ESRF	**Experiment title:** Chaperonin Structure	**Experiment number:** LS 455 BM2
Beamline: D2AM	**Date of experiment:** **from:** 26 June 1996 to: 27 June 1996	**Date of report:** 28 Feb 1997
shifts: 6	**Local contact(s):** E. Fanchon	*Received at ESRF:* 10-3-97

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Report:

Introduction

GroEL is a large multi-subunit protein consisting of fourteen, 60 kDa subunits arranged as two stacked rings of seven . It's co-chaperonin GroES is a single ring of seven subunits each of which have a molecular weight of 10 KDa. GroEL acts with GroES to assist protein folding in the cell, binding nucleotide and unfolded protein in a highly cooperative manner. Although the crystal structures of a GroEL mutant with and without bound ATP-y-S are available, the mutant fails to show the same co-operativity as the native structure, and fails to show the large conformational changes observed by EM on binding nucleotide. We have crystallized the native protein in the presence of ATP and GroES and collected a full data set on one such crystal at the ESRF.

Data Collection and Processing

Many of our best crystals were ready mounted in frozen form on an assembly ideal for attachment to a standard crystal arc. These could not readily be attached to the goniometer device on D2AM. However, a few crystals were attached to suitable steel pins before setting out for Grenoble and these were used for data collection. It is not possible to transport GroEL crystals in an unfrozen condition. The data collection proceeded very **smoothly, the**

CCD detector and beam strength decreased the data collection time by a factor of at least 10 (compared to DLSRS) making collection of a complete data set at 3.5 Å resolution possible in one session.

Data processing proceeded relatively smoothly although we feel that better quality data could have been extracted if a wider range of processing packages were available. The final data set consisted of 380 frames from 0.5" oscillations, giving 77,000 unique reflections to 4 Å resolution with a completeness of 89 %, multiplicity of 2.7, an average $I/\sigma I$ of 29.4 and an R_{symm} of 15 %.

Molecular Replacement

The structure of native GroEL was used as the search model and the AMORE package was used to perform the molecular replacement. The rotation function showed a set of 14 peaks reflecting the internal symmetry of the molecule. The highest of these was chosen for use in the translation function. The translation function was performed in both the P2 and $P2_1$ space groups. In the $P2_1$ case one peak stood out clearly from the others, whereas no single peak was dominant in P2. After refinement in AMORE the solution had a correlation coefficient of 39.48 % and an R-factor 48.93 %. Electron density maps calculated from the molecular replacement solution looked reasonable indicating deviations from the model and some asymmetry between the two rings. We are currently attempting to improve the phasing of the molecule using NCS averaging prior to refinement. The final model should confirm the crystal structure of the native protein, show the same asymmetry as seen by EM and may also suggest a mechanism explaining the complex co-operativity of the molecule.