Studies on the Oxidase Activity of Copper(II) Carboxypeptidase A

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Summary Copper(II) carboxypeptidase A catalyses the oxidation of ascorbic acid and this reaction is inhibited by α-benzylsuccinate, a known inhibitor of the thiol-esterase action of the copper enzyme; the pH dependencies of k_{cat} and k_{cat}/K_m are similar near pH 7 to those seen for the peptidase and esterase activities of native carboxypeptidase A.

CARBOXYPEPTIDASE A (CPA, E.C.3.4.2.1) is a zinc-containing metalloenzyme that exhibits peptidase and esterase activity.[1] Unlike several other metallocarboxypeptidase A species which contain divalent metal ions bound to the active site, Cu^{II} CPA shows neither peptidase nor esterase activity with the exception of its hydrolytic action on a thiol ester.[2] From the spectroscopic properties of Cu^{II} CPA, it has been suggested that the donor-atom set in this enzyme is N_2O_2 and that the co-ordination around the metal ion is significantly distorted from square planar toward a tetrahedral geometry.[3] In spite of the lack of catalytic activity towards most substrates of the native zinc enzyme, the binding site intrinsic to the native species appears to be preserved in Cu^{II} CPA since CPA inhibitors like β-phenylpropionate still bind to the Cu^{II} species. Since many copper proteins are known to function as oxidases[4] (*e.g.* ascorbate oxidase, tyrosinase, or amine oxidase), we have begun to explore the possibility that Cu^{II} CPA might act as an oxidase upon biologically important substrates. We now report that Cu^{II} CPA functions as an effective catalyst in the oxidation of ascorbic acid to give dehydroascorbic acid.

The rate of ascorbate oxidation catalysed by Cu^{II} CPA was followed spectrophotometrically at 265 nm at 25·0 °C.[5] Under conditions of substrate in excess, over the pH range 6—9, this rate became independent of the substrate concentration when these concentrations were somewhat higher than 10^{-4}M. The rate data obtained could be analysed according to the Michaelis–Menten kinetic scheme. Over the same range of ascorbate concentration the aquo-complex of Cu^{II} did not exhibit Michaelis–Menten kinetics in its far slower reaction with this substrate. Since the pK_a values for the proton dissociations of ascorbic acid are 4·17 and 11·57,[6] the ascorbate monoanion is the predominant species of the substrate in the pH range investigated.

The rate parameters k_{cat} and k_a/K_{cat} increased with increasing pH and showed sigmoidal pH dependencies from

which pK values of *ca.* 6·5 and 7, respectively, could be calculated. These values correspond reasonably well with the acidic pK values seen for ester or peptide hydrolysis catalysed by native CPA and by several of the metallocarboxypeptidases which have been interpreted as reflecting the ionization of the γ-carboxy-group of the Glu-270 residue.[1,7] DL-α-Benzylsuccinate, a strong inhibitor of the hydrolytic action of native CPA,[8] was found to inhibit the oxidation of ascorbate by Cu^{II} CPA with K_I of *ca.* 3×10^{-5} M, similar to the K_I measured for the inhibition of the thiolesterase activity of the copper enzyme. Limiting values of $(k_{cat})_{lim} = 6$ min^{-1} and $(k_{cat}/K_m)_{lim} = 2{\cdot}5 \times 10^4$ l mol^{-1} min^{-1} were obtained in the Cu^{II} CPA-catalysed oxidation of ascorbate.

Our observations demonstrate that the replacement of one active-site metal ion by another in a given protein matrix can result in the conversion of a powerful hydrolytic catalyst into an oxidase.

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