ESRF	**Experiment title:** High-resolution X-ray diffraction data collection of the soluble homologues of the ABC transporters, SecA and UvrA	**Experiment number:** LS-755
Beamline: ID2	**Date of experiment:** from: 06-Dec-97 to: 08-Dec-97	**Date of report:** 22-Feb-98
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Report:

X-ray diffraction studies on the DNA repair enzyme UvrA

We have carried out preliminary X-ray diffraction studies on crystals of the DNA repair enzyme UvrA. The UvrA crystals are small with typical dimensions of 0.8 x 0.3 x 0.03 mm and diffract to about 4.5 Å using rotating-anode X-rays. Previous tests have shown that the diffraction is significantly better when using synchrotron radiation. The objective ,of the studies at beamline ID2 at ESRF was to obtain the maximum diffraction achievable with the most brilliant synchrotron radiation available and to assess the possibilities to solve the crystal structure of UvrA at a resolution sufficiently high to answer important biological questions.

As a result of our studies at ESRF, with the high-brilliance synchrotron radiation provided by beamline ID2, we could significantly extend the diffraction limit of our UvrA crystals to about 3 Å (as compared to 3.5 Å on beamlines Al and Fl at CHESS). The high flux, however, resulted in crystal decay, so severe that we could not adjust the data collection strategy in order to obtain a complete native data set in the time allotted. Our major conclusion is that the structure determination of UvrA with our crystals is feasible, but high-brilliancy synchrotron radiation will be critical to achieve this goal.

X-ray diffraction studies on SecA

During our time on ID2 during December of 1997, we collected 2.7 to 2.8 angstrom data sets on crystals of native SecA and ligand complexes of SecA with ADP and ATP. We are currently using our best native data set from ID2 for final refinement of the structure, and we expect to publish the structure refined against this data, barring unforeseen complications. We also expect to publish the structure of the ATP complex of SecA based on the data set from ID2. However, we have not yet decided which data set will be used for publication of the structure of the ADP complex of SecA because soaks conducted in slightly different ways were collected at the ESRF and at CHESS, and the relative properties of these data sets have not yet been evaluated fully.

Our experiment at ID2 was successful, but we feel that we could have used the beamtime more effectively, if it had been possible to reduce and scale our data as they were being collected. Even at 100 K, our SecA crystals decay relatively rapidly when exposed to synchrotron levels of x-ray flux, so that the quality of the diffraction data depends on our ability to closely monitor the onset of decay. Although we had spent 12 days during 1997 collecting data from SecA crystals at CHESS, the data collection strategy we worked out at this facility was not useful on ID2 because of the substantially higher x-ray flux. We found it difficult to get reliable estimates of the relative flux of various synchrotron beamlines and its influence on crystal decay rates before actually collecting data. Visual inspection of the frames during data collection was not a satisfactory method to monitor decay. A much more objective indicator of decay would have been the inflation of scaling B-factors as data collection progressed, but it was not practical to perform the necessary calculations in time at ID2 in December 1997.

Finally, although we could tell from the crystal decay rate that the radiation flux was very substantially higher on ID2 than on the CHESS beamlines (Al and Fl), the limiting resolution of our SecA data sets was essentially the same from both sources. The I./sigma statistics at intermediate resolution were very clearly superior for the data from ID2, which is why we are using it for refinement and publication. However, with the superior flux on ID2, we would have expected some improvement in limiting resolution as well. On this basis, we feel that the CCD detectors used at CHESS my have slightly higher sensitivity at low photon count levels compared to the imaging plate detector used on ID2. Of course, this is a qualitative impression and could not be considered to be reliable in the absence of some systematic comparison. It would be very helpful if data like this were available to the user community to assist them in designing and evaluating their data collection strategies.