The Effect of Strong NH···S Hydrogen Bonds in the Copper(i) Thiolate Complex, (NEt₄)₂[Cu(o-pabt)₃] (o-pabt = o-pivaloylaminobenzenethiolato)

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Short Cu–S bonds, induced by strong intramolecular NH···S hydrogen bonds, have been established by X-ray analysis, IR spectra, and EHMO calculations for (NEt₄)₂[Cuᴵ(o-pabt)₃] (o-pabt = o-pivaloylaminobenzenethiolato) and suggest an important role for this type of hydrogen bonding in blue copper proteins.

The active site in oxidised blue copper proteins[1,2] contains copper(II) coordinated strongly to three donors and very weakly to a further two in azurin[3] and one in poplar plastocyanin.[4] In the former, strong bonds are formed to a cysteine–S with an unusually short (2.15 ± 0.05 Å) Cu–S bond and two histidine–N atoms at 2.00 and 2.08 Å. The weaker bonds are formed to a methionine–S and an O atom from the carbonyl oxygen of an amide group. The CuN₂S unit is approximately planar and this geometry is considered to be important in determining the properties of the copper site in both native proteins and model complexes.[5] A similar geometry has also been observed in the reduced form of azurin[3] but with slightly longer Cu-donor atom distances (Cu–S 2.26 Å; Cu–N 2.05, 2.13 ± 0.05 Å). We show that

although the increases on reduction are of the same order as the estimated standard deviations they are all in the same direction so that the pattern is a consistent one. In reduced poplar plastocyanin[4] the Cuᴵ adopts a trigonal planar geometry at low pH whereas at high pH a distorted tetrahedral geometry is favoured. This latter geometry is similar to that observed in the oxidised form of plastocyanin. In the model Cuᴵ thiolate complex, (NEt₄)₂[Cu(SPh)₃], a trigonal planar coordination geometry has been reported.[6]

X-ray analysis[1,7] and resonance Raman spectroscopy of blue copper proteins[2,8,9] have indicted the presence of NH···S hydrogen bonding between the S(cysteinate) ligand and an amide-N of the peptide chain. The NH···S hydrogen bonds are shortened (by ca. 0.15 Å) upon reduction of the copper in
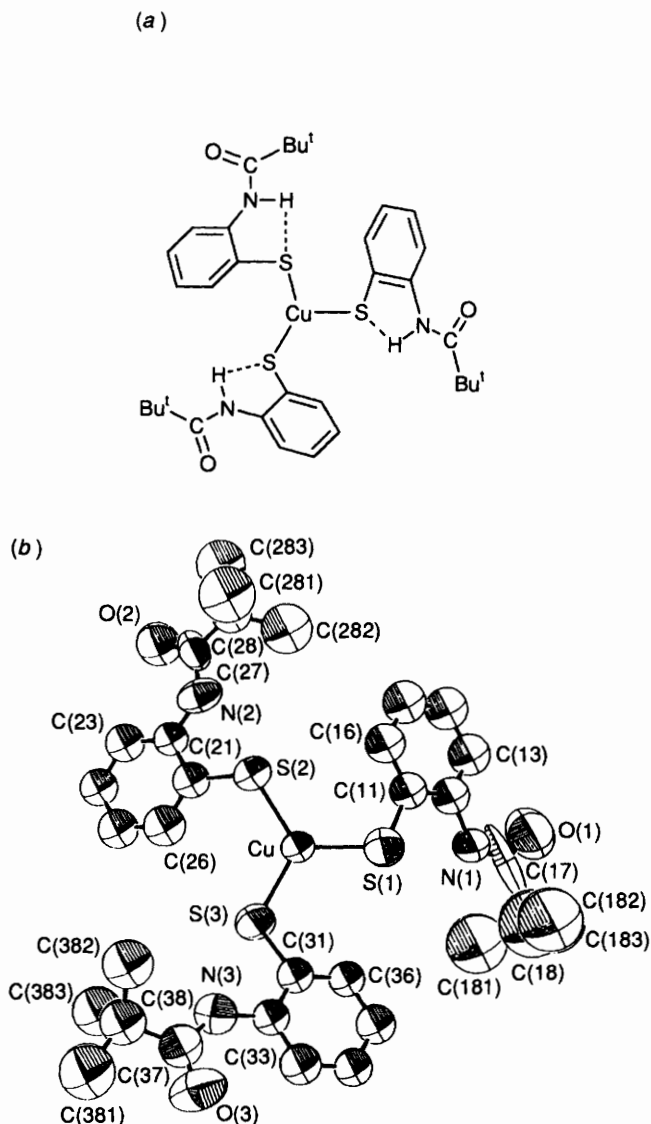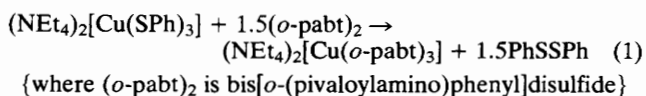
(a)

