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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 ^{1}H 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 ^{1}H 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 (m*M*)	CD max (nm)	K_d (m*M*)	CD max (nm)	DpH
	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 m*M* tris-HCl buffer. [b] CD spectra were run at 25 °C on JASCO J-20 spectropolarimeter, ADH 'Boehringer'. [c] Dye immobilized on Sepharose Cl-6B [3]; n.d., not determined; n.s.ch. no spectral change.

NAD from the adsorbent loaded by Cu^{2+}, Cd^{2+}, Zn^{2+} (purification factor subsequently – 15, 20, 17, yield – 56–68%) but not Ni^{2+}, Mn^{2+} (yield 2.2 and 15.8%) or Co^{2+}, Fe^{3+} (not eluted). This study supports the idea that the interaction of such metal ions as Cu^{2+} and Zn^{2+} (Cd^{2+}) with LRY chromophore (in contrast to the metal ions Mn^{2+} and Ni^{2+}) stabilizes a particular conformation of dye that is sterically acceptable to the NAD-binding site in ADH.

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EPR Studies of Ribulose-1,5-bisphosphate Carboxylase/Oxygenase Activated With Cu^{2+}

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Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase catalyzes the primary reaction in two metabolic pathways in plants.

A: in CO_2fixation:

RuBP + CO_2 → 2,3-phosphoglyceric acid (PGA)

B: In photorespiration:

RuBP + O_2 → PGA + phosphoglycolate

In the carboxylase reaction a 6-carbon intermediate has been identified. In the oxygenase reaction a 5-carbon peroxointermediate has been suggested. However, there is little experimental evidence for its existence [1].

RuBP carboxylase/oxygenase requires a divalent metal ion for activity. The metal ion stabilizes the carbamate formed between the enzyme and a CO_2 molecule in the activation process [2]. Whether the metal also has a function during catalysis is not clear. Mg^{2+} is the normal activator *in vivo* but the enzyme is also activated by Fe^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+}.

Cu^{2+} gives little activity and has not been much studied in carboxylase research. Despite this, Cu^{2+} may be a useful paramagnetic probe.

Our results show that Cu^{2+} in the presence of HCO_3^- (50 m*M*) is specifically bound to the enzyme. The high field part of the EPR spectrum shows nitrogen hyperfine splitting indicating that the metal in the Enz-CO_2–Cu^{2+} complex has at least one nitrogen

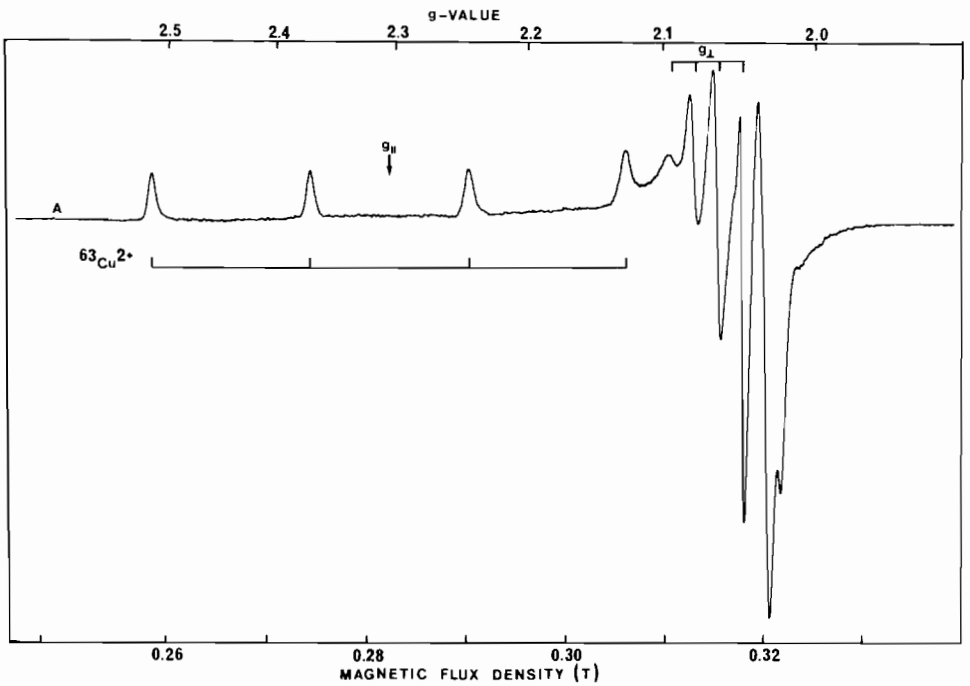

Fig. 1. Effect of addition of RuBP in the presence of HCO_3^- and Cu^{2+}. To 0.25 ml enzyme (41 mg/ml in 50 m*M* HEPPS buffer at pH 8.3) was added 0.015 ml $NaHCO_3$ (0.5 *M*) followed by 0.025 ml RuBP (0.1 *M*) and 0.010 ml $^{63}CuCl_2$ (12.5 m*M*). The spectrum was recorded at 77 K. The spectrometer gain was 1×10^5 and the modulation amplitude was 0.5 mT. From the spectrum the following parameters are obtained: $g_\parallel = 2.301$, $g_\perp = 2.055$, $A_\parallel = 15.75$ mT and $A_\perp = 2.39$ mT.