EFFECT OF METAL IONS ON PEA ALCOHOL DEHYDROGENASE

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Received February 16th, 1978

The effect of Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+} and Co^{2+} on alcohol dehydrogenase (ADH) isolated from germinating pea seeds was examined. The enzyme is inhibited approximately to the same degree if incubated 5 to 30 min with Ag^{+}, Cu^{2+}, and Cd^{2+}; the same degree of inhibition can be brought about by zinc ions only if concentration by one order higher (10^{-4}M) is used. Co^{2+}-ions do not inhibit pea alcohol dehydrogenase even at 10^{-3}M concentration. NAD and Zn^{2+} decrease the inhibitory effect of Cd^{2+} yet not the effect of Cu^{2+} or Ag^{+}. The differences in the mechanism of action of individual heavy metal ions on plant ADH are discussed.

Pea ADH (EC 1.1.1.1) is a relatively stable enzyme[1] which has been sufficiently well studied as regards its substrate specificity[2,3] and mechanism of catalysis[4].

According to Cossins[5] the molecule of this enzyme bears a zinc atom and, from the results of inhibitory experiments with agents reacting with SH-groups, also sulfhydryl groups; the latter together with zinc are essential for the redox mechanism of the reaction. In this study the effect of metals on the activity of the enzyme was investigated.

EXPERIMENTAL

Plants. The experiments were carried out with pea seeds (*Pisum arvense* L., cv. RAMAN ELITA).

Isolation of pea ADH *and determination of its activity.* The enzyme was isolated from germinating seeds by the method described in preceding papers[3]. The specific activity of the enzyme was 0·5 unit. μmol/min. mg The activity of the enzyme was determined by the method of Racker[7], modified by Leblová and Mančal[3]. When necessary to express the reaction rate in absolute units (μM s^{-1}), the molar absorption coefficient $\varepsilon \frac{\text{NADH}}{366} = 5{\cdot}7 \,.\, 10^{3}\text{M}^{-1}\,\text{cm}^{-1}$ was used.

Inactivation measurement. The metal ions were used either as sulfates ($ZnSO_4$, $CuSO_4.5\,H_2O$, $3\,CdSO_4.8\,H_2O$, $CoSO_4.7\,H_2O$) or nitrates ($AgNO_3$); all compounds were of analytical purity. The inactivation measurements were carried out in a volume of 1 ml at 20°C. The incubation time varied between 0 and 30 min. The measurements were carried out in 0·025M Tris-acetate buffer at pH 7. The buffer (0·7 ml), the enzyme solution (0·2 ml), and the inhibitor solution (0·1 ml) of appropriate concentration were pipetted into a test tube. To determine the protective effect of NAD or zinc ions, the incubation medium was treated with NAD or Zn^{2+} to a final concentration shown in the Tables.

RESULTS AND DISCUSSION

We found that pea ADH is reversibly inhibited by heavy metal ions; the degree of inhibition is approximately the same after 5- to 3-min incubation with Ag^+, Cu^{2+} and Cd^{2+} as follows from Table I. A concentration of Zn^{2+} higher by one order is necessary to achieve the same inhibitory effect which show these three heavy metals. Pea ADH is not inhibited by Co^{2+}. Long-term treatment of the incubation medium with salts brings about complete inactivation in the case of Cu^{2+} and Ag^+ yet only a 50% inactivation if zinc ions are used.

TABLE I

Inhibitory Effect of Heavy Metal Ions on Pea ADH

Experimental conditions: The activity was measured in a medium (1 ml) containing 0·1M phosphate buffer, pH 8·5; [ethanol] = 100 mM; [NAD] = 0·5 mM. The results are given in % of the original activity.

