Vol. 86, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1979 Pages 428-433

EPR NON-DETECTABLE COPPER IN PYRIDOXAL BIOGENIC AMINE SCHIFF BASE COMPLEXES

Patrick Sharrock[1], Françoise Nepveu-Juras[2],
Michel Massol[2] and Raymon Haran[3]

[1]Départment de chimie, Université de Sherbrooke, Sherbrooke, Québec, Canada J1K 2R1; [2] Laboratoire de chimie bioinorganique. Université Paul Sabatier, Toulouse, France, 31400; [3]Laboratoire de Chimie de Coordination, BP. 4142, Toulouse Cedex, France 31030

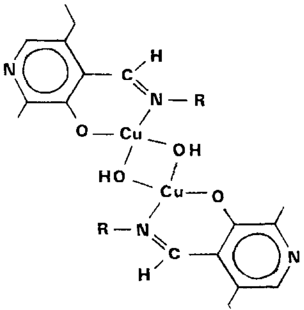
Received August 29, 1978

SUMMARY: Schiff bases formed with octopamine, pyridoxal and pyridoxal phosphate react with copper ions to give various pH-dependent species. The outstanding feature of these complexes is their absence of EPR spectra at physiological pH values. We propose dimeric dipolar coupled structures for the EPR non-detectable copper complexes, involving hydroxyde anions and vitamin B-6 Schiff bases. These results establish that EPR non-detectable copper in enzymes may arise from dipolar coupling between metal ions involved in Schiff base type complexes.

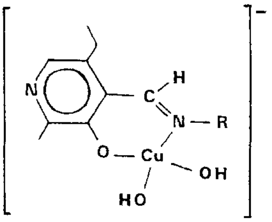
INTRODUCTION: In view of the well known pharmacological properties of biogenic amines, current interest has intensified on the molecular mechanisms of their activity. Even though little is known of the degradation processes for these amines, oxydative deamination may proceeed though enzymatic processes involving pyridoxal and metal ions, as in the case of plasma monoamine oxidases (1,2). Since the original in vitro observations by IKAWA and SNELL (3) of metal catalyzed pyridoxal dependent deamination of amino acids, research on metal complexes of such systems has been active (4,5,6). However, recent evidence points to the fact that monoamine oxidase in an iron (but no copper) dependent enzyme. Nevertheless, nutritional requirements for copper and pyridoxal phosphate have been established for diamine oxidase activity (7).

In any case, EPR can be used to study paramagnetic metals in enzymes and help understand the mechanism of their function, even when the metal is not an essential cofactor, as for example, copper in mito-

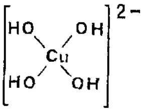
0006-291X/79/020428-06$01.00/0



I



II



III

chondrial monoamine oxidase (8) Copper (II) sites have been classified into three types based on their spectral properties (9), and recently, Copper (III) has been shown to be the catalytically active center in galactose oxidase (10). We now report EPR results on Copper (II) model complexes with unusual properties.

EXPERIMENTAL: Materials. Pyridoxal-5'-phosphate was purchased from Boehringer Mannheim. Pyridoxal (Merk) and DL-octopamine (Sigma) were used as the hydrochlorides. Spectra: EPR spectra were recorded on Varian E-9 and E109 spectrometers operating at X-band. The pH values of freshly prepared solutions were adjusted with concentrated sodium hydroxyde and measurements were performed with a flat .3 mm quartz cell (Varian E-248/v-4548).

RESULTS AND DISCUSSION: We have explored previously Schiff base formation between various amines and vitamin B-6 analogues by ^{13}C NMR (11,12). A preliminary U.V.-visible study of the pyridoxal-dopamine system revealed significant peak shifts upon addition of copper ions, characteristic of metal complex formation. The copper ion-dopamine system is further complicated by the rapid oxidation, under basic conditions, of the catechol moiety to give the corresponding dihydroquinone. In order to investigate Schiff base complex formation, we therefore chose the analogous copper octopamine system.

