THE ZINC CONTENT OF RHODANESE

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SUMMARY

The binding of zinc ion to bovine beef liver rhodanese has been investigated by nuclear magnetic resonance and emission spectroscopic methods. One equivalent of zinc ion is found to bind strongly to the enzyme; however, zinc is absent in the fully active native enzyme and the addition of zinc ion does not enhance catalytic activity. It is thus unlikely that zinc ion can serve as the cationic site in the enzyme catalyzed cleavage of the sulfur-sulfur bond of the substrate.

Rhodanese (thiosulfate:cyanide sulfur transferase E.C.2.8.1.1) catalyzes the transfer of the planetary sulfur atom of thiosulfate to cyanide by a double displacement mechanism which includes a sulfur substituted enzyme intermediate (1-4). Solvent and salt perturbation studies (5) indicate the presence of a cationic site for thiosulfate binding and enzyme kinetics studies are consistent with a partial neutralization of charge during this binding process. From polarographic and atomic absorption metal ion analyses of dialysates of the enzyme against 0.2 N HCl, Volini et al., reported the presence of one equivalent of zinc per mole of enzyme (6). In the same communication the authors stated that the enzyme may be prepared zinc free, in a note added in proof. However, Leininger and Westley (4) subsequently proposed that the zinc ion could function in the catalytic mechanism as the cationic binding site for thiosulfate and thus account for the partial charge neutralization suggested by the solvent and salt perturbation studies. Their discussion about the catalytic function of zinc would seem to be irrelevant if the enzyme is active in the absence of zinc as intimated in reference (6). This communication quantitatively confirms the results suggested in reference (6), and demonstrates the presence of one strong

site that is not directly linked to the catalytic mechanism.

The interaction of zinc ion with enzymes is difficult to observe optically, however, chlorine-35 nuclear magnetic resonance has successfully been used to study zinc in association with proteins (7,8). In the case where there is a labile equilibrium between chloride ions and an enzyme-bound zinc atom, a large increase in the chlorine-35 nmr line width is expected. In general the chlorine-35 line width, $\Delta\nu_T$, will be given by an equation of the form

$$\Delta\nu_T = P_f\Delta\nu_f + P_e\Delta\nu_e + P_m\Delta\nu_m$$

where P_f is the probability that the observed chlorine nucleus is free in solution and $\Delta\nu_f$ is its corresponding line width, P_m is the probability that the chlorine ion is bound to the metal on the enzyme, $\Delta\nu_m$ its line width at the metal site, P_e is the probability that the chloride ion is bound to the enzyme in a non-specific way and $\Delta\nu_e$ is the corresponding line width at that site or sites. For the discussion presented in this communication, it is important to notice that the line width is directly proportional to the concentration of the metal-enzyme complex.

Experimental: Nuclear magnetic resonance measurements were made on a modified Varian DP-60 spectrometer system equipped with a V-4210 variable frequency r.f. unit operating at 5.6 MHz and a Princeton Applied Research Model 121 lock-in amplifier operating at 500 Hz with the phases adjusted to display the first audio side band of the absorption signal. Line widths reported are full widths measured at half height of the absorption mode signal. Indicated errors are standard deviations of the mean for at least five spectra.

The enzyme preparation was purchased as electrophoretically homogeneous purified powder from Sigma Chemical Company. The enzyme preparation was fully active (specific activity of 220 units per mg as defined by Sorbo (1)). All other chemicals used were 'Baker' reagent grade. All substrates were checked for zinc ion contamination by emission spectroscopy. Zinc solutions were prepared by dissolution of the metal. Enzyme concentrations

were determined spectrophotometrically using an absorptivity of 1.75 cm^2mg^{-1} at 280 nm (9). A molecular weight of 19,000 was assumed for the protein (6).

Trace metal analyses: Samples of the enzyme were analyzed for the presence of metal ions by both atomic absorption and emission (D.C. Arc) spectrography at two separate laboratories (10).

TABLE I

Metal Ion Content of Beef Liver Rhodanese

Metal Ion	Moles metal ion / Mole of protein
Al	0.014
Ca	0.015
Cu	0.008
Fe	0.021
Mg	0.008
Na	0.049
Pb	0.046
Si	0.003
Zn	<< 0.003

Results and Discussion: Preliminary nmr measurements cast some doubt on the presence of zinc in the enzyme at the levels first reported. Metal analysis of the enzyme preparation by atomic absorption and emission are summarized in Table I. Dialysis of the enzyme against sodium acetate buffer prior to the lyophilization step in the preparation accounts for the presence of sodium ions (11). These data indicate that the fully active enzyme preparation contains metal ions only at trace levels.

