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Bisdipyridyl Cu(II) Iodide as a Pentacoordinate Model of the Cu(II) Bovine Carbonic Anhydrase Iodide Complex

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Bovine carbonic anhydrase (BCA) is a monomeric enzyme, molecular weight 29,000, which catalyzes the reversible hydration of CO_2 to H_2CO_3 [1]. It contains one Zn(II) ion bound to three histidyl residues and to one water molecule. The enzymic activity, which is almost unaffected by substitution of Zn(II) with Co(II), is completely lost by substitution with Cu(II). Nevertheless the Cu(II) enzyme retains many of the physical and chemical properties shown by the native and Co(II) enzymes (*cf.* ref. 2 and refs. therein). In a previous study [2] of these properties we suggested a pentacoordinate structure for the protein-bound metal in the halide derivatives of both Cu(II) and Co(II) BCA. This suggestion was further supported by an ESR study of Co(II) BCA iodide [3]. In the present study we attempted to gain more information on the structure of Cu(II) BCA iodide by comparing its spectroscopic properties with those of model bisdipyridyl Cu(II) halide complexes, $[Cu(bipy)_2X]^+$.

Results and Discussion

The crystal structure of $Cu(bipy)_2Cl_2 \cdot 6H_2O$ [4] and of $Cu(bipy)_2I_2$ [5] shows the copper ion to be in an approximately trigonal bipyramidal environment, with the two axial Cu–N bonds shorter than the two equatorial ones and one equatorial halide. The second halide is not bound to copper. In nitromethane or nitrobenzene solutions the complexes behave as uni–univalent electrolytes and their absorption spectra retain the essential features [6] of the diffuse reflectance spectra of powdered samples, indicating that a similar coordination is present.

Very similar spectra are also obtained in EtOH solution (Fig. 1 and Table I). The Cl^- complex can be prepared by mixing stoichiometric amounts of $CuCl_2$ and bipy in EtOH. The other halide complexes can be obtained by further addition of a concentrated aqueous solution of the salt, since the affinity increases in the order $Cl^- < Br^- < I^-$. The amount required to displace Cl^- is an approximately tenfold excess for Br^- and a two–threefold excess for I^-.

In Fig. 2 are reported the corresponding ESR spectra at the X-band in frozen EtOH. The relative parameters are reported in Table II; they were determined graphically, only in the highest and lowest regions of magnetic field, since the rhombic com-

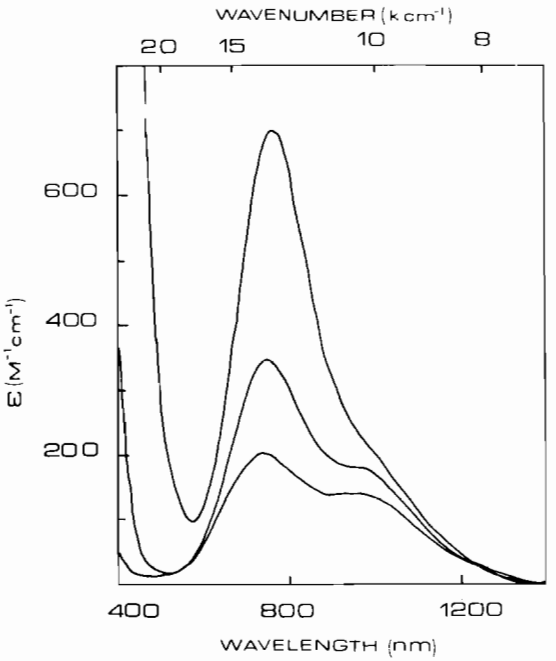

Figure 1. Optical absorption sepctra of 1.0 m*M* $Cu(bipy)_2Cl_2$ in EtOH (a); plus Na Br (b); plus NaI (c).

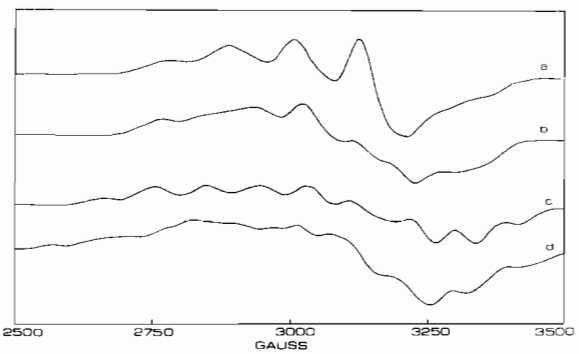

Figure 2. ESR spectra of 1.0 m*M* $Cu(bipy)_2Cl_2$ in EtOH at 100 °K (a); plus 6.0 m*M* HaBr (b); plus 2.0 m*M* NaI (c). 2.0 m*M* aqueous Cu(II)BCA plus 20 m*M* NaI (d). Microwave frequency 9.12 GHz, microwave power 20 mW, field modulation amplitude 10 Gauss.