EPR spectra of .03 M Cu^{++}, - .03 M pyridoxal and -.03 M octopamine aqueous and H_2O-CH_3OH solutions, both at room temperature and 77^{o} K revealed a surprisingly broad and vanishing signal at neutral pH values. This system was not investigated at higher concentration due to gel formation upon standing, in the interval $5.5 < pH < 10.5$.

Figure 1 shows how the epr signal tends to change with pH from the featureless absorption of $Cu(H_2O)_6^{++}$ to the typical four line pattern of molecular copper complexes. However, the signal intensity progressively decreases with complex formation and becomes undetectable at neutral pH values. Normal signal intensity is restored in basic solutions.

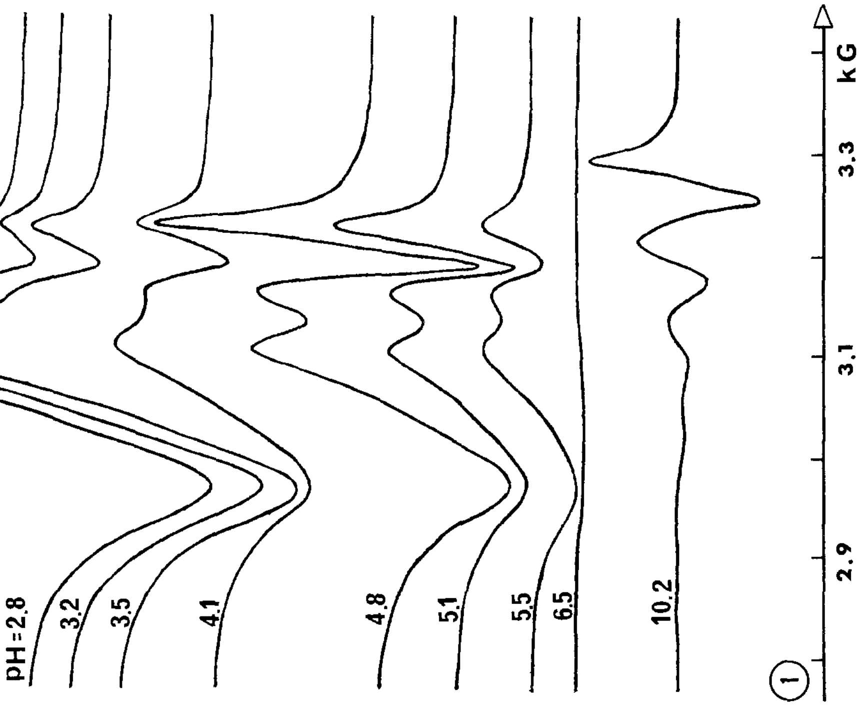
Pyridoxal phosphate forms more soluble Schiff bases, and the epr spectra of .03 M solutions containing equimolar amounts of copper and octopamine showed an unusual structure at neutral pH, reminiscent of the copper citrate system (13). Such spectra are typical of dipolar coupled copper pairs with $g_{//} < g_{\perp}$. As the pH increases, the intensity of this signal seems to increase, as shown in figure 2, but in fact, visual inspection of the spectra shows it is being replaced by another four-line spectrum which reaches its full intensity in very basic solutions.

We conclude from these facts that a dimeric or polymeric dipolar coupled copper complex exists in the pyridoxal-octopamine system. Other complexes are known to show spin coupling, even with large metal-metal distances, such as the hydrogen bonded copper (II) dimer of the diimine of acetylacetone and 2-aminoethanol (14). X-ray structure determination have established polymorphism in Schiff base Cu(II) complexes in the solid state, and the degree of association of such complexes in various