Incubation with inhibitor min	Ions, M				
	Ag^+ 10^{-5}	Cu^{2+} 10^{-5}	Cd^{2+} 10^{-5}	Zn^{2+} 10^{-4}	Co^{2+} 10^{-3}
5	64	55	80	76	100
10	54	50	75	68	100
20	54	47	62	62	100
30	50	45	61	60	93

TABLE II

Effect of Zn^{2+} and Co^{2+} on Inactivation of Enzyme Caused by Cd^{2+}, Ag^+, and Cu^{2+}

Experimental conditions: Incubation of pea ADH in 0·025M Tris-acetate buffer, pH 7·0, with individual ions (0·01 mM concentration). The activity was measured in a medium (1 ml) containing 0·1M sodium phosphate buffer, pH 8·5; [ethanol] = 100 mM; [NAD] = 0·5 mM. The results are given in % of the original activity.

Incubation min	$Cd^{2+} + Zn^{2+}$	$Ag^+ + Zn^{2+}$	$Cu^{2+} + Zn^{2+}$	$Cd^{2+} + Co^{2+}$	$Ag^+ + Co^{2+}$	$Cu^{2+} + Co^{2+}$
5	91	85	79	100	63	61
10	91	73	68	100	56	54
20	91	63	62	100	55	45
30	91	50	47	93	50	40

A common feature of all the heavy metal ions found efficient in our inhibition studies is their tetrahedric configuration. As regards the mechanism of their effect, an unambiguous explanation could provide their interaction with the SH-groups of the enzyme; the reactivity of the ions with these groups decreases in the series Ag^+, Cu^{2+}, Cd^{2+}, Cu^+, Zn^{2+}. The reactivity of the ions with the SH-groups is in good agreement with the inhibitory effect which heavy metal ions have on pea ADH. A role in the inhibitory action of the metals may also play the redox properties of silver and copper ions.

As stated in the introduction, pea ADH contains according to Cossins[5] a zinc atom in its molecule. This zinc atom has been replaced in animal ADH by a cadmium atom[8]. We have observed also with the plant enzyme that the degree of inhibition caused by cadmium ions decreases if zinc atoms are simultaneously applied. Zinc

TABLE III

Restitution of Activity of Enzyme Inhibited by Cd^{2+} after Addition of Zn^{2+}

Experimental conditions: The enzyme was incubated with 0·01 mM Cd^{2+} and Zn^{2+} (0·01 mM) were added afterwards. The remaining conditions were the same as those described in Table I and II

Incubation min	Activity, %
0	100
10	79
20	93
30	98

TABLE IV

Protection of Enzyme by NAD^+ against Inhibitory Action of Heavy Metal Ions

Experimental conditions: Incubation of pea ADH in 0·025M Tris-acetate buffer, pH 7·0, with 1 mM NAD and 0·01 mM Cd^{2+}, Cu^{2+}, and Ag^+. The remaining conditions were the same as those described in Table I and II.

Incubation min	$Cd^{2+} + NAD^+$	$Cu^{2+} + NAD^+$	$Ag^+ + NAD^+$
5	100	86	79
10	95	76	70
20	92	62	62
30	86	47	52

atoms at a 10 μM concentration applied simultaneously with Cd^{2+} prevent pea ADH from inactivation which would cause the cadmium atoms by themselves (Table II). It is important that Zn^{2+} can restore to a certain degree the activity of the enzyme partly inactivated by Cd^{2+} (Table III). Zinc atoms on the other hand do not affect at all the inhibition brought about by silver and copper ions. A similar protective effect show also Co^{2+} ions (Table II).

The coenzyme (NAD) affects the inhibitory action of Cd^{2+} yet does not decrease the inhibitory effect of Cu^{2+} and Ag^{+} (Table IV). The effect of NAD on the inhibition by Cd^{2+}-ions is concentration-dependent: a 2 mM concentration of the coenzyme prevents almost completely the enzyme from inactivation (Fig. 1).

