ESRF	**Experiment title:** X-ray data collection for the L-protein of glycine decarboxylase complex from pea leaf mitochondria	**Experiment number:** LS-607
Beamline: BM02	**Date of Experiment:** from: 08.06.97 to: 09.06.97	**Date of Report:** 12/08/97
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Names and affiliations of applicants (*indicates experimentalists):

Magali Faure*, IBS/ CNRS-CEA, Grenoble
Claudine Cohen-Addad", IBS/ CNRS-CEA, Grenoble
Michel Neuburger, PCV/DBMS/ CEA, Grenoble
Roland Douce, PCV/DBMS/CEA, Grenoble
Jacques Bourguignon, PCV/DBMS/CEA, Grenoble
David Macherel, PCV/DBMS/CEA, Grenoble

Report:

During the photorespiratory cycle in green leaves, glycine produced in the peroxisome is quickly oxidized in mitochondria by the glycine decarboxylase complex composed of four proteins named P (pyridoxal phosphate-containing protein, 2x100 kD), H (lipoate-containing protein, 14 kD), T (tetrahydrofolate-dependent protein, 43kD) and L (lipoamide-dehydrogenase, 2x50 kD). The lipoyl group of the H-protein which is bound to a lysine residue by an amide linkage plays a central role in the complex. During the reaction, the H-protein interacts with each of the other three proteins and its lipoate moiety passes three states: oxidized, loaded with a methylamine group, reduced.

Each of the component of the complex has been isolated separately and purified. The aim of the project is to determine the structure of the different components in order to understand the enzymatic mechanism at an atomic level and the protein-protein interaction within the complex. We have solved the structure of the different forms of the central H-protein. This allowed the lipoate group to be located for the first time. We are now undertaking the crystallization and structure determination of the L-protein.

The L-protein has been overexpressed from ***E.*** coli. Small crystals have been obtained by the microbatch method (dimensions 0.15x0.06x0.03 mm3) and were frozen in PEG MME 5K. Several crystals were tested and the best one diffracted to 4Å resolution on BM02 with the machine in 16 bunch mode.

Data were collected with the following conditions:
Distance crystal-detector=31Omm, θ=0°, λ=1.03 Å
Total range of phi=60", increment=O.Y per frame, time per frame=60 seconds.
The data were processed with XDS and SCALA and the results are the following:
Space group $P2_12_12_1$ a=101.6 b=108.6 c=204.1 Å
Rsym= 6.5 % up to 4.1 Å, 43558 measured reflexions, 13306 unique reflexions, completness 73.6%, redundancy 3.3.
The structure was solved by molecular replacement (Amore program) using the data from 10 to 5 Å resolution and the model of the L-protein of PDH from ***Azotobacter Vinelandii*** (R=44.6%, correlation=46.9%, R after preliminary refinement=40.1%, Rfree=41.4%).
We need new data at a higher resolution to refine the structure. A beam line with higher flux (hybrid mode or 2/3 filling mode) is essential for these measurements.
Another data collection was performed on the H-protein in the reduced form in order to obtain high resolution and accurate electron density around the reduced lipoate for comparison with the oxidized form of the H-protein.
A crystal of 200x150x100 microns was frozen in 20 % PEG 400 and the conditions of the data collection were the following:
Distance crystal-detector=1 80 mm, θ=0°, λ=1.03 Å
Range of phi=-23 to 70°, increment per frame=0.5°, time per frame=60 seconds
The data were processed with XDS and SCALA.
The results are the following:
Space group $P3_121$ a=55.68 c=134.49 Å (isomorphous to the oxidized form of the H-protein)
Rsym = 3.7 % up to 2.3 A, 53680 reflexions, 9975 unique reflexions, completness 88.5%, redundancy 5.4
The refinement is underway. The starting values R=29.6 %, Rfree=40 % have to be improved.