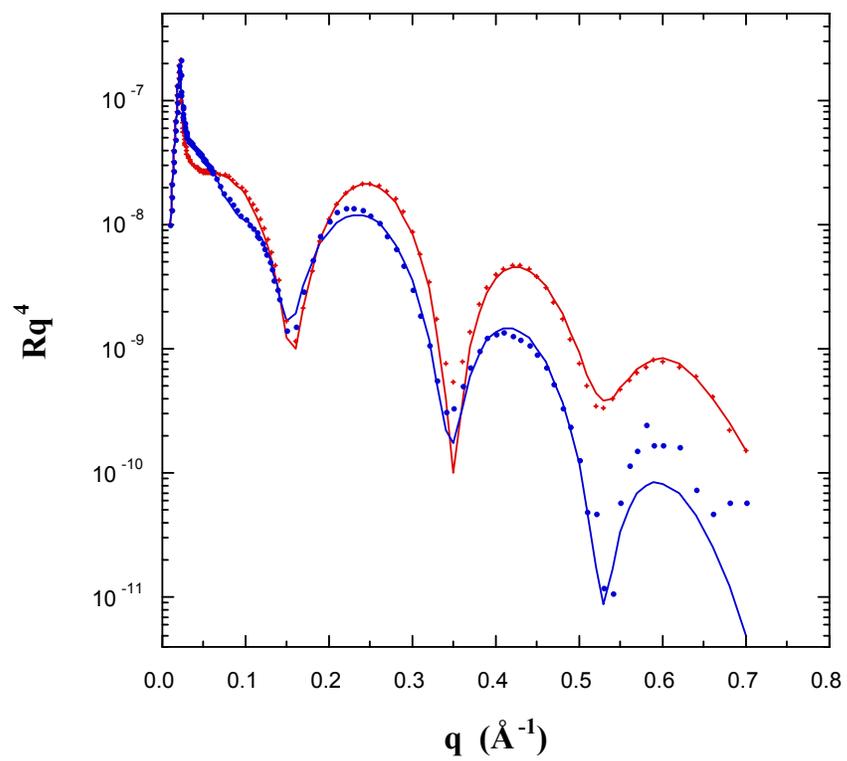


Fig. 3a. X-ray reflectivity for 100 % DSIDA on buffered H_2O subphase with and without myoglobin in the subphase. Solid lines are fits to the data.

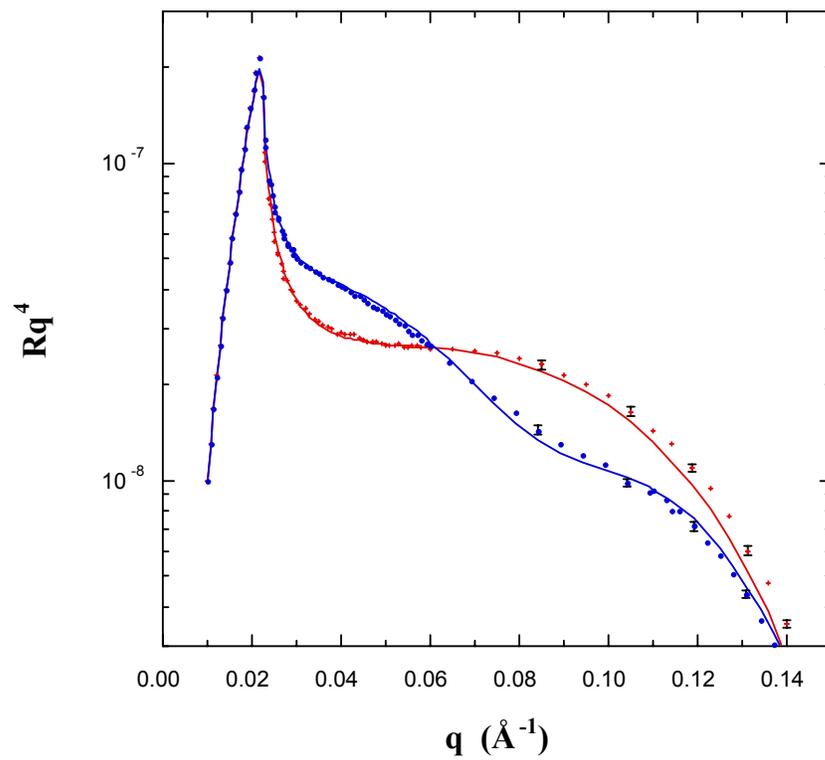


Fig. 3b. Expanded view of the low q region of the data in Figure 3a. This displays the signal due to the adsorbed protein.

