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INACTIVATION OF α-AMYLASES BY COBALT COMPLEXES*

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SUMMARY

Crystalline, bacterial α-amylase (α-1,4 glucan 4-glucanohydrolase, EC 3.2.1.1) was inactivated by incubation with a number of Co complexes. Inactivation was most effective at room temperature after incubation for at least 240 min. The extent of inactivation was governed by the stability and was unaffected by the formal charge on the Co complex. Enzyme inactivation depended both on the concentration of the Co complexes and on complex:enzyme weight ratio. The inactivation could be partly reduced by incubation of the amylase with the complex in the presence of starch.

INTRODUCTION

A number of workers[1–7] have studied the effect of chemical modifications of α-amylases (α-1,4 glucan 4-glucanohydrolase, EC 3.2.1.1) on the activity of the enzyme. The author[8] has recently reported on the effect of synthetic detergents on α-amylases of fungal, bacterial and pancreatic origin.

BELLO AND BELLO[9] have presented evidence for the formation of Co complexes of proteins in which Co is probably bound to the peptide groups. The present report deals with the effect of Co complexes on inactivation of bacterial α-amylase.

EXPERIMENTAL

The crystalline bacterial enzyme employed was purchased from Worthington Biochemical Corp., Freehold, N. J. (U.S.A.)**. The Co complexes were prepared in the laboratory according to procedures given by KLEINBERG[10]. All reagents, unless stated otherwise, were of analytical grade. The starch used as the substrate to measure the amylolytic action was Amaizo 721A—pregelatinized waxy maize starch, from American Maize Products Company, New York, N.Y. (U.S.A.).

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α-Amylase activity was measured by following the liquefying action of the enzyme[8] during 20 min at 37°.

Inactivation was studied on lots of 0.1 mg of enzyme in 10 ml 0.2% $CaCl_2$ solution mixed with 10 ml Co complexes, adjusted with 0.005 N HNO_3 or NaOH to pH 5.9. The final concentration of Co complex was 0.007 M. The extent of inactivation was computed from a calibration graph correlating the effect of enzyme concentration with starch liquefaction.

RESULTS AND DISCUSSION

The extent of enzyme inactivation depended primarily on the stability of the Co complex in the tested solutions. Thus, for example, among the Co complexes with diethylenetriamine only the trinitro complex, which is stable towards water, had no effect on amylase activity. The other diethylenetriamine complexes, which are known to hydrolyse and form more stable hydroxo and aquo complexes, inactivated the tested enzyme. Similarly, the stable $K_3[Co(CN)]_6$ or cobalt complexes containing either ethylenediamine or propylenediamine had little or no inactivating effect. The relatively more stable complexes containing NO_2^- has a smaller inactivating effect than CH_3COO^-- or Cl^--containing complexes. However, the simple Co salts such as $Co(NO_3)_2$ and $CoCl_2$ had no inactivating effect at the employed concentration despite their lability. The formal charge on the complex was of little, if any, consequence as both the electrically neutral complex $[Co(NH_3)_3(NO_2)_3]$ and $(NH_4)_2[CoCl_4]\cdot 2H_2O$ despite its negative charge on the Co complex, were highly effective in inactivating the enzyme. Similarly, the formal valence of the Co was of no consequence in governing the extent of inactivation. Both the polynuclear complex $\left[Co\left\{\begin{matrix}OH\\OH\end{matrix}\right\rangle Co(NH_3)_4\Big\}_3\right](SO_4)_3$ and Co^{II} complex $(NH_4)_2[CoCl_4]\cdot 2H_2O$ were just as effective in inhibiting enzyme activity as any of the active Co^{III} complexes. Inactivation was temperature-dependent; incubation at 5° reduced the extent of enzyme inactivation to 25% of that at 25°. None of the tested Co complexes had an appreciable inactivating effect at concentrations below 0.001 M, and with most active complexes inactivation was maximal at 0.007 M. The extent of inhibition depended on the length of incubation of the enzyme with the Co complex, and was complete after 240 min.

To determine the effect of anions added with the Co complexes, 0.007 M solutions of a number of salts were tested. $(NH_4)_2SO_4$, $NaNO_3$, $NaNO_2$, KI, KBr, NaCl, NaCN, CH_3COONa, and $NaHCO_3$ had no effect on amylase activity. $(NH_4)_2C_2O_4$ inactivated the enzyme to an extent of 81%, probably due to precipitation of Ca and absence of free Ca^{2+} ions essential for enzyme stability under conditions of prolonged incubation.