(b)

Fig. 1 (a) Schematic drawing of NH···S hydrogen bonds in **1** and (b) the molecular structure of the [Cu(o-pabt)$_3$]$^{2-}$ anion. Selected distances (Å) and angles (°) are: Cu–S(1) 2.232(8), Cu–S(2) 2.232(6), Cu–S(3) 2.217(7), N(1)···S(1) 2.86(1), N(2)···S(2) 2.83(1), N(3)···S(3) 2.92(1); S(1)–Cu–S(2) 121.1(3), S(1)–Cu–S(3) 119.9(3), S(2)–Cu–S(3) 118.9(3).

Alcaligenes denitrificans azurin.[10] However the role of NH···S hydrogen bonding in the region of the copper site is not yet clear.

As reported previously we have designed the o-acylamino-benzenethiolate (S-o-RCONHC$_6$H$_4$, R = alkyl group) ligand which contains an intra-ligand NH···S hydrogen bond. The monooxomolybdenum(v,iv), iron(ii) and cobalt(ii) complexes with this ligand show remarkably short M–S bonds compared with known benzenethiolate complexes.[11,12] The purpose of this paper is to clarify the effect of the NH···S hydrogen bonding on the Cu–S bond in trigonal planar coordination geometry.

(NEt$_4$)$_2$[Cu(SPh)$_3$] + 1.5(o-pabt)$_2$ →
(NEt$_4$)$_2$[Cu(o-pabt)$_3$] + 1.5PhSSPh   (1)

{where (o-pabt)$_2$ is bis[o-(pivaloylamino)phenyl]disulfide}

A novel copper(i) thiolate complex, (NEt$_4$)$_2$[Cu(o-pabt)$_3$] **1** was synthesised by the ligand exchange reaction shown in eqn. (1). The exchange reaction, carried out under argon, proceeds as a result of the difference in the reduction potentials of

(o-pabt)$_2$ and PhSSPh, which were observed at −1.27 and −1.92 V (vs. SCE) respectively, in acetonitrile.

The molecular structure† of **1** indicating the NH···S hydrogen bonds [N(1)···S(1) 2.86(1), N(2)···S(2) 2.83(1), N(3)···S(3) 2.92(1) Å] is shown in Fig. 1. The hydrogen bonds are all shorter than their counterparts in the Mo^{IV} and Mo^V complexes (PPh$_4$)[MoO(S-o-MeCONHC$_6$H$_4$)$_4$] and (PPh$_4$)$_2$[MoO(S-o-MeCONHC$_6$H$_4$)$_4$] [N···S range 2.965(5)–3.026(7) Å][12] and represent strong interactions. They are also significantly shorter than the corresponding NH···S approaches observed in reduced azurin (3.29–3.37 Å). However in the azurin structure[10] the S is involved in two hydrogen bonds and thus the longer distances are not unexpected. In the complex **1** the copper atom lies in the plane of the three donor S atoms with a mean value for the Cu–S bond distance of 2.227 Å [Cu–S(1), 2.232(8); Cu–S(2), 2.232(6); Cu–S(3), 2.217(7) Å]. A similar geometry is observed for the Cu in the related structure (NEt$_4$)$_2$[Cu(SPh)$_3$], **2**[6] except that the slightly longer mean Cu–S bond distance of 2.250 Å (range 2.239(2)–2.258(2) Å] is observed in the latter. In both model structures these distances are in good agreement with those determined in the reduced azurin structure having regard to the lower accuracy of the latter. Also, in **1**, the three phenyl rings of the ligand are rotated by an angle of 33° relative to the trigonal plane whereas in **2** this rotation is much smaller at only 13°.

