ESRF

Experiment title:
X-ray data collection for H/T protein complex **glycine** decarboxylase complex

Experiment number:
LS-398

Beamline: BL19

Date of Experiment: from: 28-4-96 to: 29-4-96

Date of Report: 29-7-1996

Shifts: 3

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Received at ESRF: 20 AUG 1996

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Report:

During the course of the photorespiratory cycle which occurs 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, 2x1OO kD), H (lipoate-containing protein, 14 kD), T (tetrahydrofolate-dependent protein, 43kD) and L (lipoamide-dehydrogenase, 2x57 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 undergoes three forms (lipoate moiety oxidized, charged in methylamine group, reduced).

The crystal structures of two forms of the H-protein have been determined (lipoate oxidized and charged in methylamine group). They show for the first time that the lipoic acid covalently attached to a specific lysine residue is not free to move in aqueous solvent and does not behave as a real freely swinging arm conveying substrate from one active site to another in the complex. Under these conditions, it is possible that complexes are formed between the H-protein and the other components during the enzymatic reaction.

The association between the H protein and the other components of the complex has been studied by small angle scattering data. In the case of the H/T interaction, they indicated the formation of a 1/1 ratio H-T protein association. This led us to try to crystallize the H-T complex in the 1/1 ratio.

During the first trials, we have obtained crystals of a new NDP kinase which was present as a minor component and attempts to crystallize the complex H/T were unsuccessful.

Three of the six shifts allowed on BL19 were used for the study of the third form of the H protein, the reduced form. It has been crystallized in presence of the L protein, NADH and DTT under argon. Data were collected at 100 K on BL19. A CCD detector was used at the wavelenght of 0.9 Å and data were collected up to 2.8 Å resolution, leading to a Rsym value of 5,970.

The structure is isomorphous to the structure of the oxidized form. We did not observe any difference between the oxidized and reduced form of the lipoate group which, in both cases, is flexible at the surface of the protein, while in the methylamine-charged form it is tucked into a cleft and not free to move in the solvent.

Crystallization of the other components of the complex and of their associations with the H protein is in progress.