Investigating *E. coli* copper amine oxidase by mutational and inhibitor studies

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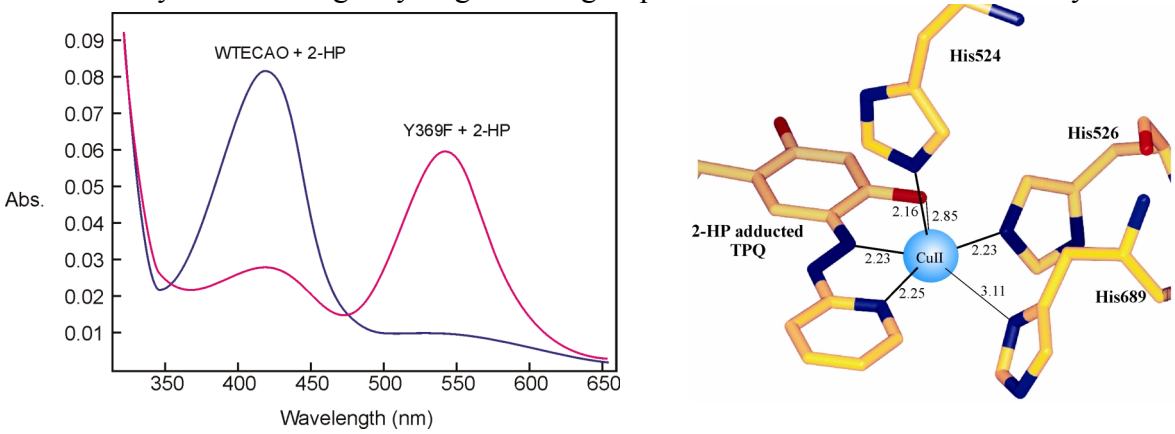
Copper amine oxidases are dimeric enzymes containing a single copper and a post-translationally modified 2,4,5-trihydroxyphenylalanine quinone (TPQ) derived from an active site tyrosine by a copper and oxygen mediated self-processing reaction. We have explored the role of the conserved Tyr369 that is involved in a short H-bond to the O4 of TPQ. The Y369F variant displays an altered activity profile across the pH range with an increased pK_a for the active site base that, together with an altered visible spectrum, indicates local alterations within the active site environment. In support of this view, the TPQ is seen to adopt a distinct conformation as revealed by X-ray crystallography. Previous studies have implicated Tyr369 as a component of a proton transfer pathway to dioxygen during the oxidative half reaction. The variant is, however, only some 50-fold less active than wild-type indicating that such a role is not essential and alternative pathways must exist in the variant.

The importance of Tyr369 in maintaining the conformation of TPQ within the active site is further highlighted by studies with a suicide inhibitor, 2-hydrazinopyridine. In the wild-type enzyme at neutral pH the predominant product is Adduct 1, representing a mixed population of the hydrazone (non-planar ring) form, stabilised by H-bonding with the active site base Asp383, and the azo form (planar, conjugated ring) in which these interactions are lost. In the Y369F variant, Adduct 1 is rapidly converted to Adduct II, postulated from model compound studies by our collaborator Dr Minae Mure (Berkeley, CA) to involve metal binding. The X-ray crystal structure of Adduct II supports this view with the predominant electron density describing a conformation in which the Adduct has rotated onto the copper with the 2-HP ring now liganded to the metal centre. These studies reveal the significant differences mediated by loss of a single hydrogen bond group within the active site of this enzyme.



(A) Absorbance spectra of 2-hydrazinopyridine adducts of wild-type and Y369F at pH 6.0 showing rapid conversion to Adduct II in Y369F. (B) X-ray structure of the copper site in the Y369F Adduct II showing the 2-HP liganded to the copper.

Reference

Murray, J.M., Kurtis, C.R.P., Tambyrajah, W., Saysell, C.G., Wilmot, C.M., Parsons, M.R., Phillips, S.E.V., Knowles, P.F. & McPherson, M.J. (2001) Conserved tyrosine-369 in the active site of *Escherichia coli* copper amine oxidase is not essential. *Biochemistry*, **40**, 12808-12818.

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