X-Ray Diffraction of a Protein Crystal Anchored at the Air/Water Interface

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ABSTRACT We report the first successful in situ x-ray diffraction experiment with a 2D protein array at the lipid/water interface and demonstrate that the order can be controlled via lateral pressure or density.

A protein (streptavidin) was bound to a monolayer of biotinylated lipid at the air/water interface, and diffraction of the protein layer could be measured to many orders. Compression of the monolayer changed the diffraction pattern drastically, indicating that the protein structure can be strongly influenced by external parameters like lateral pressure or density. From the width of the peaks, we find that aggregates consisting of as few as 100 monomers contribute to the diffraction. This indicates that the structure of even low order aggregates can be studied in situ. Grazing incidence diffraction can become a strong new method to study the crystallization and the interactions between proteins free from artifacts by staining or sample preparation.

INTRODUCTION

Many important questions in biosciences have been solved via high resolution structure analysis of protein crystals. These studies, however, are limited by the availability of sufficiently perfect crystals. This may be an inherent problem due to small energy differences of various protein arrangements and to a lack of understanding of complex interactions. The latter necessitates systematic studies of protein crystallization in defined geometry. An optimum model system for this are two-dimensional protein arrays anchored to a lipid monolayer at an air/water interface: the two-dimensional protein density can be varied via lateral compression of the film and also environmental conditions can be varied over a broad range. Monolayers of streptavidin, a tetrameric protein strongly binding to biotinylated surfaces (Darst et al., 1991) have been studied by a variety of techniques within the last years. X-ray- (Haas and Möhwald, 1993), neutron- (Vaknin et al., 1991), and visible light reflection techniques (Herron et al., 1992) to measure the protein arrangement at the interface in the direction of the layer normal have been done, fluorescence (Blankenburg et al., 1989), and plasmon microscopy (Schmidt and Knoll, 1989) has been used to study lateral arrangement with μm resolution. After transferring on a support and staining, the 2D arrays could be studied by electron diffraction in vacuum. Because the latter technique is not free of artifacts and because the structure at the fluid interface has been shown to depend on environmental conditions, in situ diffraction studies of protein arrays are of utmost importance. Surface x-ray diffraction techniques have recently been developed to study lipid monolayers at air/water interfaces (Als-Nielsen and Möhwald, 1991; Kjaer et al., 1987). Extension of these techniques to investigate 2D protein crystals without staining, however, requires another step in instrumentation and surface control because of the large unit cell, small electron density contrast and, presumably, low crystal perfection. This step has now been achieved at the undulator beamline BW1 of the synchrotron source at DESY, Hamburg, studying 2D arrays of streptavidin binding to a monolayer of biotinylated phospholipid.

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MATERIALS AND METHODS

The biotinylated phospholipid *N*-((6(biotinylamino)hexanoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (triethylammonium salt) was purchased from Molecular Probes Inc. (Eugene, OR), and the streptavidin was from Boehringer Mannheim GmbH (Mannheim, Germany).

The biotin lipid was spread from a chloroform solution onto a subphase of 0.5 M NaCl in Millipore-filtered water. The monolayer was compressed to 15 mN/m, and a solution of 1 mg/ml streptavidin in 0.5 M NaCl was injected into the subphase to a protein concentration of 2×10^{-8} M.

Synchrotron grazing incidence x-ray diffraction experiments were performed using the liquid-surface diffractometer (Als-Nielsen and Möhwald, 1991; Kjaer et al., 1987) on the undulator beam line BW1 at HASYLAB, DESY, Hamburg. A sealed and thermostated Langmuir trough ($135 \times 80 \times 16$ mm^3) with continuous Wilhelmy type pressure-measuring system was mounted on the diffractometer. The subphase volume (170 ml) and the trough depth under the investigated area were reduced (to 300 μm) to minimize the influence of vibrations. The background level was reduced by maintaining a He atmosphere inside the trough during the measurements. The Synchrotron beam was made monochromatic by Bragg reflection from a Be (002) crystal to a wavelength $\lambda = 1.364$ Å and was adjusted to strike the surface at an incident angle $\alpha_i = 0.85\ \alpha_c$, where α_c is the critical angle for total external reflection. A linear position-sensitive detector (Braun, Garching, Germany) of 100 mm length was vertically arranged and moved horizontally to measure the diffraction of the monolayers. The resolution in in-plane wave vector transfer Q_{xy} given by a Soller collimator amounted to 0.02 Å^{-1}, that in normal wave vector transfer Q_z to 0.005 Å^{-1}.