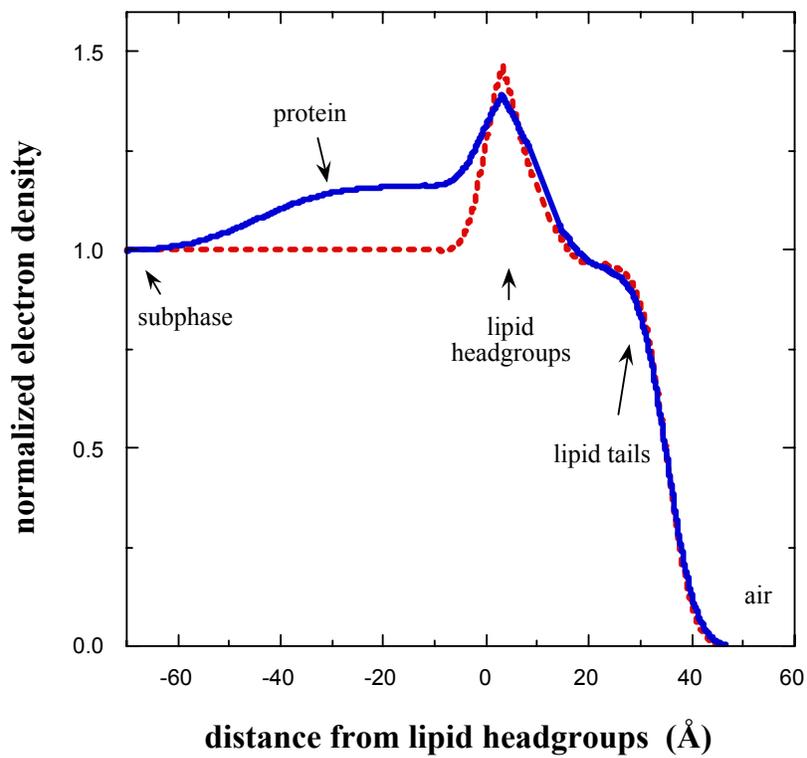


Fig. 3c. Normalized electron density profiles corresponding to the fits in Figure 3a and Figure 3b.

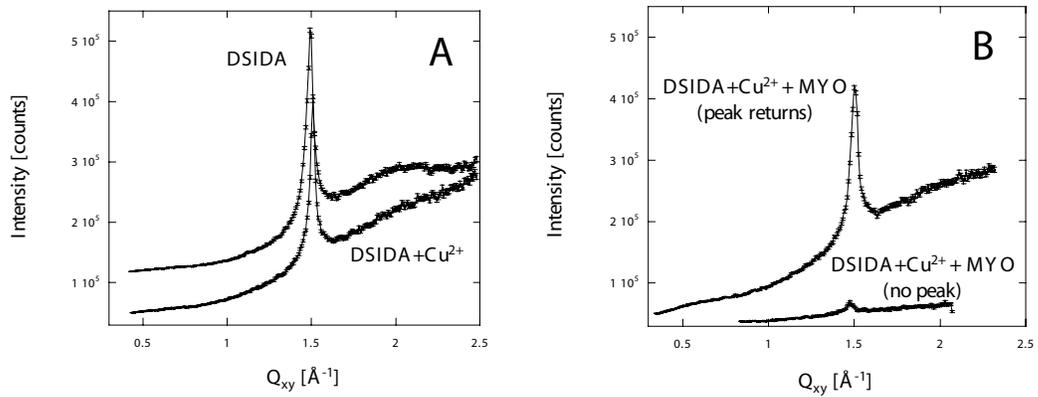


Fig. 4. Surface diffraction peak at a constant surface pressure of 40 mN/m: (a) 100 % DSIDA and also with Cu^{+2} , (b) 1.9 hr (no peak) and 4.4 hr (peak returns) after myoglobin injection.