



	Experiment title: Tyrosine phenol-lyase	Experiment number: LS1383
Beamline: ID14-4	Date of experiment: from: 22/02/99 to: 25/02/99	Date of report: 20.02.00
Shifts: BAG	Local contact(s): Julien Lescar	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Fred Antson * Keith Wilson York Structural Biology Laboratory, Chemistry Department, University of York, Heslington, York, YO10 5DD, UK		

Report:

Tyrosine phenol lyase (TPL; EC 4.1.99.2) is a PLP-dependent enzyme that catalyses the beta-elimination of L-tyrosine to give phenol and ammonium pyruvate. The molecule of TPL consists of four chemically identical subunits, each with a molecular weight of about 50 kDa, and binds 4 mol PLP per tetramer. A reaction mechanism has been proposed for TPL that proceeds through a number of intermediate steps, which include:

1. formation of the external aldimine, the Schiff's base linkage between the PLP and the substrate amino group.
2. abstraction of the C α proton by a first base with a pKa of 7.8 leading to the formation of a quinonoid intermediate.
3. abstraction of the phenol hydroxyl proton by a second base (pKa 8.0) in concert with a push of an electron from the PLP ring and donation of a proton to the C γ position by the first base leading to the formation of cyclohexadienone and subsequent removal of phenol.

The project is focused on obtaining structural snapshots for the intermediate steps of the reaction. In February 1999 data were collected from two complexes of TPL. The first complex was prepared with L-Ala using the D214N mutant of TPL. The second complex was prepared using wild-type enzyme soaked with L-Ala and pyridine N-oxide. The structures of these two complexes have been refined and electron density maps show the presence of the substrate analogues. The structural events of the two intermediate states of the reaction are now well understood and a manuscript is in preparation.