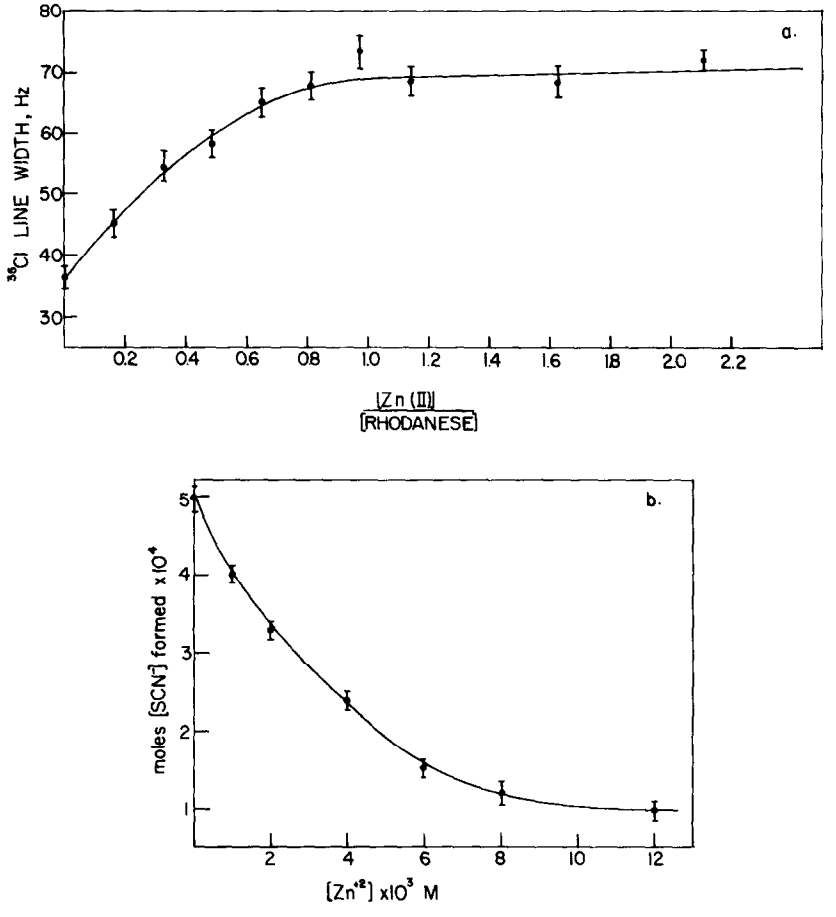

Fig. 1a. ^{35}Cl line width plotted as a function of the molar concentration ratio of zinc ion to the total protein. pH 6.5, 0.05 M phosphate 0.5 M sodium chloride and 25°C.

1b. Enzyme activity in terms of moles of SCN^- produced is plotted vs. zinc concentration. pH 6.5, 0.05 M phosphate, 0.1 M sodium chloride, 25°C. Enzyme concentration was 2.5×10^{-6} M.

In order to observe the interaction of zinc with rhodanese, a 2.46×10^{-4} M solution of the enzyme in 0.5 M sodium chloride buffered at pH 6.5 with 0.05 M phosphate was titrated with zinc chloride and the binding of the metal followed by measuring the chlorine-35 nmr relaxation time. The results are shown in Fig. 1a. The data demonstrate the presence of one strong zinc binding site on the enzyme in addition to a non-specific chloride interaction. To determine whether zinc ion affected the enzyme reaction, enzyme activity was

measured as a function of zinc concentration. The rate of formation of thiocyanate from cyanide and thiosulfate, catalyzed by rhodanese was measured at 25° C and pH 6.5 in 0.05 M phosphate buffer made 0.1 M in sodium chloride. Equimolar solutions (2×10^{-2} M) of both reactants were mixed in the presence of 2.5×10^{-6} M enzyme and allowed to react for ten minutes. The reaction was quenched by the addition of formaldehyde and the thiocyanate was assayed by recording the absorbance of the iron complex at 460 nm on a Cary 15 spectrophotometer according to the method of Sorbo (1). The zinc concentration was varried from 0 to 12×10^{-3} M. The results are presented in Fig. 1b. The zinc-free enzyme samples demonstrated normal enzyme activity; however, at high levels of zinc as much as 80% inhibition of the enzyme reaction is observed. The apparent equilibrium constant for the zinc binding reaction is estimated to be 5×10^{2}. Calorimetric measurements (12) of the zinc binding constant are in excellent agreement with this value.

The data reported here demonstrate that zinc is not required for full enzymic activity, but that there is one strong zinc binding site on the enzyme. The data also indicate that high levels of zinc cause inhibition of the enzyme reaction studied.

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