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MODELLING THE BLUE PROTEIN ACTIVE SITES: SYNTHESIS AND CHARACTERIZATION OF CuN_2S_2 COMPLEXES SHOWING RHOMBIC EPR SPECTRA AND HIGH Cu^{II}/Cu^{I} POTENTIAL

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Abstract—Two new tetradentate ligands have been synthesized by Schiff base condensation of diisobutyraldehyde disulphide with 2-mercaptoethylamine (L^1) and 2-aminothiophenol (L^2) respectively and then reducing the imine linkages with $NaBH_4$ in refluxing methanol. In the free ligands the thiolate sulphur is protected with tertiary butyl groups which are cleaved in the presence of Cu^{II}-salts to give neutral CuN_2S_2 complexes. The copper complexes show ligand field transitions at 815 and 760 nm at room temperature which are independent of the solvents used and are consistent with a pseudotetrahedral coordination around the Cu^{II} ion. The EPR spectrum of the aliphatic thiolate in MeCN glass shows significant rhombic splitting ($g_x - g_y = 0.09$ and $A_x - A_y = 60 \times 10^{-4}$ cm^{-1}) attributable to d_{z^2} mixing into the ground state wavefunction. For the aromatic thiolate complex, however, the EPR spectrum was not well resolved although the rhombic nature of the spectrum could easily be observed. Both the complexes exhibit well-defined cyclic responses in their cyclic voltammograms at RT and in acetonitrile for the Cu^{II}/Cu^{I} couple with $E_{1/2} = 0.5$ V vs SCE. This high positive value for the redox couple is also consistent with a coordination geometry much distorted from planarity. The active sites of the blue proteins which contain copper in distorted geometries exhibit Cu^{II}/Cu^{I} potential in the range 300–800 mV vs NHE at pH = 7.0.

Ligation of thiolate sulphur to copper at the active sites of quite a number of copper proteins has been established. Cu^{II}–thiolate bonding has been shown to be present by X-ray crystallography in the case of single copper proteins like plastocyanin, azurin,[2] pseudoazurin[3] and basic blue protein from cucumber[4] and in a multicopper protein, ascorbate oxidase.[5] A preliminary structure is also available [6] on the bacterial blue protein, amicyanin from *Thiobacillus versutur*. In the case of other single copper blue proteins like stellacyanin, umecyanin, rusticyanin or multicopper oxidases like tree and fungal laccases, ceruloplasmin etc., the presence of copper–sulphur bonding has been established by various spectroscopic studies.[7] The Cu_A site in *cytochrome c oxidase* also contains[8] two cysteine thiolates bonded to Cu^{II}. Moreover, for Cu^{II}-substituted proteins like horse liver alcohol dehydrogenase, this bonding is also present[9] as inferred from various spectroscopic investigations.

A major reason for the peculiar spectroscopic behaviour exhibited by these proteins is due to the presence of strong Cu^{II}–thiolate bonding.[10] Hence the bioinorganic scope of this bonding is quite considerable. Copper blue proteins, in particular, have attracted a lot of attention by chemists who are involved in synthesizing electronic structural analogues of the type 1 site which is intrinsic in nature.[11] The electronic spectrum of a blue site is dominated by an intense absorption band centred around 600 nm (molar Cu absorptivity lies in the range 2000–6000) attributable to the σ (thiolate) $\rightarrow$ Cu^{II} LMCT transition. There have been continuous efforts to

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mimic this spectral feature in small molecular weight thiolate complexes.[12–14]

EPR studies of the protein active sites have revealed that subtle yet important differences exist between blue proteins like plastocyanin, azurin, etc., on the one hand and stellacyanin, cucumber basic blue protein, etc., on the other.[15] The plastocyanin class of blue proteins show essentially axial EPR spectra; only a small rhombic splitting ($g_x - g_y = 0.017$) could be detected[16] in the Q-band spectrum of poplar plastocyanin. The stellacyanin class of proteins show significant rhombic splitting ($g_x - g_y = 0.057$ for stellacyanin). Both classes of proteins show small hyperfine coupling in the parallel region. The ground state descriptions should, therefore, be different for the two classes of proteins. For the plastocyanin class, extensive delocalization of the single d-electron onto the sulphur $p\pi$ (Cys 84) orbital results in unusual EPR parameters while in case of the stellacyanin class of proteins, about 3% mixing of the d_{z^2} orbital into the ground state wavefunction has been attributed as the principal cause for significant rhombic splitting.[15] Also, in case of pseudotetrahedral model Cu^{II} complexes, a significant amount of 'g' split has been suggested as due to d_{z^2} mixing into the ground state.[17]

