

Inhibitor studies with copper amine oxidases

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Copper amine oxidases are homodimeric and contain a single cupric ion and a post-translationally modified tyrosine cofactor; 2,4,5-trihydroxyphenylalanine quinone (TPQ). TPQ biogenesis is an autocatalytic event requiring copper and oxygen. Together with international collaborators, we have engaged in a series of studies related to inhibitor binding to amine oxidases. For some enzymes, such as the human form, the identification of inhibitors that are selective for the copper and not flavin-containing amine oxidases is likely to prove an important advance. Crystallographic studies with the *E. coli* enzyme (ECAO), as a model for this class of enzymes, has revealed the formation of a reversible inhibitory complex with one enantiomer of tranylcypromine (TCP), an anti-depressant drug (Fig. 1). The binding of this drug to human copper amine oxidase may result in unwanted side-effects of the use of the inhibitor against the flavin-containing enzymes.

This TCP structural model is similar to the structure of the irreversible inhibitor 2-hydrazinopyridine (2-HP) bound to ECAO, which is widely used to investigate the formation of the TPQ cofactor and as a suicide inhibitor to study aspects of the enzyme mechanism.

We have undertaken a wide range of biochemical, chemical spectroscopic, mutational and structural studies to characterise the inhibition of ECAO by 2-HP. The interaction of this inhibitor with the enzyme results in two spectroscopically distinct species (adduct I and adduct II) that were considered to represent the equivalent to the substrate Schiff base and product Schiff base intermediates. We have now shown that in fact Adduct I represents both of these two intermediate states and that these are spectroscopically distinguishable. The shift to Adduct II involves a substantial rearrangement in the enzyme active site to allow the inhibitor TPQ complex to co-ordinate with the active site copper, a situation that is more readily achieved in the active site

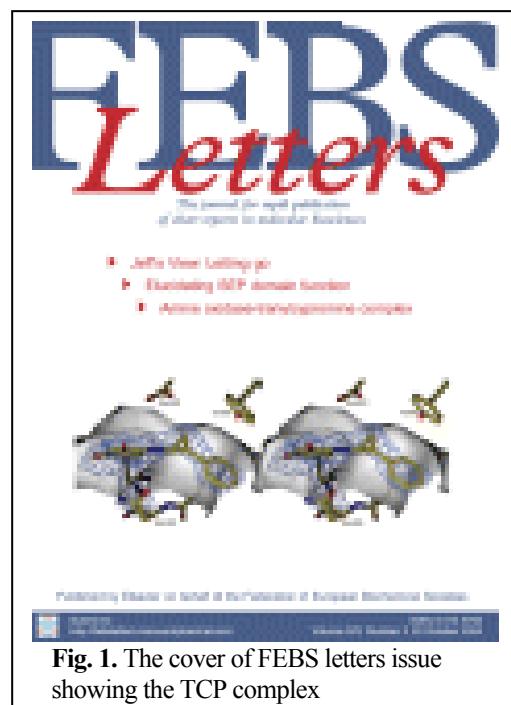


Fig. 1. The cover of FEBS letters issue showing the TCP complex

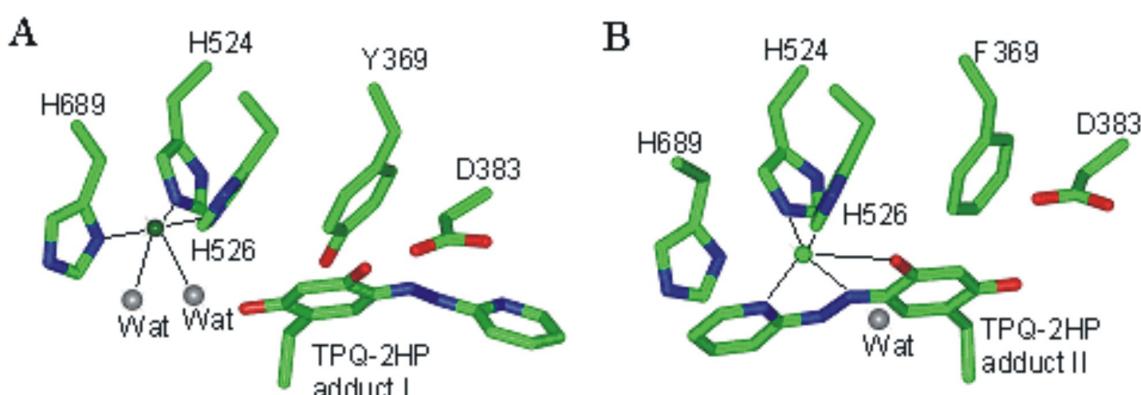


Fig. 2. Structure of 2-HP complexed with the Y369F variant of *E. coli* copper amine oxidase. (A) Adduct I with the 2-HP is the position normally occupied by substrate (B) Adduct II showing a dramatic rotation of the cofactor inhibitor complex to allow co-ordination with the copper centre.

variant Tyr369Phe which lacks a stabilizing H-bond interaction to the O4 of TPQ, thus facilitating rotation of the TPQ (Fig. 2).

We have also undertaken some work related to the human copper amine oxidase that functions as an important vascular adhesion protein. The mechanism by which this enzyme acts as a cell adhesion molecule is postulated to involve the formation of a covalent intermediate with peptide amine side chains on cell surface proteins. To test this, a series of peptides were examined for their ability to inhibit the enzyme activity. One peptide was shown to be an effective inhibitor supporting the prospect of a catalytic mechanism-based cell adhesion interaction. Such inhibitors may prove therapeutically valuable for control of inflammatory states.

Publications

Wilmot, C.M., Saysell, C.G., Kurtis, C.R.P., Blessington, A., Conn, D.A., McPherson, M.J., Knowles, P.F. and Phillips, S.E.V. (2004) Medical implications from the crystal structure of a copper-containing amine oxidase complexed with the antidepressant drug tranylcypromine, *FEBS Letters*, **576**, 301-305.

Yegutkin, G.G., Salminen, T., Koskinen, K., Kurtis, C.R.P., McPherson, M.J., Jalkanen, S. and Salmi, M. (2004) A peptide inhibitor of vascular adhesion protein-1 (VAP-1) blocks leukocyte-endothelial interactions under shear stress. *European Journal of Immunology* **34**, 2276-2285.

Mure, M., Brown, D.E., Saysell, C., Rogers, M.S., Wilmot, C.M., Kurtis, C.R.P., McPherson, M.J., Phillips, S.E.V., Knowles, P.F. and Dooley, D.M. (2005) Role of the interactions between the active site base and the substrate Schiff base in amine oxidase catalysis. Evidence from structural and spectroscopic studies of the 2-hydrazinopyridine adduct of *Escherichia coli* amine oxidase *Biochemistry*, **44**, 1568-1582.

Mure, M., Kurtis, C.R.P., Brown, D.E., Rogers, M.S., Tambyrajah, W.S., Saysell, C., Wilmot, C.M., Phillips, S.E.V., Knowles, P.F., Dooley, D.M. and McPherson, M.J. (2005) Active site rearrangement of the 2-hydrazinopyridine adduct in *Escherichia coli* amine oxidase to an azo copper(II) chelate form: A key role for tyrosine 369 in controlling the mobility of the TPQ-2HP adduct *Biochemistry*, **44**, 1583-1594.

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