ESRF

Experiment title:
Structure determination of 2D crystals of cholera toxin by grazing incidence X-ray diffraction.

Experiment number:
LS166

Beamline:
IDl0

Date of Experiment:
from: **July 20,1995** to: **Ju1y 24, 1995**

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Shifts:
11

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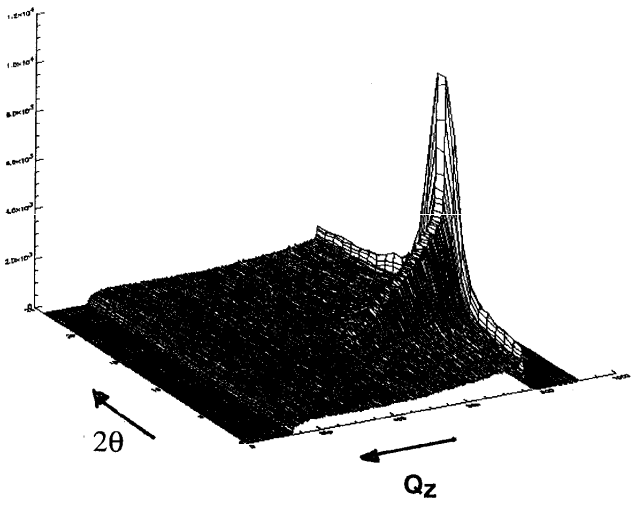
Report:

In the field of soluble proteins, a general method of 2D crystallization has been developed, based on the specific interaction between proteins and lipid-ligands incorporated in a planar lipid film at an air-water interface. Using this method, we have obtained 2D crystals of Cholera Toxin subunit B (CTB) bound to its physiological cellular receptor, themonosialoganglioside GM1. Our objective is to use grazing incidence X-ray diffraction as a complementary method to electron crystallography for analyzing the structure of protein 2D crystals. The main advantages are twofold : 1) protein-lipid films are analyzed *in situ,* in their aqueous native environment; 2) the distribution of structure factors along Bragg rods is determined directly.

Four shifts were necessary to align the ID10 intrument in the grazing incidence diffraction mode (2mrad of incidence angle on the Langmuir trough). This included the adjustments of the piezoelectric (vertically) focussing mirror and of the water level regulation system, plus the tuning of the monochromator and of the analyser to the wave-

length λ = 1.4235Å. The analyser crystal was necessary to achieve high resolution in the horizontal scattering plane. Using Gel 11, we measured an angular resolution of 9 mdeg.

The set up was then tested with a monalayer of 1 -dodecanol at the surface of water. The Figure shows that a signal/background ratio of about 15 was thus obtained. From calculations of the 2D powder diffraction intensities, resealed with the calculated and measured intensities of dodecanol. We thus expected at least 8 Bragg reflections having a signal/background ratio larger than 2 in the angular range : 2 deg c $2\theta <$ 6 deg, and for a 100% crystalline monalayer of CTB.



<1O> Bragg rod from dodecanol monolayer

During the following shifts, several monolayer of CTB bound to the lipid-ligand layer were prepared using the procedure well established in electron crystallography, but none of them produced any detectable Bragg reflection. We have identified two problems which apparently prevented the crystallisation of the protein monolayer:

(i) a first batch of lipids contained impurities affecting the stability of the lipid film; this was realized after a full day of experiment from the observation of the low rigidity of the monolayer.

(ii) the unsaturated DOPC used to ensure the fluidity of the lipid film actually polymerized under the intense X-ray beam due to the presence of double bonds : indeed, at the end of the experiment, a white streak was observable along the footprint of the beam and samples taken along this streak showed no crystalline domain by electron diffraction (performed after the experiment).