Standard Project

Experimental Report template

Proposal title: Determination of crystallographic structures of neuroglobin under high pressure and under moderate gas pressure.		**Proposal number:** 20110676
Beamline: CRISTAL FIP 30-01-887	**Date(s) of experiment:** From: 2 may 2012 to: 4 may 2012 24 -25 june 2012 and 6-7 december 2012	**Date of report:** 22 august 2012
Shifts: 6 on CRISTAL 6 on FIP	**Local contact(s): Pierre Fertey** Michel Pirrochi	*Date of submission:*

Objective & expected results (less than 10 lines):

We have studied in parallel pressure-induced and gas-induced structural modifications on neuroglobin, to understand how neuroglobin work under pressurized environment and how gases modify its function. Neuroglobin possesses a large functional cavity which is necessary during the hem sliding mechanism which occur when gaseous ligand (O_2, CO) bind. Inert gas like xenon and krypton bind within this large cavity. Pressure main structural effect reduces empty cavity volume, and gas main structural effect expands them.

Results and the conclusions of the study (main part):

Neuroglobin structures under high hydrostatic pressure.

Since neuroglobin stability under pressure was investigated for the first time, we first perform compressibility curves to determine the highest pressure we could reach while keeping diffracting crystals. We noticed that above 5000 bar (500 MPa), crystals were dead, and that above 4000 bar (400 MPa), crystals were poorly diffracting.

The analysis of the compressibility curve clearly revealed a transition around 3000 bar, with a modification of the slope (cf. Fig. 1).

Full data collections have been performed at ambient pressure, below the transition pressure (2700 bar), and above the transition pressure (3150 bar). Statistics of data collection were all of good quality, resolution of 2 A which will allow precise comparison between the three structures, a completeness above 97 %, even in the highest resolution shell, a Rmerge around 10 %, and I/Sigma(I) around 10.

The three structures have been solved using Bucanner with a reconstruction of the side chains, to avoid model bias. The native structure has been refined with no particular problem (R = 15.6 % and Rfree = 21.3 %), with a clean electronic density map at the end of the refinement. In contrary, the two structures under pressure have a lot of unknown blobs in the electron density map (R = 18.1 %, Rfree = 25.4 % for the structure at 2700 bar and R = 18.7 %, Rfree = 24.9 % for the structure at 3150 bar).

The most striking features is the huge difference in the large internal cavity, which has its volume largely reduced. Moreover, the connection between back of the cavity (putative oxygen storage location) and the hem is broken.

There is also a shift of a loop toward the hem in both high pressure structures. The B factor is a little higher in both high pressure structures. Residues close to the hem are destabilized by pressure and residues from the opposite side are stabilized by pressure. The biological meaning of all these differences is currently analysed.

Neuroglobin structures un inert gas pressure.

Data sets of neuroglobin under various pressure of xenon, nitrous oxide, krypton and argon have been collected. Xenon and nitrous oxide binds in four different sites, two solvent-accessible pockets, and two sites located in the large internal cavity behind the hem. At high pressure, xenon binds to three new sites with low occupancy. These thee new xenon binding sites are likely to be not physiological since they are located at packing interfaces. The presence of xenon or nitrous oxide in the large hem cavity expands the volume of this cavity by more than 20 %.

The structures under krypton and argon pressure have not yet been determined.

A structure of native neuroglobin at 100 K has also been collected, but not yet solved. This low temperature structure will be compared with the ambient temperature and pressure structure, to perform a comparison between the temperature-induced and the pressure-induced modifications.

Justification and comments about the use of beam time (5 lines max.):

CRISTAL : 6 shifts were a little too short to collect full data sets, but it was mainly due to three beam shutdown during this run.

FIP: 6 shifts were fine to collect data sets under pressurised gas at room temperature

Publication(s):

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