



ESRF
experimental
report

Experiment title:

Structure determination of complexes between influenza virus hemagglutinin and neutralizing antibodies

Beamline(s) used:

Beamline 4 - ID 2

Date and time of experiment:

from: 10/11/95

to: 12/11/95

Experiment number:

LS-300

Local contact(s):

J. Shaw

Date of report

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received
(completed by ESRF):

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Experiment report (if this work has been published, please give reference and abstract):

Data collection of 2 different crystal forms was attempted during run LS 300.

1 - Influenza virus hemagglutinin / HC19 antibody Fab fragment complex

We attempted to collect a complete dataset at cryogenic temperatures (100 K). Previous experiments, at the resolution attainable at the L. U.R.E. (4 Å), had indicated this might be possible; but at 3Å, which is the resolution we were aiming for, the mosaicity of cryo-cooled crystals (which was in every case higher than 0.3°) proved incompatible with sufficient spot separation - a very drastic requirement in that particular case, as the crystal form has one very long unit cell axis (515 Å).

2 - Influenza virus hemagglutinin / BH151 antibody Fab fragment complex

In preliminary attempts of data collection at room temperature, we found that crystals of this complex were very sensitive to radiation damage. We have therefore collected data at 100K on a single crystal using the cryosystem implemented at the ID2 station.

To optimize data collection, we built "perpendicular loops", where the plane of the loop was approximately set perpendicular to the spindle axis; we chose that strategy because of the special morphology of the crystals -i.e. thin plates with the highest symmetry axis perpendicular to the plate. In this way, we were able to collect a complete dataset with sufficient redundancy, using a 50° rotation about the spindle axis.

The diffraction pattern is strongly anisotropic: the observed resolution limit is 2.5 Å along the c^* axis but only 3.0 Å in the (a^* , b^*) plane. The mosaicity is relatively high (0.6°) - which is often observed with cryo-cooled crystals. On the other hand, decay of crystal diffraction is negligible and this has allowed us to collect a data set of good quality (see statistics below).

The data characteristics after processing with software MOSFLM (version 5.2-A. G. W.Leslie (1990) in 'Crystallographic computing', Oxford University Press) and programs of the CCP4 suite (Rotavata-Agrovata) areas follows:

- maximum resolution: 2.7Å
- completeness: 98%
- Rsym on intensities: 6.4%
- average redundancy: 2.5

The structure of this complex has been solved by molecular replacement and refinement is now in progress.