Binding of Zinc in Carboxypeptidase

CARBOXYPEPTIDASE is a metallo-enzyme which contains zinc. It has been reported by Coleman and Vallee[1] that the enzyme can be de-activated by removal of zinc and that activity can be restored by the addition of zinc, cobaltous, ferrous, nickel and manganous ions but not by addition of cadmium, magnesium or calcium ions. This metallo-enzyme therefore falls in the group of enzymes which have no specific metal requirement for activity[2]. In biological systems, however, a particular metal, zinc, is associated with the enzyme presumably because complex ion equilibria favour this particular association over all others. The situation is not very different from that found in the case of the enzyme enolase, where magnesium and manganous are the particular ions associated with activity in the biological system, but where other cations such as zinc can activate the enzyme *in vitro*[3]. The apparent stability constants for the association of zinc and cobaltous ions with carboxypeptidase are 2×10^8 and 1×10^6 respectively[1]. The *p*H range of activation by the different cations indicates that association with the active centre is in the order zinc(II) > nickel(II) and cobalt (II). The study of complexes of a wide variety of simple organic ligands has shown[2] that this stability sequence is observed only when one of the co-ordinating centres is sulphide, R—S^-. I conclude that zinc is bound by a sulphide group in carboxypeptidase and give the following additional evidence in support of this binding.

The cobaltous carboxypeptidase complex is highly coloured. The molar extinction coefficient at 530 mμ is 150[1]. The usual value of the extinction coefficient of cobaltous complexes is < 10, except when the cobalt ion is bound to large, polarizable ligand atoms such as chloride and sulphide[4]. Thus both cobaltous thiocyanate and thiosulphate have extinction coefficients of the order of 10^2, and cobaltous cysteine and thioglycollate complexes are highly coloured. In the complex with carboxypeptidase, the binding of the cobaltous ion is probably restricted to oxygen, nitrogen or sulphur centres, and it would appear that only in the case of binding to sulphur is the colour of the complex explicable from the known properties of model complexes. The shift of the absorption band from 512 mμ in the hydrate to 530 mμ in the carboxypeptidase complex, that is, a shift to longer wavelengths, is in the expected direction if binding to cobalt is through a mixture of oxygen and sulphur co-ordinating centres, but is in the opposite sense from the shift produced by extensive co-ordination to nitrogen.

In the metallo-enzyme enolase I have postulated that the binding of the cations is largely through oxygen atoms[2]. In this enzyme magnesium is active. Magnesium cannot activate carboxypeptidase. It is not known to bind to sulphide groups. On the other hand, cations such as lead(II), cadmium, mercuric, silver, and cupric which bind sulphur groups more strongly than zinc may be expected to be powerful inhibitors.

If cobalt is bound to sulphur in carboxypeptidase, it is possible to predict that there will be a further new absorption band absent in the complexes of the other cation complexes of this enzyme. The new band will be at about 285–300 mμ and will have an extinction coefficient greater than 10^3. Both this strong band and the band in the visible of extinction coefficient 10^2 are present in model cobalt complexes containing Co—S—R links[4]. Neither of these bands is observed in cobaltous insulin, and we can predict that in this complex the binding of zinc is to a mixture of oxygen and nitrogen groups. Both insulin and carboxypeptidase contain sulphur groups.

Note added in proof. Since this communication was submitted I have heard from B. L. Vallee and his associates that they have demonstrated Zn—S binding independently.

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A Plate Assay for Elastase

SINCE Balo and Banga[1] described the pancreatic enzyme elastase, its dissolving effect on elastin, and its possible implications in atherosclerosis, a considerable amount of interest has been focused on the quantitative measurement of its enzymatic activity.

Two methods most commonly used for the assay of elastase are based on gravimetric measurements and nephelometry. Much has been learned in regard to the interaction of elastin and elastase and its inhibitor, serum, by these two methods. However, both methods suffer from the fact that they require much time and special equipment and skilled personnel.

If a programme designed to establish and follow elastase-inhibitor levels in sera from a variety of sources, including human sera, is anticipated, a relatively easy and rapid assay for elastase and its inhibitor is essential. Such a programme is in progress in this laboratory, and an assay fulfilling these requirements has been developed.

Table 1. WATER–SODIUM HYDROXIDE ELASTIN IS USED. IT IS AUTOCLAVED FOR 10 MIN. AT 10 LB. PRESSURE. THE RESULTING *p*H IS 7

Components	Composition of elastin medium: Amount of components in stock solution	Volume of stock solutions for 100 ml. of complete medium
Inorganic salts		
K_2HPO_4	25 gm.	
KH_2PO_4	25 gm.	
Distilled water	250 ml.	0·5 ml.
Inorganic salts *B*		
$MgSO_4\,7H_2O$	10 gm.	
NaCl, $FeSO_4\,7H_2O$	0·5 gm.	
$MnSO_4\,4H_2O$	of each	
Distilled water	250 ml.	0·5 ml.
Elastin	10 gm.	
Distilled water	100 ml.	10 ml.
Distilled water		87·5 ml.
Agar		1·5 gm.

Table 1 shows the composition of elastin agar. 20 ml. of this medium is poured into a sterile 15 × 150 mm. Petri dish. The non-transparent medium (due to the presence of elastin) is allowed to harden. Six wells are made in the medium by either allowing the medium to harden in the presence of plastic molds which can then be easily removed after hardening, or the medium is allowed to harden and the molds are pushed through the agar. The resulting cylinder of agar can be teased away with an inoculating needle. The wells serve as reservoirs for elastase and its inhibitor serum. The diffusion of elastase from the wells and its subsequent dissolution of elastin result in clear zones around the wells, the zones varying in diameter with the concentration of elastase used. The resulting clear zones can be seen