The diffracting surface area was about 50×3 mm^3, which is much larger than the size of protein domains. Because these domains are not aligned, we measure a two-dimensional powder.

Diffraction was measured for the pure lipid monolayer and starting from 4 h after protein injection. Then the monolayer was compressed to 20 and 30 mN/m, and protein diffraction was measured at these points.

RESULTS AND DISCUSSION

Fig. 1 shows the diffraction intensity as a function of in-plane wave vector transfer Q_{xy} at various stages of compression

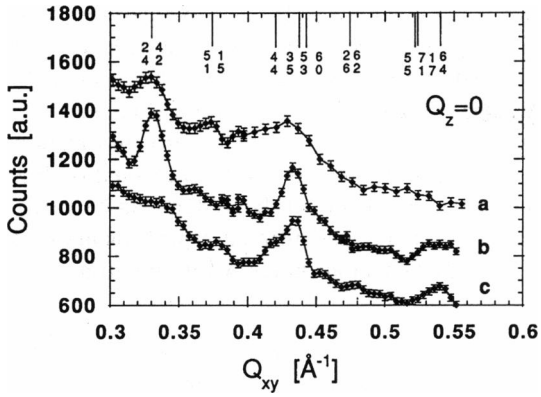

FIGURE 1 In-plane grazing incidence diffraction (GID) of streptavidin bound to a monolayer of Biotin-Caproyl-DPPE at the air/water interface at 15 mN/m (*a*) and after compression to 20 and 30 mN/m (*b* and *c*). The count rate for the middle curve is shifted by +200; the other two are the original data. The indicated lines above the plot give the positions for the allowed (hk0)-reflexes for a unit cell of C222 symmetry and with crystal axes of 84 and 85 Å as given in Darst et al. (1991).

after protein crystallization. For the initial state at 15 mN/m, by fluorescence microscopy one could observe the growth of protein domains with long range orientational order. At this pressure, one observes at least two diffraction peaks (at 0.33 and at 0.43 $Å^{-1}$) indicative of positional order. On increasing the pressure to 20 mN/m, the peaks become more pronounced, demonstrating an improved order. On further increasing the pressure, the diffraction pattern again changes. New peaks appear, e.g., at 0.38 $Å^{-1}$ and as a shoulder at 0.42 $Å^{-1}$, the peak at 0.33 $Å^{-1}$ seems to disappear. On top of Fig. 1, we indicate positions of allowed reflections for a two-dimensional centered rectangular lattice with symmetry and spacings as derived from the electron diffraction studies with this system (Darst et al., 1991). The protein arrangement at the air/water interface and the 2D symmetry that forms the basis of the peaks are depicted in Fig. 4. Apparently, the observed maxima coincide with the proposed positions. Small changes of the packing constraints strongly influence

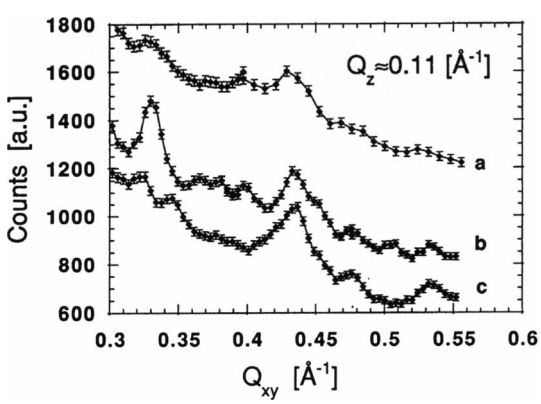

FIGURE 2 GID for the same system with $0.08 < Q_z < 0.15$ $Å^{-1}$. The main peaks split for the highest stage of compression. This may be due to an anisotropic deformation of the unit cell. The count rate for the middle curve is shifted by +200.

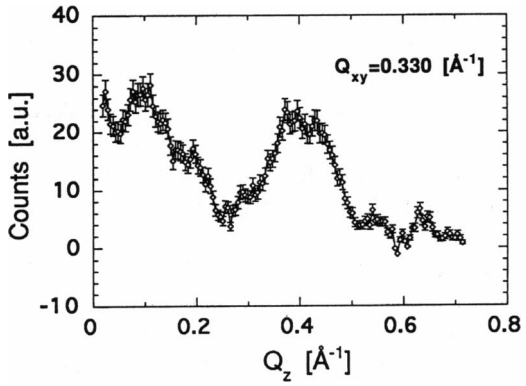

FIGURE 3 Q_z-resolved diffraction (rod scan) for the peak at $Q_{xy} = 0.330$ $Å^{-1}$.