The high redox potentials observed for the blue sites are also reflective of their structures much distorted from a regular planar geometry favoured by Cu^{II}. This distorted structure is a compromise geometry acceptable to both oxidation states so that their interconversion can be achieved easily. The Cu^{II}/Cu^{I} potential may be raised[18] by any one or a combination of the two factors which lead to lowering of LFSE: (i) decreasing the ligand σ-donor ability and (ii) constraining the geometry of the metal centre to the one that favours Cu^{I}, i.e. tetrahedral. The presence of π-acceptor ligands also favours the Cu^{I} state. With strong donors like amino nitrogen and thiolate sulphur, a Cu^{II} complex with the geometry distorted from planarity is expected to show a high positive value for the Cu^{II}/Cu^{I} couple and for small molecular weight Cu^{II}-thiolates this has been observed.[13,18–21] In order to address the peculiar EPR spectral characteristics as well as high redox potential values exhibited by the blue proteins, synthesis of Cu^{II}-thiolates having coordination geometry much distorted from planarity is thus highly desirable.

Studies of molecular models show that with an acyclic tetradentate ligand, introduction of a large bite angle for two donors can enforce a pseudotetrahedral geometry overcoming the ligand field driven tendency of Cu^{II} to form a square planar complex. Thus we designed and synthesized the two new ligands having N_2S_2 donors with large bite angles between the two nitrogens.

EXPERIMENTAL

Materials

Sulphur monochloride, isobutyraldehyde, 2-mercaptoethylamine hydrochloride and 2-aminothiophenol (Aldrich) were of reagent grade and were used as received without further purification. All the solvents were purified prior to use following standard procedures.[22] Physical measurements were made as reported earlier.[23]

Synthesis of ligands and complexes

The ligand syntheses were achieved in several steps as illustrated in Fig. 1.

Di-isobutyraldehyde disulphide. This was prepared by homolytic cleavage of the S—Cl bond by isobutyraldehyde following a published procedure.[24] Sulphur monochloride (20 g; 0.15 mol) in dry CCl_4 (25 cm^3) was added dropwise to a solution of freshly distilled isobutyraldehyde (21.4 g; 0.30 mol) mixed with 40 cm^3 of dry CCl_4, maintaining the temperature at 40–45°C throughout. As soon as sulphur monochloride came into contact with isobutyraldehyde, copius evolution of HCl gas took place which was driven out of the reaction vessel by occasional purging with N_2 gas. Total addition time was 2 h after which the reaction mixture was maintained at 40°C for 48 h for completion of the reaction. Then CCl_4 evaporated off and the resulting pale yellow thick liquid was washed several times with distilled water to remove dissolved HCl and finally allowed to distil under reduced pressure. The dialdehyde was obtained as a pale yellow oily liquid (15 mm; 90–100°C) in 81% yield. ^{1}H-NMR (80 MHz; $CDCl_3$; ppm): 1.1 (s, 12H), $4CH_3$; 9.0 (s, 2H), 2CHO.

S-t-Butylmercaptoethylamine. This was synthesized following the method of Pastuszak and Chimiak.[25] 2-Mercaptoethylamine (7.7 g; 100 mmol) was allowed to reflux with a mixture containing *t*-butanol (15 cm^3) and 2 N hydrochloric acid (50 cm^3) for 15 h using a long reflux condenser. The mixture was then allowed to distil under atmospheric pressure to remove water, acid and *t*-BuOH to leave behind the hydrochloride salt of the desired product as a colourless solid. The free base could be obtained by treating the salt with aqueous KOH followed by extraction with $CHCl_3$. ^{1}H-NMR (80 MHz, $CDCl_3$, TMS; ppm): 5.2 (sb, 2H, NH_2); 2.6 (t, 4H, CH_2); 1.2 (s, 9H, $3CH_3$).

