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|---|---|---|
| **ESRF** | **Experiment title:**<br>MAD experiment on heme Fe-absorption edge of neural Hb-like hemoprotein from *Cerebratulus lacteus* | **Experiment number:**<br>**LS1933** |
| **Beamline:**<br>ID29 | **Date of experiment:**<br>from 23-06-2001 to 24-06-2001 | **Date of report:**<br>09-07-01<br>*Received at ESRF:* |
| **Shifts to BAG: 6** | **Local contact(s):**<br>Andy THOMPSON |  |

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A hemoprotein very loosely related to Hb occurs in circulating red blood cells, neural tissue and body wall muscle tissue of the nemertean worm *Cerebratulus lacteus*. The proteins isolated from neural and body wall muscle are composed of 109 residues only, which makes them the smallest naturally occurring Hb-related proteins known. This finding should make the *C.lacteus* Hb particularly valuable for studies of folding and stability, besides shedding light on the recently discovered role of "neural hemoglobins".

*Cerebratulus* Hb has a relatively high oxygen affinity and is present at high concentration within the neural tissue. This suggests that the Hb also may function as oxygen store when the animal encounters anoxic conditions during burrowing. However, other functions *in vivo* have also been considered.

Multiple sequence alignment with 'truncated Hbs' from the unicellular green alga *Chlamydomonas eugamentos* and the protist *Paramecium caudatum* (121 and 116 amino acid residues per chain, respectively), does not show sufficient similarities to suggest neatly comparable 3D structures.

A MAD experiment has been performed on *Cerebratulus lacteus* neural Hb (CerHb), based on the anomalous signal of the heme Fe-atom. This experiment is the continuation of previously native and heavy atom data collections on the same protein, performed at ESRF (for details see the experiment report LS1803, beamline ID14-3 of 28-04-2001).

CerHb crystals belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters: a=42.72 Å, b=43.17 Å, c=60.11 Å, and one molecule per asymmetric unit.

The MAD experiment was successful, and an interpretable electron density map was obtained using the SOLVE and RESOLVE programs. The initial phases (at 1.95 Å) and the high resolution remote data set were directly fed into the wARP program, used to optimise the initial phases, to extend them from 1.95 Å up to 1.5 Å resolution and then for automated building of all the main chain and side chains. The R-factor after wARP is 19.8%. The refinement of CerHb structure is currently in progress.

**Table**: Summary of *C.lacteus* Hb data collection statistics:

| | **Absorption peak** | **Inflection point** | **Remote** |
|---|---|---|---|
| Wavelength (Å) | 1.74 | 1.74 | 0.915 |
| Resolution (Å) | 35-1.95 | 35-1.95 | 35-1.5 |
| Completeness (%) | 93.4 | 93.5 | 97.1 |
| Rsym (%) | 4.8 | 4.9 | 5.4 |
| Mosaicity (°) | 0.4 | 0.4 | 0.4 |