the relative intensity, so for an unambiguous indexing the complete structural information of the protein is needed. The development of the peaks on compression could be further illuminated by measuring also diffraction with normal wave vector transfer $Q_z > 0$ (Fig. 2). One clearly observes a splitting of the dominant peaks. This would be expected for a transition from a quadratic to a rectangular lattice, like a unidirectional compression of the lattice as depicted in the upper part of Fig. 4. For $Q_z \approx 0.5$ $Å^{-1}$ (not shown here), a splitting is also observed for the peak at 0.38 $Å^{-1}$. Information on the electron density distribution along the layer normal is expected from a measurement of the diffraction intensity along Q_z (Bragg rod). This is given in Fig. 3 for the peak at $Q_{xy} = 0.33$ $Å^{-1}$. One realizes a pronounced structure with decaying intensity for $Q_z > 0$ and a second maximum at $Q_z = 0.4$ $Å^{-1}$. From the FWHM of the peaks, one can estimate the extension L of the scattering unit according to

$$\text{FWHM} = 2\pi/L.$$

From the width of the most pronounced maximum at $Q_z = 0.4$ $Å^{-1}$, one derives a thickness of about 40 Å. This is close to the protein thickness calculated from reflectivity

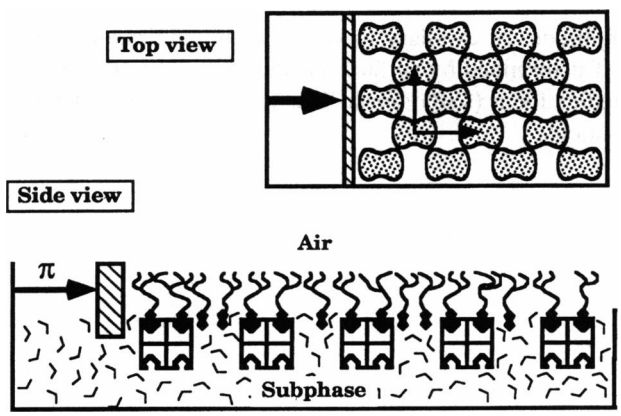

FIGURE 4 Sketch of the arrangement of the protein at the air-water surface. The side view shows the protein binding to the lipid to form a monolayer with regular spacings. In the top view the lateral arrangement (Darst et al., 1991) is depicted from which the diffraction lines in the upper part of Fig. 1 were derived.

data (Haas and Möhwald, 1993) and supports our conjecture that we indeed observe diffraction from the protein. Taking the protein as a vertically aligned cylindrical scatterer, one would expect only the first maximum at $Q_z \approx 0$. Because we find a second maximum and the rod scans are different for different peaks, a more detailed analysis should yield rich information about the electron density distribution throughout the protein.

From the peak width in Q_{xy}, one derives a lateral positional correlation length in the order of hundreds of Angstroms.

The experiments have shown that by compression the quality of the ordering in a protein array can be improved and the structure can also be changed in symmetry. This is in agreement with reflectivity studies leading to the conclusion that the lateral protein density can be varied by nearly a factor of two without destroying the orientational order (Haas and Möhwald, 1994). It raises the question whether these domains are crystals or liquid crystals with low lateral mobility. The mesophase picture is supported by the rather broad in-plane diffraction peaks measured in this work. From the peak widths, one estimates a positional correlation length of some hundreds of Angstroms, which is characteristic for a mesophase.

CONCLUSIONS

Although this study has not yet revealed a very high resolution protein structure, it opens many perspectives.

We have shown that 2D protein arrays can be studied in situ by x-ray diffraction. This is possible even if there are broad peaks, i.e., even aggregates containing only about 100 monomers are detectable.

We have observed more peaks, although faint, at lower Q_{xy} on a large background and need to observe even more for a high resolution. This is now a matter of optimization having recorded the first maxima. Also, at higher Q_{xy} more peaks should be found. It will become technically easier once lines become narrower because of better crystal perfection.

In normal direction, the limiting resolution is given by the amplitudes of capillary waves. The amplitudes are about 3 Å if determined by surface tension. However, they may be smaller for a (stiff) crystal because of a reduced bending elasticity.

To achieve the latter, we have shown that the monolayer at an air/water interface is a well suited system. The structure can be varied via the lateral density, and this should also lead to a structure optimized for diffraction studies.

Finally, this study promises that a protein function, e.g., ligand binding or hydrolysis can be measured on line. The environmental parameters of the system can be varied with time scales of seconds, and diffraction peak changes are measurable with sufficient accuracy within this time scale.

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