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

H_2O_2 / site	Copper / Enzyme	Zinc / 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 / Enzyme	% I	% II	% III
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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