Zn IN YEAST D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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GAPD*-s isolated from mammalian muscle or from yeast are Zn-proteins (Vallee et al. 1956,Keleti et al. 1962).Zn in the mammalian enzyme is essential for enzymic activity as well as for the maintenance of the native steric structure (Keleti and Telegdi 1959,Keleti et al. 1962).In the native enzyme isolated from mammalian muscle Zn cannot be exchanged with ^{65}Zn *in vitro*.But after the modification of the steric structure of the enzyme by blocking some of its SH groups with PCMB,Zn becomes exchangeable (Keleti 1964). After blocking all of the SH groups with PCMB (Keleti 1964) or with Ag ions (Keleti and Boross 1966) the protein becomes unable to bind Zn ions.

In the present paper we report the incorporation *in vivo* as well as the exchangeability *in vitro* of Zn in GAPD isolated from yeast.

3 kg of yeast were cultivated in 6 lit. fully synthetic culture medium containing 4.5 mC of ^{65}Zn isotope,at 20°C,during 3 days.The incorporation of ^{65}Zn in yeast cells was followed by measuring the decrease of radioactivity in the medium.The yeast cells were separated from the medium when 75 per cent of the initial radioactivity was taken up.

*Abbreviations used:GAPD=D-glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD oxidoreductase,phosphorylating,EC.1.2.1.12); PCMB=p-(chloro)mercuri-benzoate;ADH=alcohol dehydrogenase (EC.1.1.1.1)

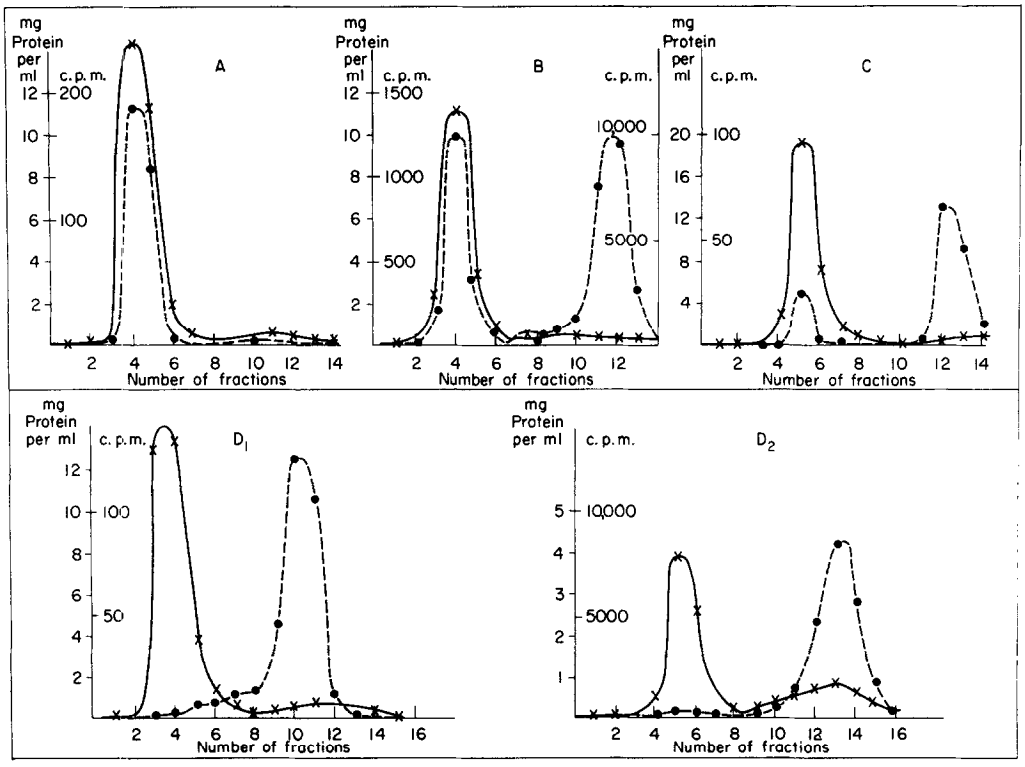

Fig.1. In vitro exchange of Zn in yeast GAPD

Solid line, protein content as measured by the absorbancy at 280mμ*. Dotted line, radioactivity in counts per min. The incubation medium was 0.1 M glycine buffer, pH 8.5. Incubation at +4°C, during 12-16 hours. After incubation the samples were gel-filtered on a Sephadex G-50 (fine) column (8 g) with 0.1 M glycine buffer, pH 8.5, 10-20 minutes after treatment with 200 eq. of cysteine.

A: Gel-filtration profile of GAPD containing *in vivo* incorporated ^{65}Zn. 3 ml of 59.1 mg/ml protein solution was gel-filtered.