An EHMO calculation shows that coplanarity of the three phenyl rings with the CuS$_3$ coordination plane increases the π-bond character of the Cu–S bonds as estimated from the overlap population.‡ These results thus predict that the observed orientation of the phenyl rings in **1** decreases the Cu–S π-bond character in comparison with **2**, a result which is contrary to that of X-ray analysis. We conclude therefore that a further interaction must contribute to the Cu–S bond shortening and suggest that this is due to the NH···S hydrogen bonds.

IR spectral data support the presence of strong intramolecular hydrogen bonds. Complex **1** shows an N–H stretch at 3226 cm^{-1} which is significantly lower than the value of 3389 cm^{-1} observed for the free N–H group in the disulfide reference compound and even lower than the value of 3251 cm^{-1} for the N–H intramolecularly hydrogen bonded to the carbonyl group in the same reference compound.[12] As

well, the C=O stretch at 1667 cm^{-1} is only slightly shifted relative to that for the non-hydrogen bonded carbonyl in the reference (1679 cm^{-1}). These results suggest that the frequency of N–H absorption is caused not by the intermolecular interaction NH$\cdots$O=C, but rather by intramolecular NH$\cdots$S bonds.

Voltammetric oxidation of the copper thiolate complexes, 1 and 2, is irreversible as copper(II) causes the rapid oxidation of thiolates to form copper(I) and disulfides.[15] The oxidation peak potentials, under conditions of cyclic voltammetry at a glassy carbon electrode with a scan rate of 100 mV s^{-1}, for 1 and 2 were observed at +0.30 and +0.18 V (relative to SCE) in acetonitrile. As has been demonstrated in other biological[16] and reversible synthetic systems,[11,12] the relatively more positive oxidation peak potential of 1 is in accord with a NH$\cdots$S hydrogen bond contribution to the redox potential of the copper active site. These results suggest that the presence of a NH$\cdots$S hydrogen bond is a factor that must be considered to contribute to the magnitude of redox potentials in metalloproteins. For example in the blue copper protein, azurin from A. denitrificans, just as weak axial interactions with the copper atom have been suggested to fine-tune the redox potential,[9] so must NH$\cdots$S hydrogen bonds to the sulfur of the cysteinate ligand be also considered.

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References

1 E. T. Adman, *Adv. Protein Chem.*, 1991, **42**, 145.
2 J. Han, E. T. Adman, T. Beppu, R. Codd, H. C. Freeman, L. Huq, T. M. Loehr and J. Saunders-Loehr, *Biochemistry*, 1991, **30**, 10904.
3 W. E. B. Shepard, B. F. Anderson, D. A. Lewandowski, G. E. Norris and E. N. Baker, *J. Am. Chem. Soc.*, 1990, **112**, 7817.
4 J. M. Guss, P. R. Harrowell, M. Murata, V. A. Norris and H. C. Freeman, *J. Mol. Biol.*, 1986, **192**, 361.
5 N. Kitajima, K. Fujisawa, M. Tanaka and Y. Moro-oka, *J. Am. Chem. Soc.*, 1992, **114**, 9232.
6 C. D. Garner, J. R. Nicholson and W. Clegg, *Inorg. Chem.*, 1984, **23**, 2148.
7 E. N. Baker, *J. Mol. Biol.*, 1988, **203**, 1071.
8 Y. Mino, T. M. Loehr, K. Wada, H. Matsubara and J. Sanders-Loehr, *Biochemistry*, 1987, **26**, 8059.
9 E. W. Ainscough, A. G. Bingham, A. M. Brodie, W. R. Ellis, H. B. Gray, T. M. Loehr, J. E. Plowman, G. E. Norris and E. N. Baker, *Biochemistry*, 1987, **26**, 71.
10 W. E. B. Shepard, PhD Thesis, Massey University, 1991.
11 N. Ueyama, T. Okamura and A. Nakamura, *J. Chem. Soc., Chem. Commun.*, 1992, 1019.
12 N. Ueyama, T. Okamura and A. Nakamura, *J. Am. Chem. Soc.*, 1992, **114**, 8129.
13 NEC PC-98 EHMO program obtained from the Kodansha Sci. Co.
14 P. K. Mehrotra and R. Hoffmann, *Inorg. Chem.*, 1978, **17**, 2187.
15 J. M. Downes, J. Whelan and B. Bosnich, *Inorg. Chem.*, 1981, **20**, 1081.
16 C. W. Carter, Jr., *J. Biol. Chem.*, 1977, **252**, 7802.