ESRF	**Experiment title:** Structure determination of the L-protein of glycine decarboxylase complex from pea leaf mitichondria	**Experiment number:** LS-807
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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 was overexpressed in E. ***coli*** as inclusion bodies. It was denatured with guanidine and refolded in presence of the FAD cofactor.Crystals are obtained by the microbatch or hanging drop method (thick plates of dimensions 0.2x0.1x0.05 mm^3) in presence of PEG monomethylether 5K at pH 5. The space group is $P2_12_12_1$ with cell dimensions of a=101, b=108, c=204 Å. .

Data collections were performed on several crystals cocrystallized with different cofactors:

- crystals 1 and 2 with the lipoic acid
- crystal 3 with TCEP, a reductive agent
-crystal 4 with NADH

These experiments would allow us to determine the structural modifications induced by these cofactors on the active site of the protein.

The experimental conditions were the following:

λ=0.9475, distance crystal-detector 178 mm, increment per frame 0.5°, exposure time 30,45 or 60 seconds, phi range 80 to 120°.

The results of the integration of the different data are the following:

- crystal 1 Rsym= 6.3%, resolution 3.15 A, 114825 measured reflexions,
  33527 unique reflexions, completness 86.7%, redundancy 3.4
- crystal 2 Rsym= 6.4% resolution 3.30 A, 112174 measured reflexions,
  32292 unique reflexions, completness 94%, redundancy 3.5
-crystal 3 Rsym= 7.8%, resolution 3.3 Å, 107719 measured reflexions,
  29638 unique reflexions, completness 89.8%, redundancy 3.6

(integration of data collected on crystal 4 in progress)

The structure was solved by molecular replacement using previous data collected on BM02 and the known structure of the L-protein from *Azotobacter Vinelandii*. The model is presently under reconstruction using the data of crystal 1.