REPORT ON VISIT TO MAJOR RESEARCH FACILITY

DATE OF REPORT:	FACILITY:	BEAMLINE/INSTRUMENT (if applicable)
18th November 1997	ESRF, Grenoble, France	D2AM
EXPERIMENT NO: LS741		

TITLE:	DATE OF EXPERIMENT
Crystallographic studies of a cytokine receptor	from 31st October 1997 to 5th November 1997
APPLICANTS + EXPERIMENTALISTS (name + affiliation/contact phone number)	CONTACT AT THE FACILITY: Dr Eric Fanchon
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ABSTRACT (200 words in plain English):

The cytokine interleukin-5 (IL-51 is well established as the primary regulator of eosinophil growth, differentiation and activation *in vivo*. Eosinophils appear to play an important role in the pathogenesis of asthma and certain parasite infections. IL-5 and its receptor may well prove to be important targets for drugs to treat allergic diseases.

We have produced single crystals of the extra cellular domain of the β subunit of the IL-5 receptor. This subunit is responsible for signal transduction across cellular membranes in response to ligand binding by IL-5. The crystals we have diffract weakly and data can only just be recorded above the poise level on a rotating anode X-ray source, requiring *ca*. 10 days to collect a 4.0 Å dataset from a cryogenically cooled crystal.

Previous trips to synchrotron radiation facilities have shown that we can reduce data collection times to a couple of hours per crystal and that the resolution and quality of the data are both improved when compared to conventional X-ray data collection. These were improved still further at this third generation synchrotron.

On this visit datasets were collected on crystals which had been soaked in various heavy atom reagents at two different pH values. Experiments were performed on crystals of the native protein and two genetically mutated forms. Cross-linking experiments were also undertaken.

Has this work been published (references)?

EXPERIMENTALREPORT

X-ray diffraction patterns were collected from our crystals at a wavelength of 0.97Å and a temperature of 100 K. These data were collected on beamline D2AM (French CRG) at the ESRF operating at 6 GeV in 16 bunch mode. Data were collected on a CCD detector during small (typically 1°) rotations of the crystal. The maximum resolution of the data obtained under these experimental conditions was increased to 2.9Å. This compares with 3.3A obtained at second generation synchrotron and 3.5 - 4.0Å on a conventional source.

Previous experiments with a range of heavy atom reagents indicated a lack of reactivity of a targeted cysteine residue at pH 6.5. We therefore collected data from eleven crystals which had undergone heavy atom soaking at pH 7.5. There is some evidence in the literature that cysteine reactivity can be enhanced at this pH especially towards platinum compounds. Nine of these crystals diffracted to 3.781 or better and were used to collect between 30 and 120 degrees of data.

Two genetically engineered mutant forms of the protein have been produced. These were treated with a reducing solution to recover any partial oxidation of cysteine residues and subsequently soaked in a number of mercurial containing solutions. Of these two crystals produced good diffraction and complete datasets were collected.

A series of soaking experiments were also performed after a cross-linking treatment was applied to crystals of wild type protein. Seven heavy atom solutions which had previously been shown to react, but cause damage to, native crystals were perfused in after cross-linking. In all cases the diffraction limit of the data collected was not noticably worse than that of the cross-linked native crystal.

In total 1304 X-ray images were collected from 34 crystals including wild type and two genetically engineered mutants. The data have been processed and shown to be of good quality. Scaling between datasets have shown some differences which will be exploited for the calculation of difference Pattersons and where possible calculation of initial phases to be used for structure solution.