The results given in Table I were obtained by using solutions of Co complexes adjusted to pH 5.9 by 0.005 N HNO_3 or NaOH. This has been found necessary in view of the fact that the enzyme was inactivated by solutions having a pH below 4.5. Selecting Co complexes which had a pH value within the range of enzyme stability (pH 5–9) without adjustment of pH in the incubation mixture, gave results practically identical with those given in Table I.

The results given in Table II show that employing a uniform concentration of Co complexes (0.007 M), the extent of inactivation is governed to a large extent by the

TABLE I

EFFECT OF Co COMPLEXES ON BACTERIAL α-AMYLASE

20 ml of 0.007 M Co complex (pH 5.9) containing 0.1 mg amylase, kept at 25° for 18 h; en = ethylenediamine, pn = propylenediamine, dien = diethylenetriamine.

Reagent	*Inactivation (%)*
$[Co(en)(NH_3)(NO_2)_2]Cl$	0
$[Co(en)_2C_2O_4]Cl$	0
$[Co(en)_2C_2O_4]_2NO_3$	0
$[Co(en)_2CO_3]CO_3$	0
$[Co(en)_2(SCN)_2]SCN$	0
$[Co(en)_2Cl_2]Cl$	45
$[Co(en)_3]I_3$	0
$[Co(en)_3]Br_3$	0
$[Co(dien)(NO_2)_3]$	0
$[Co(dien)(NH_3)(NO_2)_2]Cl$	10
$[Co(dien)Cl(NO_2)_2]$	63
$[Co(dien)Cl_3]$	100
$[Co(pn)_2Cl_2]Cl$	10
$[Co(pn)_3]Cl_3$	0
$K_3[Co(CN)_6]$	0
$[Co(NH_3)_3(NO_2)_3]$	82
$[Co(NH_3)_4(H_2O)Cl]SO_4$	100
$[Co(NH_3)_4CO_3]NO_3 \cdot \frac{1}{2} H_2O$	100
$[Co(NH_3)_4CO_3]_2SO_4$	100
$[Co(NH_3)_5NO_2](NO_3)_2$	93
$[Co(NH_3)_5H_2O]Br_3$	100
$[Co(NH_3)_5H_2O]I_3$	100
$[Co(NH_3)_5C_2H_3O_2](NO_3)_2$	100
$[Co(NH_3)_5CO_3]NO_3$	100
$[Co(NH_3)_6]Cl_3$	100
$(NH_4)_2[CoCl_4] \cdot 2\ H_2O$	100
	100
$CoCl_2 \cdot 6\ H_2O$	0
$Co(NO_3)_2 \cdot 6\ H_2O$	0

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF BACTERIAL AMYLASE IN 20 ml OF 0.007 M $[Co(NH_3)_5CO_3]NO_3$ ON ENZYMIC ACTIVITY (18 h AT 25°)

Bacterial enzyme (mg)	*Inactivation (%)*
10.00	0
6.67	80
3.33	85
1.00	96
0.10	100

complex:enzyme ratio. Adding starch to the incubation mixture reduced the extent of enzyme inactivation (Table III), apparently due to the protective effect of formation of an enzyme–substrate complex.

The molecular weight of bacterial α-amylase is of the order of 100 000. No equimolar stoichiometric relation seems, ther fore, to exist between inactivation by Co complexes and α-amylase. The large excess of Co complexes required to completely inactivate the enzyme seems to point to the possibility of a nonspecific binding which might bring about changes in spatial configuration of the enzyme and result in loss of enzyme

TABLE III

EFFECT OF STARCH ON INACTIVATION

Inactivation of 0.1 mg bacterial amylase was measured after 18h at 25° in 20 ml 0.007 M Co-complex solution containing starch as indicated.

Starch (mg)	*Inactivation (%) by*		
	$[Co(NH_3)_5H_2O]Br_3$	$[Co(NH_3)_5CO_3]NO_3$	$[Co(NH_3)_4(H_2O)Cl]SO_4$
100	100	89	100
250	93	71	95
500	95	60	91
1000	64	56	76

activity. It is possible that the inactivation is related to the presence of at least 1 mole of firmly bound Ca per mole of enzyme. This atom of Ca seems to stabilize the configuration necessary for activity of the enzyme.

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