S-t-butyl-2-mercaptoaniline. The hydrochloride

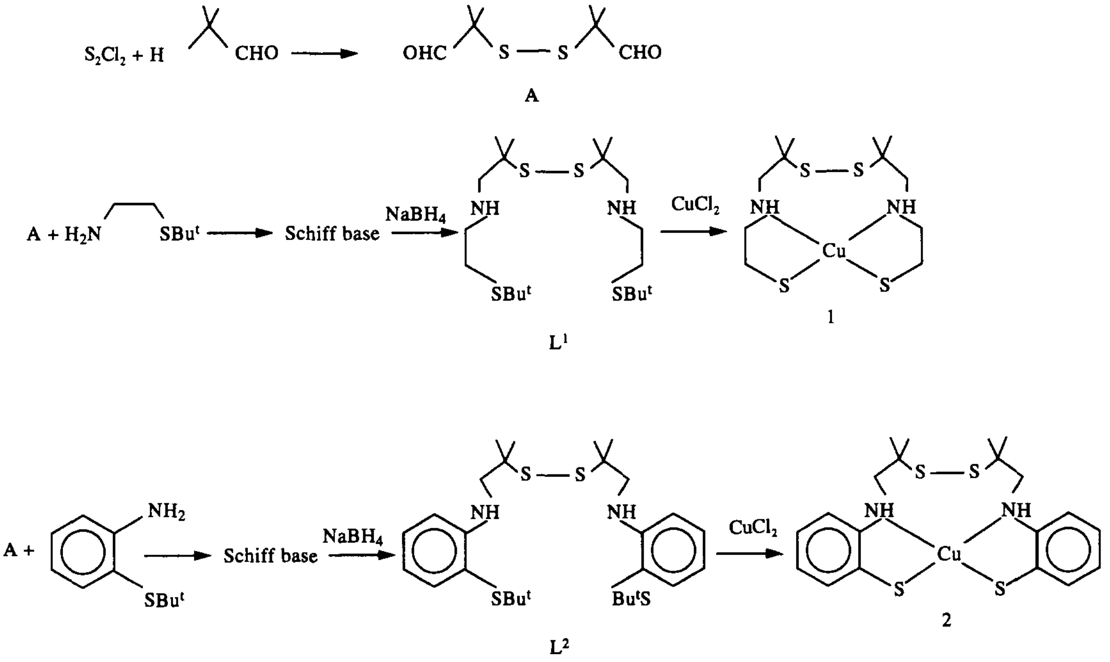

Fig. 1. Synthetic scheme for the ligands L^1 and L^2.

salt was isolated as above from which the free base was obtained as a pale yellow thick oil by treating with KOH and then extracting with $CHCl_3$. ^{1}H-NMR (80 MHz; $CDCl_3$; ppm): 7.2 (m, 4H, aromatic); 4.4 (sb, 2H, NH_2); 1.2 (s, 9H, $3CH_3$).

Synthesis of L^1. Di-isobutyraldehyde disulphide (1.2 g; 5.7 mmol) was allowed to reflux with S-protected mercaptoethylamine (1.5 g; 11.4 mmol) in dry ethanol (20 cm^3) for 6 h. The Schiff base that formed was reduced with $NaBH_4$ by refluxing for 2 h. After the reflux, EtOH was removed in a rotary evaporator, the residue shaken with 20 ml of water, followed by extraction with $CHCl_3$ and finally removal of $CHCl_3$ under reduced pressure yielded L^1 as a pale yellow thick liquid. Yield 87%. ^{1}H-NMR (80 MHz, $CDCl_3$; ppm): 4.5 (sb, 2H, 2NH); 3.4 (m, 8H, $4CH_2$); 2.3 (s, 4H, $2CH_2$); 1.2 (s, 18H, *t*-butyl); 1.0 (s, 12H, $4CH_3$). This crude product was used subsequently for complexation without further purification.

Synthesis of L^2. The ligand L^2 could be obtained in 83% yield as a pale yellow oily liquid following the above procedure starting from S-*t*-butyl-2-mercaptoaniline and di-isobutyraldehyde disulphide. This product was used for complexation without further purification. ^{1}H-NMR (80 MHz, $CDCl_3$, ppm): 7.1 (m, 8H, aromatic); 4.5 (s, 4H, $2CH_2$); 3.8 (sb, 2H, 2NH); 1.2 (s, 18H, t-butyl); 1.0 (s, 12H, $4CH_3$).

Synthesis of $Cu(L^1)$ **1**.

