Galactose oxidase precursor processing studies

Susan J. Firbank, Ramon H. Guerrero, Peter F. Knowles, Simon E.V. Phillips,
Malcolm A. Halcrow and Mike J. McPherson.

The monomeric, copper-containing enzyme, galactose oxidase, possesses a thioether link between the active site Cys228 and Tyr272, providing a built-in cofactor associated with a radical, necessary for catalysis. The mechanism of formation of this active site feature is a major focus of our research effort. Expression of galactose oxidase, under copper-free conditions, has allowed the purification of a precursor form of the enzyme that retains an additional 17 amino acid N-terminal pro-sequence. Addition of copper under aerobic conditions is sufficient to mediate the removal of the 17 amino acid pro-sequence and to form the thioether bond with formation of the radical. We routinely monitor the formation of processed enzyme by the differential migration of distinct intermediates by SDS-PAGE. Recent crystallographic studies have revealed the structures of precursor forms of galactose oxidase, providing a basis for understanding the mechanisms associated with the concerted pro-sequence cleavage and thioether bond formation. In particular, it has been observed that Cys228 is oxidised and evidence from spectroscopic studies on addition of copper indicate the presence of a sulphenic acid. This raises the possibility that the oxidised Cys228 acts as an electrophile to attack the Tyr272 ring. Mutagenesis and further solution studies are underway. This project represents a collaboration between Leeds and the group of Professor D M Dooley in Montana State University, Bozeman, USA.

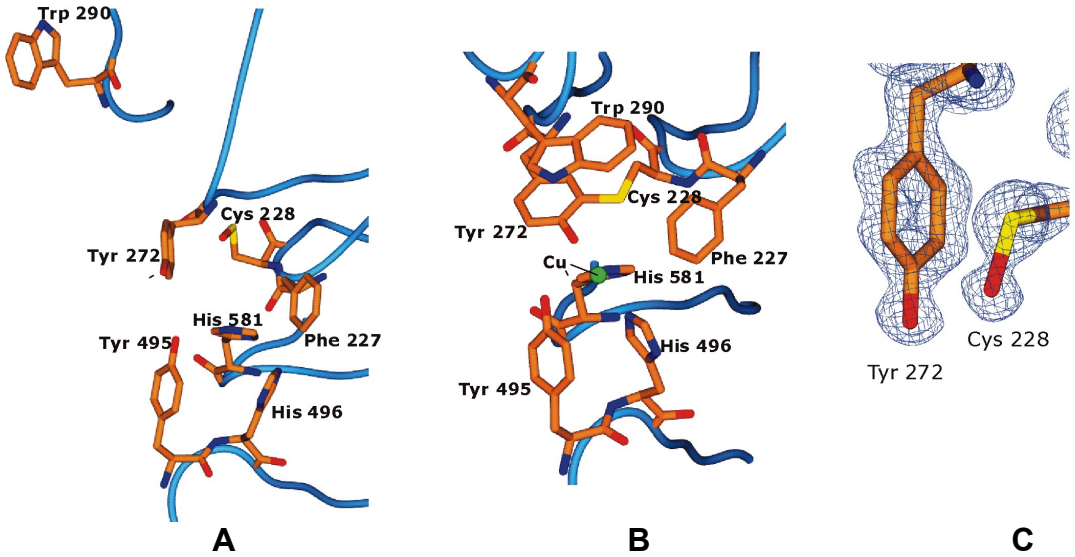

Fig. Active site residues in precursor galactose oxidase **(A)** and in the mature protein **(B)** showing the copper ligands (Tyr-272, Tyr-495, His-496, and His-581), Cys-228 that forms the thioether bond to Tyr-272, the tryptophan that stacks over it (Trp-290), and Phe-227. The S of Cys-228 in the precursor is situated directly above C2 of Tyr-272. In the precursor the sulphur of the cysteine appears to have been oxidized to a sulphenic group. **(C)** Final 2Fo - Fc electron density map for Cys-228 and Tyr-272.

Reference

Firbank, S.J., Rogers, M.S. Wilmot, C.M., Dooley, D.M., Knowles, P.F., McPherson, M.J. & Phillips, S.E.V. (2001) Crystal structure of the precursor of galactose oxidase: an unusual self-processing enzyme. *Proceedings of the National Academy of Sciences,* **98**, 12932-12937.

Funding

We acknowledge support from BBSRC and EPSRC.