B: Gel-filtration profile of GAPD incubated with ^{65}Zn. 3 ml of 27.5 mg/ml protein solution was incubated in the medium containing 1 ml of 0.28 mg/ml $^{65}ZnCl_2$ solution, 7.73 μC/ml. The radioactivity in the low molecular weight fractions is referred to the right ordinate.

C: Gel-filtration profile of GAPD containing *in vivo* incorporated ^{65}Zn, incubated with non radioactive Zn. 3 ml of 50.8 mg/ml protein solution was incubated in the medium containing 0.2 ml of 2.8 mg/ml $ZnCl_2$ solution.

D: Gel-filtration profiles of GAPD-s blocked with 7 eq. of PCMB.

D_1: GAPD containing *in vivo* incorporated ^{65}Zn, incubated with non radioactive Zn. 3 ml of 59.4 mg/ml protein solution was incubated in the medium containing 0.2 ml of 2.8 mg/ml $ZnCl_2$ solution.

D_2: GAPD incubated with ^{65}Zn. 3 ml of 14.6 mg/ml protein solution was incubated in the medium containing 1 ml of 0.28 mg/ml $^{65}ZnCl_2$ solution, 7.73 μC/ml.

*The second minor peak of the solid line in the low molecular weight fractions is a non-protein component of yeast GAPD which has maximum absorbancy at 260 mμ as described earlier (Boross et al. 1960). The previous removal from the enzyme of this component does not effect the exchange of bound Zn *in vitro*.

GAPD was isolated according to the method of Krebs (1955). During the isolation procedure and recrystallizations of the enzyme the specific radioactivity of the crude extract increases 4.75 fold (despite the removal of ^{65}Zn not incorporated in proteins and of different Zn-proteins, e.g. ADH).

The three times recrystallized enzyme was gel-filtered and as Fig.1.A. shows the protein peak contained radioactivity while no radioactivity was detected in the low molecular weight fractions. Thus ^{65}Zn is bound to the protein in a non dializable form.

In the next series of experiments we examined whether the firmly bound Zn of yeast GAPD is exchangeable with Zn in solution. We incubated non-labelled yeast GAPD for 12-14 hours in a solution containing ^{65}Zn. After gel-filtration the protein fractions contained radioactivity and the Zn content amounted to 2.7 atoms per mole enzyme (molecular weight taken as 140 000) (Fig.1.B.). On the other hand, if GAPD containing *in vivo* incorporated ^{65}Zn was incubated in a solution containing non radioactive Zn, the gel-filtered protein contains only 0.5 to 0.7 ^{65}Zn atom per mole of protein (Fig.1.C.).

These experiments indicate that a real exchange between Zn in the medium and that in the enzyme occured.

Blocking the SH groups of the enzyme with 2 mole equivalents of PCMB or $AgNO_3$ did not alter the exchangeability in either case (2.4 Zn atom per mole of protein).

If, however, all of the SH groups (i.e. 6 to 7 moles per mole of protein as determined with the spectrophotometric method) are blocked with

PCMB or Ag ion, yeast GAPD is no longer able to bind Zn. Following mercaptidation GAPD incubated in a medium containing ^{65}Zn, as well as the enzyme containing *in vivo* incorporated ^{65}Zn and incubated in a solution containing non radioactive Zn, did not contain any radioactivity (Fig. 1. D.).

The present results show that the firmly bound Zn in yeast GAPD can be exchanged even in the native state of the protein. Binding of Zn in mammalian GAPD seems to be much tighter since Zn cannot be exchanged without modification of its native steric structure (Keleti 1964).

The fact that fully mercaptidated yeast GAPD cannot bind Zn may be accounted for either by the role of some SH groups in the linkage or by a gross alteration of the steric structure of the enzyme.

The number of Zn atoms bound to the enzyme confirms our previous analytical results on the Zn content of yeast GAPD (Keleti et al. 1962).

References

Boross, L., Keleti, T., Telegdi, M.: Acta Physiol. Hung. 17 153 (1960)
Keleti, T.: Biochim. Biophys. Acta 89 422 (1964)
Keleti, T., Boross, L.: Acta Biophys. Biochim. Hung. 1 (1966) in press
Keleti, T., Györgyi, S., Telegdi, M., Zaluska, H.: Acta Physiol. Hung. 22 11 (1962)
Keleti, T., Telegdi, M.: Acta Physiol. Hung. 15 281 (1959)
Krebs, E.G.: in Colowick, S.P., Kaplan, N.O.: Methods in Enzymology. Vol. 1. Acad. Press, New York. p. 407 (1955)
Vallee, B.L., Hoch, F.L., Adelstein, S.J., Wacker, W.E.C.: J. Am. Chem. Soc. 78 5879 (1956)