

P021 pH dependence of kinetic isotope effects in monoamine oxidase A

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The mammalian monoamine oxidases are flavoproteins localised to the outer mitochondrial membrane, and contain a covalently linked FAD via the 8α -methyl group to an active site cysteine residue. A common feature to all the proposed mechanisms is the initiation of catalysis with the deprotonated form of the amine substrate. However, recent kinetic studies led to an alternative proposal in which it is the protonated form of the substrate that binds to the enzyme. This study reports on the pH dependence of recombinant human liver monoamine oxidase A as characterised by both steady state and stopped flow techniques. For all substrates there is a macroscopic ionisation in the enzyme-substrate complex attributed to a deprotonation event required for optimal catalysis with a pK_a of 7.4-8.4. Deuteration of the substrate benzylamine causes an increase in the substrate pK_a which is reflected as a small alkaline shift in the pH dependence of k_{red} , resulting in a decrease in the observed KIE from approximately 13 to 8 with increasing pH. Due to the pH dependence of the KIE the deprotonation event was assigned to that of the bound amine substrate. The pK_a of the amine substrate is greatly perturbed upon binding to the active site. The perturbation of the amine pK_a has been seen with other amine oxidases, and may be a general feature of these enzymes, allowing the efficient functioning at physiologically relevant pH.