

**Experiment title:** Structure Determination of Gonadotropins

**Experiment number:**

LS412

**Beamline:**

BL4(ID2)

**Date of Experiment:**

from: 23/3/96

to: 24/3/96

**Date of Report:**

25/2/97

**Shifts:**

6

**Local contact(s) :**

A. Aberg

*Received at ESRF:*

10 MAR. 1997

**Names and affiliations of applicants** (\*indicates experimentalists):

Neil Isaacs, Jeremy Beachamp\*, Constantina Fotinou\*, Paul Emsley\*

**Report:**

Hexagonal FSH crystals can be grown routinely in our laboratory, but diffract weakly with our rotating anode source and only a little better at the Daresbury SRS. Other rodlike FSH crystals had been newly obtained at the beginning of March and were to be tested at the ESRF together with two further forms of FSH crystals grown from ammonium sulphate. Very small (<0.05mm) crystals of the first, hexagonal form were also to be tested, having been grown in slightly different conditions.

Crystal growth, using known conditions at the ESRF, was attempted to eliminate the stress of transportation on the crystals. This was ultimately unsuccessful, probably due to differences in temperature between the Glasgow and ESRF and the short time (two days) available for crystal growth.

The four crystal forms and conditions were tested for diffraction using both cryocooled and conventional mounting techniques. Exposure times between 15sec and 1 minute were used. One of our untested crystal forms turned out to be salt, while the other three crystal forms were all protein. All the crystals transported to the ESRF were used with no significant diffraction obtained beyond 9Å resolution. This resolution is not significantly better than that obtainable at Daresbury and is not worthwhile collecting. We assume that the disorder in the crystals of all sizes prevents high resolution data-collection and this is confirmed by the experiments at the ESRF. More work is needed to improve the crystals before further diffraction experiments are justified.

*Antigen-binding fragment of the monoclonal antibody 3A2 : Fab3A2.*

Fab3A2 is the antigen-binding fragment of the monoclonal antibody 3A2 raised against human Choriionic Gonadotropin. Our purpose was to collect high resolution cryocooled data (beyond 1.8Å.)

We tried several crystals until we found one diffracting to 1.33Å (Fig.1), with 80sec exposure. We tried various distances and rotated the detector by  $28 = 7''$  in order to separate the spots and reduce interference from the beam-stop mounting. Unfortunately, with this exposure time low resolution data

were saturated. To avoid problems with scaling therefore we decided therefore to set  $\theta = 0^\circ$  and the exposure time to 40sec, to collect to  $1.5\text{\AA}$  resolution. Higher resolution were going to be collected later. We collected 95 frames at these settings. We started then collecting higher resolution data at  $2\theta = 10^\circ$ , exposure time = 100sec. We observed a shutter problem in which the shutter closed after about 20sec (not consistently). We did what was recommended in the station manual and manage to solve the communication/network problems which had appeared, but could not repair the shutter. It was then midnight on Sunday and in the absence of further assistance we decided to continue the data collection under those adverse conditions. It is quite likely that the shutter problem existed in the previous data set we had collected, but we hadn't noticed.

At Glasgow we were unable to process the data using DENZO due to many rejections and high mosaicity ( average higher than  $3.5^\circ$ ) We observed that the images had a near concentric circles of varying background intensity around the center of the plate (Fig.2).

At the Computing School in Bellingham we showed a few images to Dr. Wladek Minor and asked his help. His opinion was : a) Misaligned scanner , b) Shutter problem.

We have noted some problems with the shutter and with data-processing that have prevented us from using the data collected at the ESRF. However, we would like to say that in general, the facilities were excellent and the beamline staff were very helpful.

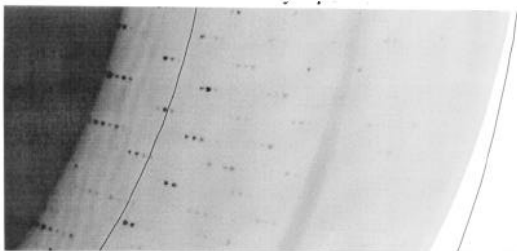


Fig.1: Diffraction of the Fab3A2 crystals to  $1.33\text{\AA}$  Outer circle  $1.3\text{\AA}$  .

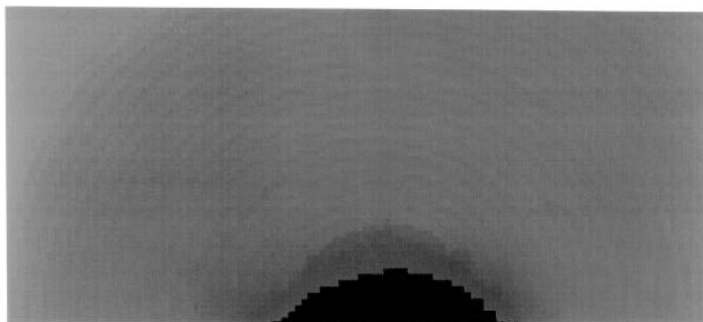


Fig.2: A part of the observed concentric circles around the center of the plate