When $CuCl_2 \cdot 2H_2O$ (1.0 g; 5.8 mmol) dissolved in 20 cm^3 ethanol, was allowed to reflux for 30 mins in an argon atmosphere with L^1 (2.5 g; 6.1 mmol) in 25 cm^3 ethanol, the S-butyl linkage was broken due to Lewis acidity of Cu^{II} to result in a dark green solution. After cooling to room temperature, the green solution was filtered and ether allowed to diffuse into it inside a desiccator at room temperature. Overnight a dark green crystalline solid settled at the bottom which was collected by filtration and dried *in vacuo*. Yield 58%.

Synthesis of $Cu(L^2)$ **2**.

This was synthesized in a likewise manner as a dark green solid in 63% yield. Both **1** and **2** could be synthesized using other cupric salts as well. Analytical data for the complexes are collected in Table 1.

RESULTS AND DISCUSSION

The complexes are air-stable in the solid state. They are soluble in common organic solvents and are stable for at least 2 weeks when stored under nitrogen. In air, these solutions showed no appreciable decomposition for at least 5 h as monitored spectroscopically. After that period, they slowly decompose. Both behave as non-electrolytes in dichloromethane, acetonitrile or in methanol. Qualitative analysis for the chloride ion was negative in each case.

The electron transfer properties of the two complexes were probed in acetonitrile medium as both

Table 1. Cyclic voltammetric[a] and analytical[b] data for the complexes

Complex	Cu^{II}/Cu^{I} couple		Analysis %			
	$E_{1/2}$ (V)	ΔE_p (mV)	C	H	N	S
1	0.51	120	36.4(36.9)	6.5(6.7)	7.2(7.2)	32.4(32.9)
2	0.55	100	49.1(49.4)	5.7(5.4)	5.8(5.8)	26.2(26.4)

[a] Cyclic voltammetric data given are those taken in acetonitrile (*ca* 1×10^{-3}M) with scan rate of 50 m s^{-1} using a glassy carbon working electrode at the scan rate of 50 mV s^{-1} and at 298 K. Ferrocene–ferrocenium couple was located at 0.4 V under the present experimental conditions.

[b] Numbers in parentheses are the calculated ones.

the complexes were found to be insoluble in water. Each of the complexes shows a well-defined response in its cyclic voltammogram as shown in Fig. 2. The redox data in acetonitrile at room temperature (RT) are collected in Table 1. The following results were obtained. For **1**, the response is quasi-reversible ($\Delta E_p = 120$ mV) with $E_{1/2} = 0.51$ V vs SCE. The ratio ip_a/ip_c is equal to 1.0 within experimental error and independent of scan rates (25, 50, 100, 200 mV s^{-1}); the ΔE_p value increases steadily although by a small magnitude as the scan speed is increased and finally the ratio $ip_c/v^{1/2}$ (where v is the scan rate) is found to be practically constant. For **2**, the response is again quasi-reversible ($\Delta E_p = 100$ mV) with $E_{1/2} = 0.55$ V vs SCE and is independent of scan rates employed; the ratio ip_a/ip_c is 1.0 within experimental error for all the scan rates while the ratio $ip_c/v^{1/2}$ is again practically constant. The peaks are assignable to the Cu^{II}/Cu^{I} couple as shown below

$$[Cu^{II}L^1] + e^- = [Cu^{I}L^1]^- \qquad (1)$$

$$[Cu^{II}L^2] + e^- = [Cu^{I}L^2]^- \qquad (2)$$

The ligand based nature of this redox couple is ruled out by the fact that the $E_{1/2}$ value does not differ to any significant extent for the two complexes in spite of L^2 having two aromatic groups while L^1 has none.

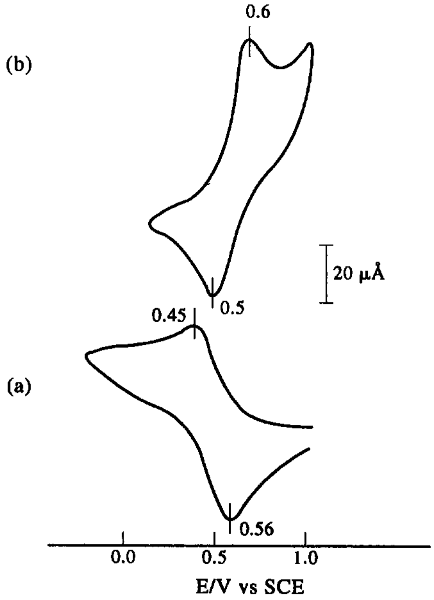

Fig. 2. Cyclic voltammograms for the complexes **1** (a) and **2** (b) recorded at RT in acetonitrile (*ca* 1×10^{-3} M) at the scan rate of 50 mV s^{-1}.

