Effect of Polyamino Carboxylic Acids on Catalase

CATALASES are known to be inactivated by the formation of compounds with cyanide[1], azide and hydroxylamine[2], so we thought it probable that the iron of the enzyme could be complexed with chelating agents, the effect being also to inactivate the enzyme. The chelating agents used were ethylenediamine tetraacetic acid (EDTA), cyclohexanediamine tetraacetic acid (CDTA), diethylenetriamine pentaacetic acid (DTPA) and NN′ethylene*bis*(2{*o*-hydroxyphenyl}) glycine (EHPG). The rate of decomposition of hydrogen peroxide by bovine-liver catalase in the presence of each of the above chelating agents was followed by a rapid titration method.

A decrease in the rate of destruction of hydrogen peroxide by the catalase was observed in the presence of all the chelating agents. With EDTA and DTPA, the inhibition reaches a maximum at a *p*H of about 8·5 and then decreases again with increasing *p*H. With CDTA, the inhibition increases slowly with increasing *p*H, whereas with EHPG the increase is rapid, although measurements could not be made at *p*H values greater than 10 due to a rapid decrease in the activity of the catalase, probably due to protein denaturation. In conclusion, it must be noted that the inhibition of catalase by these chelating agents is small compared to that of hydrogen cyanide, which has a value of K_i of $4 \cdot 6 \times 10^{-6}$ M at *p*H 7 (ref. 3), whereas at *p*H 8·5 (*p*H of maximum inhibition of catalase in the presence of EDTA and DTPA) the K_i values are $9 \cdot 2 \times 10^{-2}$ M, $9 \cdot 3 \times 10^{-2}$ M, $2 \cdot 6 \times 10^{-1}$ M, and $3 \cdot 3 \times 10^{-1}$ M for EDTA, DTPA, CDTA and EHPG respectively.

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Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis

DEXTRANSUCRASE is a representative member of the general class of enzymes known as transglycosidases. In this particular example, the glucosyl group from sucrose is transferred to a suitable acceptor, which causes chain initiation, and dextran is formed. Polymer-formation is governed by many factors such as the nature of the acceptor molecule, *p*H and temperature[1].

This work was directed at the role of metal ions in enzyme catalysis[2]. This was initiated by some previous work which had suggested that magnesium plays a part in dextran synthesis[3,4]. These investigations had shown that a relatively linear molecule of dextran could be isolated from a magnesium-deficient diet inoculated with *Betacoccus arabinosaceous* (Birmingham). This is contrasted with the branched dextran isolated from a normal medium. With this in mind, it was felt important to study the effect of metal ions on the enzyme and to carry out a detailed kinetic investigation.

Using fructose-production as a measure of enzyme activity, the conversion of sucrose to dextran was examined under the influence of several metal cations. The details of the enzyme preparation and the analytical procedures have been reported[2]. In addition to testing the effect of the metal ions on the catalysis, the role of metal chelating agents, tetrasodium ethylenediamine tetraacetic acid (EDTA) and oxalic acid were investigated. All experiments were conducted in a 0·05 M acetate buffer of *p*H 5·2 and the reaction was carried out for 0·5 hr. in a constant temperature water bath at 25° C.

Of the metal ions tried as activators of this particular enzyme, notably calcium, zinc, magnesium, manganese and copper, only the first was effective. The reciprocal plot of initial velocity against calcium ion at various substrate concentrations is shown in Fig. 1. From the common intercept of these lines, a value for the dissociation constant of the enzyme metal complex may be evaluated. This turns out to be $9 \cdot 4 \times 10^{-5}$ M. The action of EDTA is shown in Fig. 2. The graph for oxalic acid was similar, except

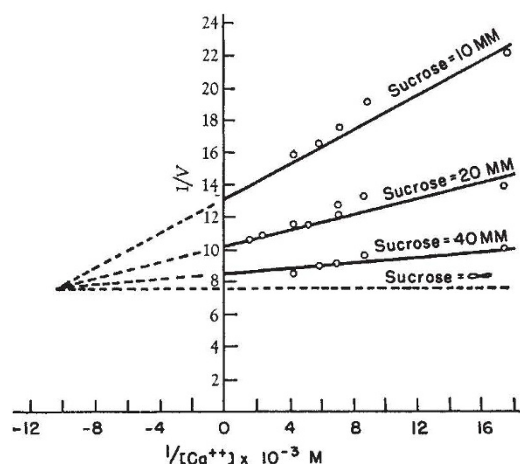

Fig. 1. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against calcium ion concentration. The dashed curve representing sucrose = ∞ was drawn through the intersection of the three other curves and the value for $1/V$ calculated from another experiment using enzyme and sucrose and an excess of calcium ion

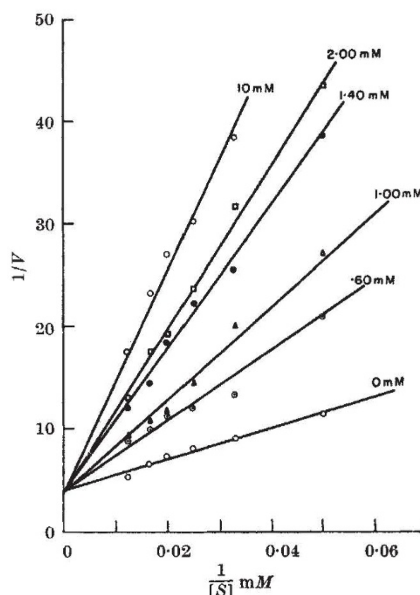

Fig. 2. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against sucrose concentration at various levels of EDTA

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