INFLUENCE OF THE NATURE OF THE METALIC PROSTHETIC GROUP ON THE BIOSYNTHESIS AND ENZYMATIC PROPERTIES OF THE D-LACTIC DEHYDROGENASE IN YEAST

Andrée Curdel

Institut de Biologie Physicochimique, Paris, and

Laboratoire de Génétique Physiologique du C.N.R.S.

Gif-sur-Yvette, Essonne, France

Received December 20, 1965

D-lactic dehydrogenase (D-LDH) of anaerobically grown yeast is a metalloenzyme (1). The inactive apoenzyme can be prepared by removing zinc with a chelator or by acid treatment (1, 2) ; it can be recombined in vitro with several divalent metals, e.g., Zn, Co, Mn, Cd, or Ni, with differing affinities (3). Combination with Zn, Co, or Mn, yields active enzyme. The enzyme formed by zinc addition is indistinguishable from the holoenzyme isolated from the cells ; the Co^{++} and Mn^{++} enzymes show new characteristics (4, 5). The cobalt enzyme exhibits both a 70 % lower V_{max} than obtained with zinc, and a lower K_m for D-lactate (4). The variation in K_m with pH for the cobalt and zinc enzymes are different, thus suggesting to us that cobalt may be linked by one less ionizable ligand than is zinc (6). The ease with which Zn^{++} can be removed from the enzyme and replaced by cobalt has prompted us to investigate the following questions.

1) To what extent does the concentration of zinc in the growth medium affect the synthesis of holoenzyme and apoenzyme D-LDH ?

2) Is it possible to synthesize in vivo a D-LDH linked to a metal other than zinc ? If so, are the properties of the enzyme formed in the

presence of cobalt, for example, identical to the properties of the D-LDH formed during cultivation in the presence of zinc ? If the properties are different, the question can be asked whether they correspond to the holoenzyme obtained in vitro by recombination with Co^{++} of the apoenzyme synthesized by cells growing in the presence of Zn^{++}.

In order to answer these questions, yeast was grown in a synthetic medium (7) in which the concentration of zinc and other metals could be controlled. The medium was prepared with salts previously crystallized three times in the presence of EDTA, using water twice distilled in quartz. The metals were spectroscopically pure (Johnson Matthey) and precautions were taken to prevent metal contamination during harvest of the cells and extraction of the enzyme. The D-LDH was extracted according to Somlo (8) ; i.e., the yeast cells were washed at 0° with bidistilled water and broken in a Nossal (9) shaker 2 x 30" in 40 mM phosphate buffer, pH 7.3 containing 1.2 mM lactate and the debris removed by centrifugation for 10 minutes at 1200 x g. Enzyme activity was measured spectrophotometrically in a Beckman DU at 27° in mM phosphate buffer, pH 7.3, containing sodium D-lactate 15 mM in a volume of 3 ml and activity expressed as the change in extinction of ferricyanide (67 mM) at 420 mμ and specific activity related to protein content as measured by biuret.

The anaerobic growth of the yeast is influenced very little by the omission of zinc from the medium, by its replacement with equimolar cobalt, or by presence of both metals, as measured by biuret protein. The total growth was respectively 89 %, 103 %, and 120 % of the growth with 5.10^{-6} Zn^{++}.

Effect of zinc deficiency. We have measured the activities of D-LDH extracted from zinc-deprived and sufficient cells. The only difference in the growth media was the addition of 5.10^{-6} M zinc sulfate to the "sufficient medium". Previously, Bertrand et al., (10) have shown a requirement for zinc in glucose-6-phosphate dehydrogenase and 6-phospho-

TABLE 1: INFLUENCE OF ZINC DURING ANAEROBIC CULTIVATION OF YEAST

Experiment	Growth Medium	D-LDH ACTIVITY			
		Assay		Activity Change	
		Without Zn^{++}	After 45 min incubation with 10^{-4} M Zn^{++}	Due to *in vivo* Zn^{++} deficiency	Reactivation by Zn^{++} *in vitro*
		Specific Activity of Extract		%	%
I	with Zn^{++}	111	122	-	10
	without	76	152	32	100
II	with Zn^{++}	135	134	-	0
	without	91	156	33	72
III	with Zn^{++}	196	184	-	0
	without	71	130	64	83

gluconic dehydrogenase from Aspergillus niger. Price has reported that Euglena cells grown in zinc-deficient media oxidize D-lactate only feebly (11).

In each of three experiments (Table 1) we have observed that zinc-deprived cells yield less D-LDH specific activity, than cells grown with excess zinc. The K_m for D-lactate is the same for both enzymes, Figure 1. Thus, as reported previously, Zn^{++} is considered to be the native metal of yeast D-LDH ; the enzyme formed in zinc-deprived medium very probably results from zinc contamination in the medium.

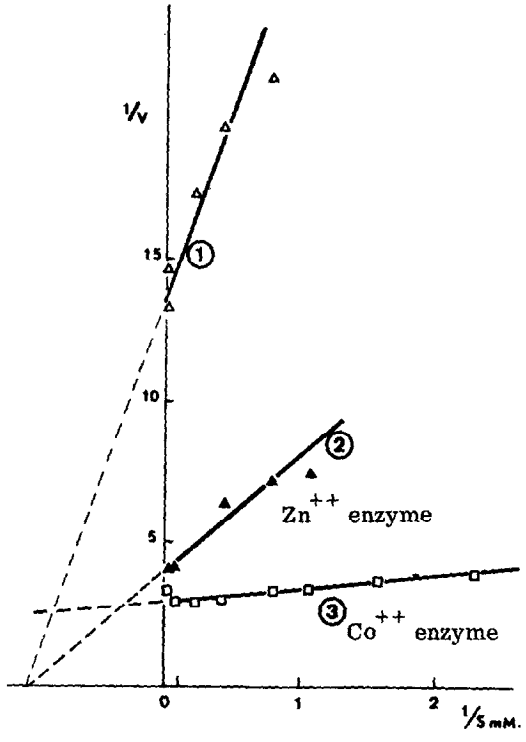

Fig. 1. Influence of the nature of the metal present during cultivation on D-LDH in yeast.