Besides, in the case of **1**, the only other redox active parts will be the thiols or the disulphide groups. Thiols will not give reversible cyclic voltammograms due to bond making/bond breaking processes involved. An N_4-analogue of the ligand L^1 where the two thiols were replaced by aliphatic amines was prepared. The Cu^{II} complex of this ligand was found to be pseudotetrahedral[26] which showed a quasi-reversible Cu^{II}/Cu^{I} couple with $E_{1/2} = 0.28$ V vs SCE and $\Delta E_p = 80$ mV. Had it been due to a sulphide group, both the CuN_4 and the present CuN_2S_2 complexes would have given similar cyclic voltammograms. The high positive value and quasi-reversibility for the Cu^{II}/Cu^{I} couple is attributable to the pseudotetrahedral coordination geometry present in both the complexes. The $E_{1/2}$ values obtained for the present complexes are substantially higher compared to known CuN_2S_2 complexes having square planar geometry[18,27,28] and very similar to the type 1 copper.[7] The high redox potential values observed in case of the type 1 copper has been attributed to the substantially distorted geometry at the active site.[29]

On scanning further in the positive side, no well-defined peak could be observed. However, when scanned in the negative side, a small broad cathodic peak appears at about −0.8 V and on scan reversal an anodic peak of similar nature appears near −0.5

V. Also, a sharp anodic peak appears at −0.2 V. We did not probe any further the cathodic and anodic peaks which appear at −0.8 and at −0.5 V respectively. The sharp peak at −0.2 V is due to oxidation of metallic copper deposited at the electrode surface. Examples of this behaviour are known.[18,30,31] We have not tested the electron transfer properties of the complexes in other non-aqueous solvents. As both the complexes were readily soluble in acetonitrile, we have used this solvent to study the spectroscopic and electrochemical properties of the complexes. This solvent has been widely used[32] for studying electrochemical properties of model Cu^{II} complexes with different geometries. Coulometric data could not be collected for either complex as they were unstable in the coulometric time-scale.

Electronic spectral data for the complexes at room temperature are collected in Table 2. To probe possible solvent coordination, dichloromethane, acetonitrile and DMF were used as solvents. In acetonitrile or in DMF, the spectral features were same as in non-coordinating dichloromethane. Therefore, if there is any solvent coordination, it must be very weak. The bands with λ_{max} at 815 and at 760 nm are assigned as ligand field transitions. These are significantly red-shifted compared to those for CuN_2S_2 chromophores having almost planar geometry (Table 2). Tetracoordinate Cu^{II} can exist in a large number of stereochemistries.[33] As the geometry distorts from planarity (dihedral angle 0°) towards tetrahedral (dihedral angle 90°) for the same chromophore, the *d*-orbital splittings decrease and consequently the ligand field bands shift towards lower energies.[34,35] Attempts have been made[36] to relate the band maxima with the value of the dihedral angle. Any conclusions based on such correlations should be made only for closely related ligands. As the data shown in Table 2 indicate, *d–d* band positions in **1** and **2** occur at energies[35,36] as in case of CuN_2S_2 chromophores where the dihedral angle is near 50° or slightly more. Somewhat higher intensities observed for the ligand field bands in the present cases are possibly due to intensity borrowing from nearby charge-transfer transitions.

Charge-transfer transitions involving amines and Cu^{II} are available.[37] The parent complex, $[Cu(en)_2](ClO_4)_2$ absorbs at 240 nm attributable to the $\sigma(N) \rightarrow Cu^{II}$ LMCT transition. Alkylation of the nitrogen atoms lowers the ionization potential[38] of the amine and results in a systematic red-shift of this band showing a linear relationship between the two parameters.[37a] Therefore, the strong peak with λ_{max} at 280 nm in **1** is attributed to $\sigma(N) \rightarrow Cu^{II}$ LMCT absorption. In case of **2**, the peak at 290 nm is similarly assigned. For **2**, the intensity of this band is very high because the free ligand (L^2) absorbs strongly in this region. Aliphatic disulphides absorb[39] at energies higher than 280 nm. The prominent absorption at 350 nm and the weaker one at 470 nm are too low in energy to be $\sigma(N) \rightarrow Cu^{II}$ LMCT bands originating from a secondary amine. When a thiolate is bonded to Cu^{II}, the

