ON THE ZINC CONTENT OF HORSE LIVER ALCOHOL DEHYDROGENASE

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In 1955 Theorell et al., prompted by the discovery by Vallee and Hoch (1955) that yeast alcohol dehydrogenase contains four atoms of zinc per molecule, analyzed horse liver alcohol dehydrogenase (LADH) for metal content. They found (in cooperation with Dr. W. Rutter and Dr. B.L. Vallee) LADH to contain two atoms of zinc per molecule, in agreement with the two molecules of coenzyme that can be bound to the enzyme. Later, Vallee and Hoch (1956) confirmed their results and, through inhibition by metal chelating compounds, could show that zinc in all probability was an enzymatically functional part of the enzyme.

This conclusion is now well established (see e.g. review by Sund and Theorell, 1963) and there is hardly any doubt that there are two enzymatically active zinc atoms in LADH. As regards the total zinc content, all analyses reported so far (Vallee and Hoch, 1957; Vallee, Coombs and Williams, 1958; Vallee, Williams and Hoch, 1959) have given values ranging between 2.12 and 2.43 atoms of zinc per molecule of LADH, based on a molecular weight of the latter of 84,000.

We have now considerable evidence, however, that pure LADH contains a total of four atoms of zinc per molecule. In connection with structural work on LADH going on in this laboratory we attempted to exchange the native zinc for other metals, starting with radioactive zinc. By careful dialysis at pH 6.5 against ^{65}Zn-chloride in tris buffer two

zinc atoms could be exchanged for ^{65}Zn. A similar exchange of two zinc atoms was recently reported by Li and Drum (1964). On total zinc analysis, using the dithizone method developed by Vallee and Gibson (1948), we found to our surprise that even after prolonged dialysis against 10^{-4} M EDTA at pH 7.3 there were four atoms of zinc per molecule of LADH. Attempts to exchange the zinc atoms for cobalt or cadmium, using the same technique, were unsuccessful: cobalt could be completely removed from the enzyme by dialysis against EDTA while cadmium caused precipitation and could not be completely removed. In both cases, however, the analyses still showed the presence of four atoms of zinc per molecule of enzyme.

After these findings we started to analyze our pure LADH-preparations routinely for zinc, checking the analytical accuracy by addition of internal standards and in one instance by neutron activation analysis on the same sample. The zinc analyses of seven different preparations, including one commercial one, are given in Table I. All analyses, except the one of preparation 5a, were made in duplicate or triplicate.

It has not been possible to remove any of the four zinc atoms without a concomitant loss of activity. Dialysis against 0.1 M phosphate buffer, pH 5.5 at 0°C for 40 hours, using zinc-free solutions and vessels, as well as prolonged dialysis against 10^{-4} M EDTA at pH 7.3, did not remove any zinc from the enzyme. Below pH 5.5, in phosphate buffer, there is an abrupt decrease of activity with pH. After 20 hours dialysis at 0°C against 10^{-4} M EDTA in phosphate buffer, pH 6.8, about 25% of the activity is lost. Dialysis for 20 hours at 0°C against phosphate buffer, pH 6.0, with 10^{-4} M EDTA, led to an 80% loss of activity while the zinc content decreased to 1.95 atoms per molecule of enzyme.

Table I

Determination of Zinc in LADH

No. of preparation	Method of preparation	Gr atoms of Zn per mole of LADH	
		Chemical analysis	Activation analysis
1[x]	Dalziel(1958)	4.18 ± 0.06	
2[x]	Dalziel(1958)	4.10 ± 0.20	
3[xx]	Bonnichsen and Brink (1955)	4.03 ± 0.06	
4[x]	Dalziel(1958)	4.02 ± 0.28	
5	Dalziel(1961)	4.04 ± 0.13	
5a[xxx]		4.22	
6	Dalziel(1958)	4.16 ± 0.16	
7[x]	Dalziel(1958)	4.25 ± 0.10	4.22 ± 0.13

[x] Chromatographic step omitted.

[xx] Commercial preparation from Worthington Biochem. Corp., Freehold, New Jersey.

[xxx] Same preparation as 5 but a small (a few per cent), also active component (see Dalziel, 1958, 1961), removed by free electrophoresis.

It is difficult to interpret these results in any way other than that all four atoms of zinc are essential components of the enzyme molecule. Two of these, probably the exchangable ones, are enzymatically functional. The other two perhaps contribute to the maintenance of the tertiary structure of the protein molecule.

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