The fact that NAD protects pea ADH against inactivation by Cd^{2+} yet not against inactivation by Ag^{+} and Cu^{2+} seems to suggest that the coenzyme prevents the enzyme from the replacement of the zinc atom in its molecule by Cd^{2+} rather than protects its SH-groups. Since NAD blocks this replacement, we may assume that the coenzyme interacts with the metal component of the enzyme forming a binary E-NAD complex. This assumption has been verified in our previous experiments with *o*-phenanthroline, a strong chelating agent: *o*-phenanthroline acts as a strictly competitive inhibitor with regard to NAD and NADH, respecitively[9]. We found moreover that Zn^{2+}-ions can influence also the reversible reaction of pea ADH with phenanthroline since when added to the enzyme which has been inhibited to 40% by this reagent they markedly increase the activity of the enzyme, up to 84% of the original activity.

We may therefore conclude from our measurements carried out with metal ions that Cu^{2+}, Ag^{+}, Zn^{2+}, and Cd^{2+} ions are inhibitors of pea ADH whereas Co^{2+} have no inhibitory effect. The effect on the SH-groups of the enzyme of all the inhibitors tested should also be considered. We have shown in previous experiments that the SH-groups can be prevented from alkylation by iodoacetate by the addition of NAD: NAD protects the SH-groups against alkylation[10] and this may indicate

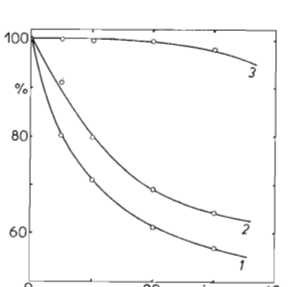

FIG. 1

Protection of Pea ADH by Coenzyme against Inactivation by Ions

Inactivation of *1* Cd^{2+}, *2* Cd^{2+} and 1 mM NAD, *3* Cd^{2+} and 2 mM NAD.

that the SH-groups which are alkylated by iodoacetate could participate on the formation of the binary E–NAD complex. By contrast, it follows from our experiments that the SH-groups which react with heavy metal ions participate in maintaining the three-dimensional structure of the enzyme rather than play a role in the mechanism of catalysis. If the sulfhydryl groups of the active center were the site of the primary reaction with the metal ions then the protective effect of NAD against the inhibitory action of heavy metal ions should be observed with all the ions tested and not merely with Cd^{2+}-ions.

We cannot exclude the possibility, however, that cadmium ions react with the SH-groups of the enzyme; however, the results of our experiments indicate rather a primary exchange reaction with zinc bound in the active center. This conclusion seems to be supported both by the decrease of the inhibitory effect of cadmium ions in the presence of zinc ions (or even by the elimination of this inhibition by the addition of Zn^{2+}) and also by the observation that the Cd^{2+} are a much milder inhibitor (if at all) of the binary E–NAD complex which probably prevents the exchange of zinc for cadmium.

We wish to acknowledge gratefully the stimulating discussion of the present paper by Dr L. Jenšovský, Department of Inorganic Chemistry, Charles University, Prague.

REFERENCES

1. Leblová A., Lapka R., Nováková N.: This Journal, in press.
2. Eriksson C. F.: Acta Chem. Scand. *21*, 304 (1967).
3. Leblová S., Mančal P.: Physiol. Plant. *34*, 246 (1975).
4. Lapka R., Leblová S.: This Journal *42*, 1262 (1977).
5. Cossins E. A., Kopala L. C., Blawacki E., Spronk A. M.: Phytochemistry *7*, 1125 (1968).
6. Leblová S., Hlochová J.: This Journal *40*, 3220 (1975).
7. Racker E.: J. Biol. Chem. *184*, 313 (1950).
8. Drum D. E., Vallee B. L.: Biochim. Biophys. Res. Commun. *41*, 33 (1970).
9. Stiborová M., Lapka R., Nováková N., Leblová S.: This Journal, in press.
10. Lapka R., Leblová S.. Physiol. Plant. *39*, 86 (1977).

Translated by V. Kostka.