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Adducts of Co(II) and Cu(II) Bovine Carbonic Anhydrase with Bidentate Ligands

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The Zn(II) ion of bovine carbonic anhydrase can be substituted with Co(II), a useful spectroscopic probe, without loss of enzymic activity. The Co(II)-enzyme binds bidentate anionic ligands such as oxalate [1], 2-pyridinecarboxylate [2] and N,N-diethyldithiocarbamate [3], giving pentacoordinate metal derivatives. The ligand displaces the water molecule bound to the metal in the unreacted enzyme, causing a decrease in the ^1H NMR relaxation rate to about the same value as in the native diamagnetic Zn(II)-enzyme.

The Cu(II)-substituted bovine carbonic anhydrase has no catalytic activity, but is able to bind the same type of ligands as the Co(II)-enzyme. The ^1H NMR relaxation rate of the Cu(II)-enzyme, which is almost unaffected by binding of monodentate anions [4], is decreased by binding of the bidentate ones [1, 3, 4]. The substantial residual relaxivity, about 20% that of the unreacted enzyme, may indicate that water coordination is retained in these adducts as it is in the case of monodentate anions, the decrease being related to a different geometry, involving a longer Cu–O distance. EPR spectra of the adducts with bidentate ligands are compatible with a hexacoordinate tetragonal geometry.

The spectroscopic properties of the bicarbonato-derivatives of both Co(II) [1] and Cu(II) [5] enzymes suggest that bicarbonate, the real enzyme substrate, does behave as a bidentate ligand.

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Role of Metal Ions to Specific Binding of Yeast Alcohol Dehydrogenase by Free and Immobilized Dyes

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A publication recently revealed that metal ions promote the binding of proteins to a number of immobilized triazine dye affinity adsorbents [1]. This paper reports that metal ions of the first row transition series in the system of free or immobilized dye and yeast alcohol dehydrogenase (ADH) have not only promoted but induced effect on the specific binding of enzyme to dye.

Dye–metal ion dissociation constants K_d are determined by difference spectroscopy [1] of reactive yellow 2KT (Y) and light resistant yellow 2KT (LRY). In the latter case as shown in Table I the order of stability for free dye complexes correlates well (except the Cd^{2+}) with the relative stability of immobilized dye complexes which are determined chromatographically as pH decomplexation (DpH) following [2].

The CD spectra of Y and LRY free of metal ions with ADH are not detected. The CD spectrum is induced by LRY in the presence of Cu^{2+} and Zn^{2+} , but not in the presence of Mn^{2+} and Ni^{2+} with ADH. When the coenzyme NAD was added to any of the ADH–dye–metal complexes the CD spectra decreased. The results of CD are in good agreement with ADH chromatography on LRY adsorbent. ADH (7.2 U/mg) can be specifically eluted with

TABLE I. Dye–Metal Ion Dissociation Constants K_d^a , CD Spectra Maximum of Dye–Metal–ADH Complexes^b and DpH of Metal Loaded LRY Affinity Adsorbents.^c

Metal ion	K_d	CD max	K_d	CD max	DpH
	(mM)	(nm)	(mM)	(nm)	
	Light resistance yellow 2KT		Yellow 2KT		
–	–	n.s.ch.	–	n.s.ch.	–
Cu^{2+}	0.084	+480	0.841	+390	1.80
Cd^{2+}	0.061	n.d.	n.s.ch.	n.d.	2.80
Zn^{2+}	0.098	+480	1.390	n.s.ch.	3.27
Ni^{2+}	0.105	n.s.ch.	n.d.	n.d.	3.85
Mn^{2+}	0.568	n.s.ch.	n.s.ch.	n.d.	4.05

^a K_d is determined at 25 °C in 10 mM tris-HCl buffer. ^bCD spectra were run at 25 °C on JASCO J-20 spectropolarimeter, ADH 'Boehringer'. ^cDye immobilized on Sepharose Cl-6B [3]; n.d., not determined; n.s.ch. no spectral change.