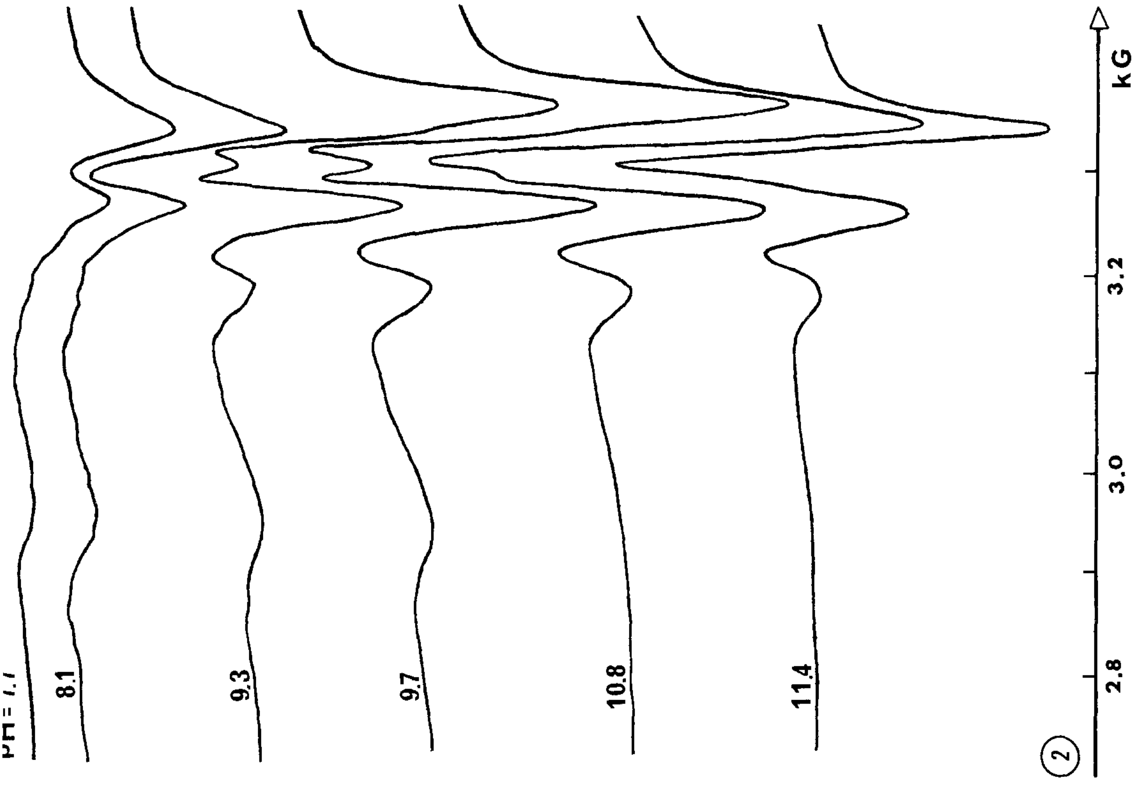
---

Figure 1. Variation with pH of the EPR spectrum of the copper-pyridoxal-octapamine system. $\nu = 9.438$GHz. At pH = 2.8, $g_0 = 2.19$; pH = 10.2, $g_0 = 2.14$, $A_0 = 80$ G.

Figure 2. Variation with pH of the EPR spectrum of the copper-pyridoxal phosphate-octapamine system. $\nu = 9.460$GHz. At pH = 11.4, $g_0 = 2.11$, $A_0 = 86$ G.



①



②

solvents has been researched previously (15,16). Therefore we can propose for our complexes several possible polynuclear structures involving the Schiff base ligands. Recent work suggests that the carbinolamine intermediate in Schiff base formation may be stabilized by Cu^{++} ions, especially under basic conditions (17). Another possibility is the formation of hydroxy bridged species I which would dissociate under basic conditions either to a square planar copper Schiff base dihydroxy anion II or further to a copper hydroxyde anion III (18). The g values obtained (see legends to figures), are close to those recently reported for $Cu(OH)_4^{2-}$ and $Cu(OCH_2\text{-}CH_2O)_2^{2-}$ (19).