Table 2. Electronic spectral data for **1**, **2** and related systems

Chromophore	Dihedral angle°	λ_{max}, nm(ε_{max})[a]	Assignment	Ref.
$CuN_2S_{2'}$ (**1**)		815(510)	ligand field	This work
		760(540)	ligand field	
		415(2825)	π(thiolate) → Cu^{II}	
		345(3415)	σ(thiolate) → Cu^{II}	
		280(5000)	σ(amine) → Cu^{II} + ligand transitions	
$CuN_2S_{2'}$ (**2**)		810(515)	ligand field	This work
		670(930)	ligand field	
		470(2420)	π(thiolate) → Cu^{II}	
		335(3125)	σ(thiolate) → Cu^{II}	
		290(5040)	σ(amine) → Cu^{II} + ligand transitions	
CuN_2S_2	21	545	ligand field	33a
CuN_2S_2	52.8	850	ligand field	34b
		760	ligand field	
CuN_2S_2	57.1	1090(20)	ligand field	34a
CuN_3S	90	996	ligand field	13
CuS_2O_2	0	830(20)	ligand field	27
		550(300)	ligand field	

[a] ε_{max} in $dm^3\ mol^{-1}\ cm^{-1}$ at RT in acetonitrile.

Table 3. EPR spectral data for **1**,[a] **2** and related systems[b]

Complex	g_x	g_y	g_z	A_x	A_y	A_z	Ref.
1	2.014	2.111	2.235	67	11	97	This work
2	2.10	(g_{av})					This work
CuN_2Cl_2	2.042	2.144	2.365	45	28	65	17b
Stellacyanin	2.018	2.077	2.287	57	−29	−35	44
CBP	2.02	2.08	2.21	60	10	55	4

[a] EPR spectral data for **1** reported are those taken in acetonitrile glass.
[b] A^{Cu} unit is 60×10^{-4} cm^{-1}.

LMCT involves[16] three transitions: one intense σ (thiolate) → Cu^{II} flanked on either side by weaker π (thiolate) → Cu^{II} bands. For CuN_2S_2 complexes the σ (thiolate) → Cu^{II} LMCT transition occur near 350 nm and hence, the peaks near 350 nm are similarly assigned both for **1** and **2**. In that case, the weaker absorption near 470 nm is assignable to the π (thiolate) → Cu^{II} LMCT transition. The higher energy transition involving thiolate and Cu^{II} is not seen in either case.

Magnetic moment values (Table 3) for the complexes after diamagnetic corrections are indicative of discrete Cu^{II}-complexes.[40] Complex **1** in the solid state or in solution at liquid nitrogen temperature exhibits an EPR spectrum which is significantly rhombic-distorted (Table 3). The EPR spectra of **1** in the solid state and in acetonitrile glass are shown in Figs 3 and 4 respectively. For complex **2**, a resolved spectrum was not obtained either in the solid state or in acetonitrile glass. The solvent molecules are not bonded to Cu^{II} in either of the complexes as the analysis of electronic spectral data showed (*vide supra*). The EPR parameters for **1** listed are those obtained by spectral simulation. The highest symmetry appropriate for the CuN_2S_2 complex that should show rhombic splitting is C_{2v}. In this point group, both $d_{x^2-y^2}$ and d_{z^2} orbitals transform to the A_1 representation. Therefore, the metal part of the ground state wavefunction should be a mixture of both these orbitals. In this symmetry, the g shifts (from the free electron value) are given by:[17a,41]

$$g_x = -2\lambda k_x^2(a-3^{1/2}b)^2/E_{xz} \qquad (3a)$$

$$g_y = -2\lambda k_y^2(a+3^{1/2}b)^2/E_{yz} \qquad (3b)$$

$$g_z = -8\lambda k_z^2(a^2/E_{xy}) \qquad (3c)$$

Here, $k_{x,y,z}$ are the orbital reduction factors,[42] λ is the spin-orbit coupling of Cu^{II} ion, a and b are the coefficients of $d_{x^2-y^2}$ and d_{z^2} in the ground state wavefunction respectively, while the denominators represent *d–d* transition energies: E_{xy} is the energy difference between the ground state and d_{xy} and so on. As these equations indicate, three possible mechanisms can split g_x and g_y, which are: (1) differences in E_{xz} and E_{yz} values, (2) differences in reduction factors (excited state delocalization) and (3) magnitudes of a and b (d_{z^2} mixing into the ground state wavefunction). Detailed calculations[17b] on Cu^{II}-doped Zn(1,2-dimethylimidazole)$_2$Cl$_2$ host lattice having a pseudotetrahedral structure suggest that for a difference of about 125 nm between E_{xz} and E_{yz} values, the difference ($g_x - g_y$) can be as much as 0.004. In the absence of polarized electronic spectral data on **1**, it is not possible to designate the two ligand field transi-

