

Interaction of metal ions on the transient reductive half reaction of xanthine oxidase

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Transient reductive half reaction of xanthine oxidase (XO) with xanthine has been investigated anaerobically by stop-flow kinetic measurements in the presence and absence of Cu^{2+} and Hg^{2+} ions. The effect of the interactions of these metal ions was found to be inhibitory towards the oxidoreductase activity of the enzyme. It is observed that in the presence of these metal ions, the half reaction kinetics shows single exponential behaviour. This indicates least perturbation of redox potential of Flavin adenine dinucleotide (FAD) centre which takes part in the intramolecular electron transfer of the enzyme. The wavelength dependence of the transient reaction kinetics shows marked change in electron accepting ability of the redox centres of metal bound enzyme. Our measurements show the transfer of electrons from the electron introduction site of the enzyme to copper bound site. The studies suggest that the blocking of the oxidative half reaction of the enzyme due to the presence of the metal ions is responsible for the steady state inhibition of the enzyme. The differences in the wavelength dependence of the transient reaction kinetics of Cu^{2+} and Hg^{2+} bound enzyme have been discussed. The investigations on the mechanism of the reductive half reaction due to the interactions of the metal ions show considerable change in the binding of the substrate and dissociation of the enzyme-intermediate complex. The pseudo first order rate constant (K_{obs}) at 600 nm increases with the increase in Cu^{2+} concentration but it decreases with increasing concentration of Hg^{2+} ion. The kinetic parameters associated with the native and metal bound enzyme, and the uneven steady state inhibition by these metal ions are discussed.

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