



	Experiment title: Structural studies on truncated Hb mutant from unicellular green alga <i>Chlamydomonas eugametos</i>	Experiment number: LS1933
Beamline: ID14-3	Date of experiment: from 10-06-2001 to 10-06-2001	Date of report: 09-07-01
Shifts to BAG: 6	Local contact(s): Stephanie MONACO	<i>Received at ESRF:</i>
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Chlamydomonas eugametos “truncated hemoglobin” (ChHb) is encoded by a gene family associated with photosynthesis and nonphotosynthetic photoreceptors, activated in response to the changes in light conditions. ChHb constitutes the first evidence for the presence of Hb in chloroplast and raises question about the function that a Hb could play in a high oxygen environment such as in the chloroplast. ChHb is a monomeric hemoprotein of 121 amino acids.

ChHb 3D structure has been previously determined in our lab. ChHb is a ‘truncated hemoglobin’, whose fold is based on a subset of the classical globin fold, characterized by a two-over-two α -helical sandwich. One of the most striking structural feature characterizing truncated Hbs is the presence of an almost continuous tunnel through the protein matrix, connecting the heme distal pocket to the protein surface at two distinct sites (1, 2). We suggest that this tunnel facilitates the ligand diffusion (O_2 , but also NO) from the protein surface to the heme, where an enzymatic conversion (to yield NO_3^-) may take place.

In order to better understand this unique feature of truncated hemoglobins, an Ala15→Val ChHb mutant has been crystallized, as a try to close the tunnel entrance. Due to small dimensions of the ChHb mutant crystals, they diffracted in house up to 3.2 Å resolution and synchrotron radiation was needed. We collected a data set up to 1.8 Å resolution (see Table for data collection statistics) and the ChHb mutant refinement is in progress.

Table: Summary of Ala15→Val ChHb mutant data collection statistics:

Space Group	P2 ₁ 2 ₁ 2 ₁
Unit cell (Å)	$a=34.7$, $b=53.0$, $c=66.9$
Resolution (Å)	30-1.8
Completeness (%)	97.2
Rsym (%)	3.2
Mosaicity (°)	0.85

References:

- (1) Pesce, *et al.*, (2000) *EMBO J.*, **19**, 2424-2434.
- (2) Milani, *et al.*, (2001) *EMBO J.*, in press.

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