TABLE I. Absorption Spectra of $[Cu(bipy)_2X]^+$ and Cu(II)BCA-I Complexes.

	Solvent	Charge Transfer Bands				d–d Bands	
		Λ	(ϵ)	λ	(ϵ)	λ	(ϵ)
$[Cu(bipy)_2Cl]^+$	EtOH	–	–	–	–	735	(200)
$[Cu(bipy)_2Br]^+$	EtOH	–	–	–	–	750	(350)
$[Cu(bipy)_2I]^+$	EtOH	350	(3500)	440	(1250)	770	(700)
$[Cu(bipy)_2I]^+$	H_2O	310[a]		405[a]		770[a]	
Cu(II)BCA-I		355	(3200)	445	(2800)	790	(400)

[a]From difference spectra; the molar extinction coefficient is omitted since complete formation could not be achieved.

TABLE II. ESR Parameters[a] of $[Cu(bipy)_2X]^+$ and Cu(II)BCA-I Complexes.

	$g_{H_{max}}$	$g_{H_{min}}$	$A_{H_{max}}$(Gauss)	$A_{H_{min}}$(Gauss)
$[Cu(bipy)_2I]^+$	1.97	2.32	79	95
$[Cu(bipy)_2Br]^+$	2.00	2.25	68	87
$[Cu(bipy)_2Cl]^+$	2.00	2.20	79	120
Cu(II)BCA-I	1.97		89	

[a]Approximate values obtained from graphical evalution.

ponent is not clearly resolved. They differ to some extent from those reported for the powdered [6] and single crystal [7] samples, but they still permit the assignment of a d_{z^2} ground state, consistent with a compressed trigonal bipyramidal coordination. In fact they show a very low value of the lowest g-factor, very near to that of the free electron [8, 9] and a small and similar absolute value of the hyperfine constants A_{11} and $A_\perp$ [10]. Very few complexes of this geometry have been studied in solution, they are of general formula $Cu(R_6tren)X_2$ (R_6tren = $R_2N(CH_2)_3N$, with R = H, CH_3, C_2H_5; X = OH, Cl, Br, I, ClO_4, NO_3); their ESR parameters are strictly comparable with those in Table II, *i.e.* the $g_\parallel$ value is lower than g_e especially for the iodide derivatives. This value cannot be explained by the perturbation theory $g_\parallel = g_e$; $g_\perp = g_e - 6r_1^2\lambda/E(^2\Gamma_8 - {}^2\Gamma_8)$ since the above equations [7] are not a good approximation when the ligand spin–orbit coupling constant is very large as in the case of the halogens [10]. Recently [11] it has been shown that the inclusion of the halogen spin–orbit coupling constant in a M.O. scheme allows a better approximation, since it causes a reduction of $g_\parallel$ below g_e and an increase of $g_\perp$. It can be noted that the trend of our $g_\perp$ values (Cl < Br < I) and $g_\parallel$ values (I < Br ≤ Cl) follows the order of the halogen spin–orbit coupling constant.

In Fig. 2 is also reported the ESR spectrum of Cu(II) BCA-I*. This spectrum is very complicated and not well resolved so that it cannot be readily assigned to any single species. However its shape is unaffected by changes of pH from 6 to 7.5, by changes in I^- concentration from 10^{-2} to 0.2 *M* (protein concentration 2×10^{-3}*M*) and by changes in temperature in the range −160 °C to +20 °C. This may indicate the presence of a slightly different iodide binding site in different protein molecules. The presence of two iodide binding sites was observed in $[Cu(Me_6tren)I]^+$ doped in the zinc isomorphous salt [11], which was expected to allow only a single site. The phenomenon, persisting in solution [11], may also account for the apparently rhombic ESR spectrum of $[Cu(bipy)_2I]^+$. The general shape of the Cu(II)BCA spectrum and its more resolved portion at higher field are very similar to that of $[Cu(bipy)_2I]^+$, so we propose for the enzyme a similar pentacoordinate structure, which is also supported by recent NMR data [12]. The optical spectra of the two iodide derivatives are also very similar, both in the d–d and in the charge-transfer region (see Table I). It may be noted that in H_2O as solvent the chargetransfer bands of $[Cu(bipy)_2I]^+$ are shifted to lower wavelength by about 40 nm and that the stability of the complex is considerably lower. Since the properties of the enzyme metal site are better simulated by $[Cu(bipy)_2I]^+$ in EtOH solution, the metal environment in the protein is likely to be considerably hydrophobic.

*The preparation of the enzyme and of its Cu(II) derivative is reported in Ref. 2.

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