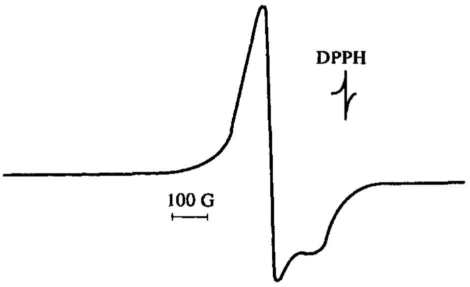

Fig. 3. EPR spectrum of **1** in the solid state at liquid nitrogen temperature.

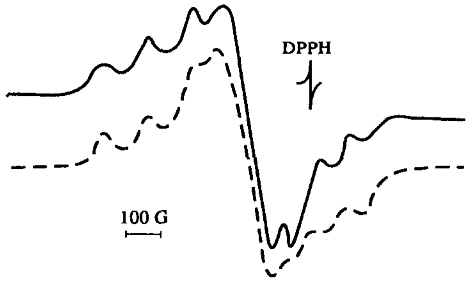

Fig. 4. EPR spectrum of **1** in acetonitrile (*ca* 1×10^{-3} M) glass. The dashed curve represents the simulated spectrum.

tions observed. In any case, the band positions put an upper limit of about 55 nm between the two transition energies, namely E_{xz} and E_{yz}. Therefore, point (1) above cannot account for such a large splitting in the g values. The major contributing factor(s) towards the rhombic splitting for **1** should be either due to d_{z^2} mixing into the ground state wavefunction or extensive excited state delocalization or both.

In probing the EPR spectral characteristics of plastocyanin, it has been argued in favour of an anisotropic delocalization over the cysteine sulphur p-π orbital.[15] However, the extent of g splitting in plastocyanin is small (0.017) compared to stellacyanin (0.057). The extensive g splitting in stellacyanin cannot be accounted for considering orbital reduction factors alone due to their small effects. On the other hand, this splitting is quite sensitive to the amount of d_{z^2} mixing. For stellacyanin, the observed g split could be explained satisfactorily taking into consideration about 3% mixing of d_{z^2} into the ground state. This amount of mixing can also account for the differences between A_x and A_y values observed in stellacyanin. To evaluate the extent of d_{z^2} mixing, neglecting the orbital reduction factors, a parameter R_g has been defined[43] as

$$R_g = \frac{2(g_x - g_y)}{g_x + g_y}.$$

For stellacyanin, R_g is 0.9 while for the Cu^{II}-doped Zn(1,2-dimethylimidazole)$_2$Cl$_2$ this value is 1.1 and for complex **1** the value of R_g is calculated to be 1.6. This parameter is a steep function[17b] of the extent of d_{z^2} mixing. For **1** a mixing of the order of 4–5% can satisfactorily account for the g split observed. For the Cu^{II}-doped complex above, about 3% mixing is suggested for the observed g split (0.102). For a 4–5% mixing, the difference, $A_x - A_y$, should be about 60×10^{-4} cm^{-1} which is very close to the value obtained for **1**.

CONCLUSION

Two neutral CuN$_2$S$_2$ complexes have been synthesized which are forced to adopt a pseudo-tetrahedral geometry due to the large bite angle of the two nitrogens. The distorted geometry raises the $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple and makes the EPR spectra rhombically distorted by a significant amount. Pseudotetrahedral model Cu^{II} thiolates can provide an important data base for studying the electronic structure and bonding of the blue protein active sites. The amount of d_{z^2} mixing into the ground state wavefunction for the stellacyanin active site is estimated to be about 3%. This necessitates increased ligand field strength along the z-direction which is possible if the local symmetry is more like C_{2v}. CBP although having the same donor set as present in plastocyanin gives a rhombic EPR signal. Studies of EPR characteristics of these blue sites along with their redox behaviour are likely to be facilitated by model Cu^{II} thiolates where close to C_{2v} symmetry exists.

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