

 ESRF	Experiment title: DNA GYRASE	Experiment number: LS-453
Beamline: ID2-BL4	Date of Experiment: from: 18/2/96 to: 19/2/96	Date of Report: 12/2/97
Shifts: 3	Local contact(s):	<i>Received at ESRF :</i> 12 FEB. 1997

Names and affiliations of applicants (*indicates experimentalists):

*D.B. Wigley,
Laboratory of Molecular Biophysics,
University of Oxford.

Report:

DNA topoisomerases are essential for the replication of DNA. There are four topoisomerases in E.coli. We have crystallised the B subunit of DNA gyrase. This protein has a subunit molecular weight of 70kDa. The crystals are cubic, 123 with unit cell dimensions $a = b = c = 250.1\text{\AA}$, and contain one dimer in the asymmetric unit. The very weak diffraction made the high intensity beam at ESRF essential for this project. The beamtime allocated during 1996 was used to collect data from native and selenomethionine substituted crystals. The crystals could not be frozen, and crystals were very sensitive to radiation damage. Consequently, data were collected from over 90 crystals, with just one or two degrees from each crystal. Part of the structure has similarity to the N-terminal region of the DNA gyrase B protein and a molecular replacement solution was found to

position that part of the structure. However, we required heavy atom data to improve the maps and get phase information for the remainder of the structure. We used low resolution data for a mercury derivative and the higher resolution selenium data from ESRF to solve the structure. These data were not used for MAD phasing, but were useful as a conventional derivative but with good anomalous measurements. The selenium sites also positioned all of the methionine residues in the structure and facilitated the model building. The refinement is progressing well, and the structure should be completed in the very near future.