ESRF /FIP	**Titre de l'expérience :** **Dihydroorotate Dehydrogenase from E.Coli**	**Numéro de l'expérience :** 30.01.004
Beamline: BM30 / FIP	**Date de l'expérience:** du : 25.6.99 au : 28.6.99	**Date du rapport:** 27.7.99
Shifts: 9	**Local contact(s):** M. Roth	*Reçu par le CRG FIP:* 28 FEV. 2000

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Dihydroorotate dehydrogenases (DHOD's) catalyse the oxidation of (S)-dihydroorotate to orotate, the fourth step in the *de novo* biosynthesis of pyrimidine nucleotides. Two major families have been identified which appear to differ substantially in their reaction mechanism. The famliy 1 enzymers are cytosolic. The *E.coli* DHOD belongs to the menbrane associated family 2 enzymes and previous attempts to solve the structure by molecular replacement were not successful. After numerous efforts the spacegroup of the crystals had been determined as $P4_12_12$ or $P4_32_12$ with cell parameters a=b=119.7, c=295.97 and 4 to 8 molecules in the a.u.. MAD data was then collected at the Se absorption edge, at 3 different wavelengths, using crystals of the Se-methionine substituted enzyme. 90 degrees were collected with an oscillation step of 0.5 degrees per image. The resolution obtained was 2.5 Å. After integration and scaling the R_{merge} for the 3 wavelengths was between 8 - 8.5 % with a completeness around 98 %. The spacegroup was confirmed to be $P4_12_12$. The program SOLVE [1] enabled us to find 24 Se-sites corresponding to 2 dimers in the a.u.. Using SHARP [2] and DM [3] initial maps of good quality were calculated. We have by now been able to trace the Cα-backbone and are working on the refinement of the structure.

[1] Terwilliger & Berendzen (1999) *Acta Cryst.*, D55: 849-861.

[2] LaFortrelle *et al.* (1997) in Crystallographic Computing 7

[3] CCP4 (1994) *Acta Cryst.*, D50:760-763.