V_m, K_m relative to D-lactate

(1) : medium without added Zn^{++}

(2) : medium with added (5.10^{-6} M) Zn^{++}

(3) : medium with added (5.10^{-6} M) Co^{++}

Measurements in phosphate buffer 66 mM pH 7.7 for the same quantities of each enzyme extract at the same step of purification.

The biosynthesis of apoenzyme seems not to be influenced by zinc deficiency, since addition of 10^{-4} M Zn^{++} to extracts from the deficient cells restores the total D-LDH activity to the level found in extracts from cells grown with excess zinc. The same addition to extracts from the sufficient cells is without effect.

Effect of cobalt. In two experiments cells grown in the presence of 5×10^{-6} M Co^{++}, in a zinc-deficient medium, gave extracts with higher specific activities of D-LDH than those grown with excess zinc ; in a third experiment, the values were about equal (Table 2). Furthermore, the enzyme formed in the presence of cobalt had a different K_m for D-lactate than the enzyme formed in the presence of zinc, Figure 1. As stated previously (4, 5), the enzyme formed *in vitro* by adding Co^{++} to apoenzyme prepared from zinc grown cells has a lower V_{max}, *ca.* 30 % of that obtained when recombined with zinc. If the Co^{++}-apoenzyme complex formed during growth in the presence of Co^{++} were of the type and level formed *in vitro*, the opposite result would be found. That is, cells grown in the presence of cobalt should have a low specific activity for D-LDH. The disparity between the *in vivo* and *in vitro* results could be explained by :

1) A stimulation by Co^{++} of the rate of synthesis of the apo-D-LDH.
2) A different structure or conformation of the apo-D-LDH synthesized in the presence of Co^{++} than that formed in the presence of Zn^{++}.
3) The Co^{++} complex of apo-D-LDH synthesized *in vivo* is different from the one formed *in vitro*.

In order to test the first possibility, we have incubated 10^{-4} M Zn^{++} with the D-LDH extracted from Co^{++} grown cells, Table 2. The total D-LDH activity was decreased by this treatment ; also the variation with pH of the K_m toward D-lactate for the Co^{++}-enzyme formed *in vivo* and that prepared *in vitro* by addition of cobalt to the apoenzyme

TABLE 2: INFLUENCE OF COBALT DURING ANAEROBIC CULTIVATION OF YEAST

Growth Media	D-LDH ACTIVITY			
	Assay		Activity Change Due To	
	Additions as shown	After 45 min incubation with 10^{-4} M Zn^{++}	Co^{++} in vivo / Zn^{++} in vivo	Zn^{++} in vitro
	Specific Activity of Extract		%	%
Experiment I				
without added metal	76			
with " Zn^{++}	111		-	
with " Co^{++}	248		+123	
Experiment II				
without added metal	91			
with " Zn^{++}	135	134	-	-1
with " Co^{++}	220	156	+65	-29
Experiment III				
without added metal	71			
with " Zn^{++}	196	184	-	-6
with " Co^{++}	208	157	+6	-25

prepared from cells grown in the presence of zinc are different. Thus one is led to conclude that the growth of yeast in the presence of cobalt leads to the formation of D-LDH altered from the one formed during growth in the presence of excess zinc.

Effect of manganese and nickel. The presence of 5×10^{-6} M Mn^{++} did not notably influence either the growth or the specific activity of the D-LDH formed in the presence or absence of Zn^{++} ; similar growth with Ni at 5×10^{-6} M decreases slightly the specific activity of the D-LDH in the extracts. Thus neither Mn^{++} nor Ni^{++} appears to be linked in vivo to apo-D-LDH.

Acknowledgements. We are greatly indebted to M. Somlo for help, advice and stimulating discussions ; we wish also to express our gratitude to Pr P.P. Slonimski, Pr R. Wurmser, and Dr F. Labeyrie for interest and encouragement throughout this work ; and Mr B. Gendrault for very capable technical assistance. The work was supported by a grant of D. G. R. S. T. and by the Centre National de la Recherche Scientifique.

REFERENCES

(1) A. Curdel, L. Naslin, F. Labeyrie. Comptes Rendus. 249 (1959) 1959.
(2) M. Iwatsubo, A. Curdel. Biochem. Biophys. Res. Com. 6 (1962) 385.
(3) A. Curdel. Comptes Rendus. 254 (1962) 4 092.
(4) A. Curdel, F. Labeyrie. Biochem. Biophys. Res. Com. 4 (1961) 175.
(5) A. Curdel, (to be published).
(6) A. Curdel, F. Labeyrie, M. Iwatsubo. Communication to the Sixth Intern. Cong. of Biochem., New York, 1964.
(7) P. Galzy, P. P. Slonimski, Comptes Rendus. 245 (1957) 2 423.
(8) M. Somlo. (manuscript in preparation).
(9) P. M. Nossal. Australian J. Exptl. Biol. Med. Sci. 31 (1953) 583.
(10) D. Bertrand, A. de Wolf. Comptes Rendus. 245 (1957) 1 179.
(11) C. A. Price. Biochem. J. 82 (1961) 61.