hance the catalytic activity of DPO as assayed with L-epinephrine. In some experiments we obtained no stimulation, or even inhibition, of the enzymatic activity at a cation concentration ten times higher than that used for Table I.

There are no marked differences when L- or D-DOPA are used as substrates (results not shown).

Studies are in progress in order to better characterize the DPO enzyme.

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Reaction of Copper–Zinc Superoxide Dismutase with Hydrogen Peroxide: a Possible Source of Heterogeneity?

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The reaction of hydrogen peroxide with copper–zinc bovine superoxide dismutase at ratios of 0.2 to 4.0 per active site at pH 10.0 results in the formation of distinct electrophoretic forms of the enzyme. The mobilities of these forms are identical to the mobilities of the forms present in heterogeneous, untreated native samples in two different electrophoretic systems, pH 6.96 (TRIS-acetic acid) and pH 8.38 (TRIS-glycine) (Fig. 1). Increasing ratios of hydrogen peroxide result in an apparent increase in this heterogeneity in addition to progressive inactivation (Fig. 2). Inactivation of the dismutase and loss of histidine was reported by Hodgson and Fridovich [1]. Furthermore this reaction results in the loss of copper from the dismutase at all ratios of added peroxide and the loss of zinc at the higher ratios (Table I). The loss of copper parallels the increase in the faster moving species suggesting the possibility that this species is copper-deficient. However, in the pH 6.96 electrophoretic system, the mobility of this faster form does not coincide with the mobility of a copper-deficient form prepared by reconstituting the apoenzyme with equimolar copper and zinc [2]. If it is copper-deficient, the faster electrophoretic form must be modified by the reaction with peroxide. It should be noted that this form is not capable of giving rise to native enzyme upon the addition of copper and zinc. In addition the reaction with peroxide forms distinct electrophoretic forms of the enzyme at the more physiological pH 7.0. Analysis of several purified preparations of superoxide dismutase have indicated that a

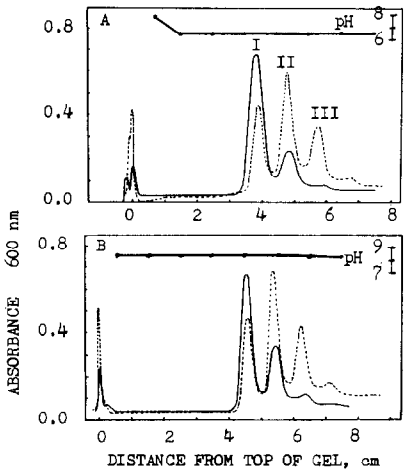

Fig. 1. Scans of polyacrylamide gels of native enzyme (——) and peroxide-treated enzyme (- - - -) (2.2 per site). (A) pH 6.96 0.022 *M* TRIS–0.02 *M* acetic acid; (B) pH 8.38 0.05 *M* TRIS–0.38 *M* glycine.

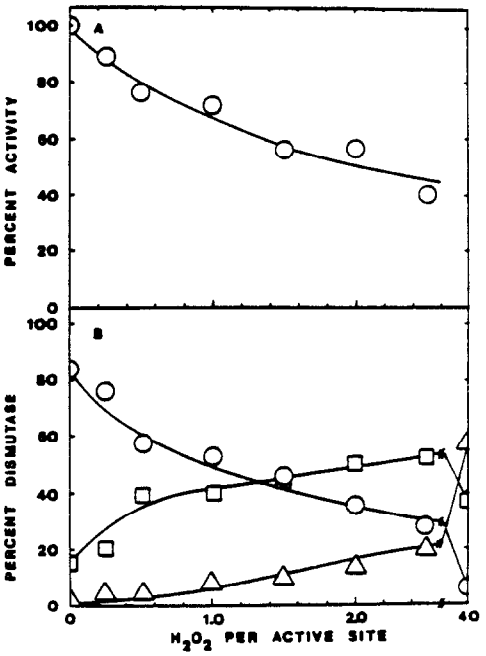

Fig. 2. (A) Percent dismutase activity remaining in the reaction with hydrogen peroxide at various ratios; (B) Percent dismutase species as a function of added peroxide: —○—, I; —□—, II; and —△—, III.

lower copper content is correlated with an increased heterogeneity (Table II). For all samples listed but one, this heterogeneity was not altered by the addition of copper and zinc.

In view of these observations we would like to suggest that the reaction with hydrogen peroxide *in vivo* may be the source of the heterogeneity so often seen in purified preparations of the copper–zinc enzyme. Hydrogen peroxide is the product of the enzyme-catalyzed dismutation reaction of superoxide anion and its reaction with the dismutase, though slow, could lead to the accumulation of altered proteins

TABLE I. Loss of Copper and Zinc from Superoxide Dismutase in the Reaction with Hydrogen Peroxide at pH 10.0.

H_2O_2	Copper	Zinc
site	Enzyme	Enzyme
0.0	1.30	1.92
0.49	1.23	1.94
0.98	1.18	1.69
1.46	1.16	1.79
1.96	1.00	1.23
2.96	0.87	–
3.96	0.85	1.08

TABLE II. Copper Content and Heterogeneity in Preparations of Bovine Superoxide Dismutase.

Sample	Copper	% I	% II	% III
	Enzyme			
Sigma [1]	1.89	83	15	2
DDI	1.83	83	16	1
Sigma [2]	1.75	66	29	5
DDI	1.61	71	24	5
Malta	1.42	40	58	2
Sigma [2] dialyzed	1.30	52	34	14

that copurify with the native enzyme in classical isolation procedures.

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Mixed Liganded Species in Native β-Helix *p*-Hemocyanin

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It is known that addition of low concentrations of cyanide to oxy hemocyanin causes a rapid bleaching of the absorption band, which has been related to the displacement of oxygen [1–3]. Removal of copper is known to require higher concentrations of cyanide and occurs with different kinetics in the presence or absence of other ligands at the site [4, 5].

In this communication, preliminary results of a study concerning the competition between O_2, CO and CN^- for the active site of Helix *p*-hemocyanin are reported. The replacement of these ligands occurs in different ways and may be related to the different modes of binding at the active site. In the case of oxygenated hemocyanin a hyperbolic displacement of oxygen by cyanide is observed, the apparent partition coefficient being independent of hemocyanin concentration in the range 10–50 μM. In the case of CO displacement by cyanide, the shape and position of the curve depend on protein concentration and at higher hemocyanin concentration a 'lag-phase' followed by a hyperbolic displacement is observed.

This behaviour has been tentatively interpreted assuming that at low concentrations of cyanide binding of the ligand to CO-saturated hemocyanin may occur to one of the two metals in a site, while CO remains bound to the other. The results of experiments in which the addition of small amounts of KNC to hemocyanin partially saturated with O_2 and CO causes an increase of CO-emission intensity and a concomitant decrease of O_2–copper absorption band are fully consistent with this hypothesis. The mixed-liganded species displays luminescence properties similar to those of CO-saturated hemocyanin [6] and the formation of the complex is reversible on dialysis or oxygenation. Since mixed-liganded intermediates have also been observed in the case of half-met hemocyanin, in which one of the metals is cupric [7], it is suggested that the geometries of the active site of such species and of native hemocyanin may be quite similar.

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