CONCLUSION: The exact nature of supposedly dimeric copper complexes with biologically important ligands is of current interest, as two types of copper enzymes can be modelled by such systems (9): magnetically coupled dimers account for the epr non-detectable type III copper, and mixed valence Cu(I)-Cu(II) dimers offer promising possibilities for studying the unusual properties of blue copper proteins (the so-called type I copper (20). Recently, magnetic studies on exchange-coupled copper dimers of small peptide-like amino acids were reported (21) and shown to be solvent-dependent. The results herein reported show that the magnetic properties of copper (II) can be greatly influenced by pH. Therefore, following copper by EPR spectroscopy in such enzymes as monamine oxidases where its function is questionned will remain a delicate problem.

ACKNOWLEDGMENTS: Thanks are due to Mrs. D. Olivier, of Dr. Che's laboratory, Université Paris VI, for help in obtaining EPR spectra on the Varina E109. P.S. is thankful for a post-doctoral fellowship from the Ministère de l'Education du Gouvernement du Québec.

REFERENCES.

1. YAMADA, H. and YASUNOBU, K.T. (1962) J. Biol. Chem., 237, 3077.
2. BUFFONI, F. and BLASCHKO, H. (1964) Proc. R. Soc. Lond. B. Biol. Sci. 161, 153.

3. IKAWA, M. and SNELL, E.E.,(1954) J. Am. Chem. Soc., 76, 4900.

4. HOLM, R.H. (1973) Inorganic Biochemistry, G.L. Eichorn (Ed.), Elsevier, vol. 2, 1137.

5. RAJAN, K.S.; SKRIPKUS, A.; MARKS, G.E. and DAVIS, J.M. (1976) Bioinorg. Chem., 6, 93.

6. PURDY, E.R. (1976), Copper Complexes of Schiff Bases Derived from Biologically Active Amines. S.U.N.Y. at Stony Brook, Ph.D.; see also Diss. Abstr. Int., B37, 3406 (1977).

7. SOURKES, T. and MISSALA, K. (1976). "Monoamine oxidase and its inhibition", Ciba Foundation Symposium 39, Elsevier, Excerpta Medica, p. 83.

8. SOURKES, T. (1972) Pharmacol. Rev., 24, 349.

9. OCHIAI, E.I. (1977), Bioinorganic Chemistry, an introduction, Allyn and Bacon, Toronto, Chap. 9, 213.

10. HAMILTON, G., ADOLF, P.,de JERSEY, J., DUBOIS, G., DYRKACZ, G. and DANIEL LIBBY, R., (1978), J. Am. Chem. Soc., 100, 1899.

11. NEPVEU-JURAS, F., Thèse de Doctorat de troisième cycle, (1977), Toulouse, France.

12. HARAN, R., MASSOL, M. and NEPVEU-JURAS, F. La vitame B_6 et ses dérivés. III. Etude par RMN ^{13}C de systèmes fournis par le phosphate de pyrodoxal ou le pyrodoxal avec l'octopamine. Submitted.

13. BOAS, J.F.; DUNHILL, R.H.; PILBROW, J.R.; SRIVASTAVA, R.C. and SMITH, T.D. (1969), J. Chem. Soc. (A), 94.

14. BERTRAND, J.A. and ELLEN, P.G. (1976) Prog. Inorg. Chem., 21, 29.

15. CLARK, H.C., WATERS, J.M., WATERS, T.N. and WILLIAMS, G.J. (1977), J. Inorg. Nucl. Chem., 39, 1971.

16. CLARK, H.C., O'BRIEN, R.J. (1961) Can. J. Chem., 1030.

17. HERSHEY, S.A. and LEUSSING, D.L. (1977), J. Am. Chem. Soc., 99, 1992.

18. GRAY, Jr., E.T., TAYLOR, R.W. and MARGERUM, D.W., (1977), Inorg. Chem., 16, 3047.

19. CHAO, Y.Y., and KEARNS, D.R. (1977) J. Phys., 81, 666.

20. GAGNE, R., KORAL, C., and SMITH (1977), J. Am. Chem. Soc., 99, 8367.

21. SHARROCK, P., THIBAUDEAU, C. and CAILLE, A. Magnetic Studies on Two new copper hippurate dimers. Inorg. Chem., in press.