Cu^{II} Carboxypeptidase A-catalysed Hydrolysis of *S*-(*trans*-Cinnamoyl-L-α-mercapto-β-phenylpropionate

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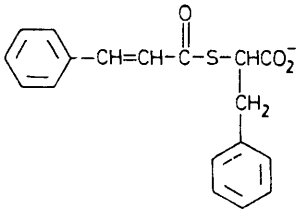
Summary Cu^{II} carboxypeptidase A has been found to catalyse the hydrolysis of *S*-(*trans*-cinnamoyl)-L-α-mercapto-β-phenylpropionate at pH 7·5 and 25 °C at a rate similar to that of the catalytic hydrolysis of the thiolester by the native zinc enzyme; this is the first observation of catalytic activity for the copper form of the enzyme and makes possible mechanistic investigations of this metallocarboxypeptidase species.

REPLACEMENT of the active site Zn^{II} ion with Cu^{II} in carboxypeptidase A (CPA) is known to result in a complete loss of the catalytic activity of the enzyme in the hydrolysis of both peptide and ester substrates.[1,2] The lack of catalytic activity of the Cu^{II} species is in marked contrast to the behaviour of metallocarboxypeptidases containing other divalent metal ions which have been found to retain either peptidase or esterase or both activities.[1,2] Some evidence exists against the hypothesis that Cu^{II}CPA is catalytically inactive for reasons associated with strong distortions of the arrangement of the ligands around the metal ion from a nearly tetrahedral toward a planar geometry.[3,4] The alternative proposal[4] that the Cu^{II} enzyme is catalytically inactive toward peptides and esters because certain of the important protein side chains which participate in the catalytic action of the native enzyme have shifted from their optimal positions in the case of the Cu^{II} species also lacks definitive support.

Recently, we reported that the hydrolysis of both enantiomers of *S*-(*trans*-cinnamoyl)-α-mercapto-β-phenylpropionate (I) was catalysed by native carboxypeptidase A.[5] We also presented evidence for a difference in mechanism between the reactions with CPA of L-(I) and D-(I).[6] We now report that Cu^{II}CPA shows a substantial degree of catalytic activity in the hydrolysis of L-(I). When assayed at 310 nm with $0{\cdot}8 \times 10^{-4}$ M *O*-(*trans*-*p*-chlorocinnamoyl)-



(I)

L-β-phenyllactate at pH 7·5 and 25 °C in 0·5 M NaCl and 0·05 M Tris buffer, the activities of apoCPA obtained by the treatment of CPA_{α}[1] with 1,10-phenanthroline, Cu^{II}CPA obtained by the treatment of apoCPA with $CuCl_2$, and CPA obtained by the reconstitution of apoCPA with $ZnCl_2$ were found to be 2·4%, 2·1%, and *ca.* 100% compared to that of native CPA. However, measurements at 310 nm indicated that the Cu^{II}CPA-catalysed hydrolysis of L-(I) occurs readily in 0·5 M NaCl, and 0·05 M buffer at pH 7·0 and 25 °C. Values of k_{cat}/K_m, k_{cat}, and K_m for the action of Cu^{II}CPA on L-(I) which were obtained from the analysis of the rate data under these conditions with $E_0 >> S_0$ were $386 \pm 27\ M^{-1}\ s^{-1}$, $0{\cdot}0363 \pm 0{\cdot}012\ s^{-1}$, and $(9{\cdot}42 \pm 3{\cdot}77)$

$\times 10^{-5}$ M, respectively. The value of k_{cat}/K_m obtained for the Cu enzyme is about 45% of that for the action of native Zn^{II}CPA on L-(I) at the same pH.[7] When the reaction of Cu^{II}CPA toward D-(I) was examined at pH 8·0 under conditions where the native enzyme shows considerable activity toward the D-enantiomer no catalytic activity was found in the case of the copper enzyme.

The activity manifested by Cu^{II}CPA exclusively on the L-(I) thiolester species is in marked contrast to the lack of activity of the copper enzyme on *O*-acyl esters of L-α-hydroxy acids or peptides derived from L-amino acids. The nature of the binding of the L-thiolester in the active site region of the copper enzyme remains to be elucidated, but the similarity in the value of k_{cat}/K_m to that found for the action of the native enzyme on the L-thiolester suggests that the productive binding modes in the reactions of the thiolester with the copper and native enzymes may be quite similar. Having discovered a way in which the catalytic activity of Cu^{II}CPA can be studied, we are now engaged in studies to compare its mechanism of action to those of other metallocarboxypeptidases.

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[1] J. E. Coleman and B. L. Vallee, *J. Biol. Chem.*, 1961, **236**, 2244.
[2] J. E. Coleman, P. Pulido, and B. L. Vallee, *Biochemistry*, 166, **5**, 2019.
[3] F. A. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.*, 1971, **25**, 1.
[4] R. C. Rosenberg, C. A. Root, P. K. Bernstein, and H. B. Gray, *J. Amer. Chem. Soc.*, 1975, **97**, 2092.
[5] E. T. Kaiser, T.-W. Chan, and J. Suh, in 'Protein—Metal Interactions,' ed. M. Friedman, Plenum Press, 1974, p. 59.
[6] J. Suh and E. T. Kaiser, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 863.
[7] T. W. Chan, Ph.D. Thesis, The University of Chicago, 1974.