

Dicopper(II/II) complexes of an amine phenol hexadentate ligand showing μ -bis(tridentate) coordination: EPR spectral model of binuclear Cu_A centre of nitrous oxide reductase

Manindranath Bera, Sanjit Kumar Mahato and Debashis Ray*

Department of Chemistry, Indian Institute of Technology, Kharagpur 721 302, India

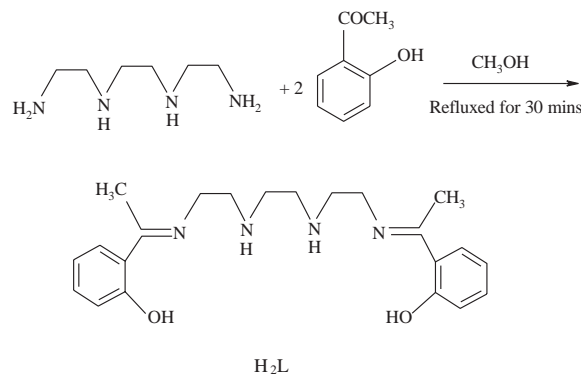
A new family of binuclear Cu(II)-Cu(II) complexes with a hexadentate Schiff base ligand containing imine-phenol end groups has been studied as an EPR spectral model of the binuclear Cu_A centre of nitrous oxide reductase. The complexes have been characterised by elemental analysis, solution electrical conductivities, IR, UV-vis, EPR spectral, magnetic studies and geometrical optimisation by molecular mechanics (MM2) method.

Keywords: copper(II)/(II), nitrous oxide reductase, EPR, hexadentate amine phenol

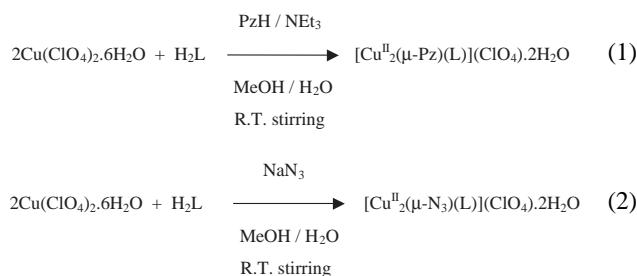
Synthetic dicopper complexes show promise as spectroscopic models for structure and ligand specific binding patterns of biocatalytic functions. One such binuclear copper centre has recently been identified in the Cu_A site of nitrous oxide reductase (N_2OR) in *Pseudomonas nautica* 617, which catalyses the conversion of N_2O to N_2 , in the last step of the complete denitrification pathway in denitrifying bacteria.¹ The required two electrons for the catalytic reaction are supplied by the metal ions. Besides these biological model aspects, magnetic exchange interactions in dinuclear complexes² have been investigated for a long time. Design and synthesis of heterocyclic ring grafted binucleating ligands are important to bind two same or different metal ions.^{3,4} Use of simple hexadentate ligands of ethylenediamine bridging for the synthesis of dicopper complexes have not been reported in the literature. This paper describes the synthesis of a new symmetrical hexadentate Schiff base ligand H_2L and its binding in a μ -bis(tridentate) fashion in dinuclear copper (II) complexes. This new acyclic ligand H_2L gives singly bridged, binuclear Cu(II)-Cu(II) complexes (**1a** and **2a**) in reasonable yields where the fourth coordination site of each copper atom is satisfied by the exogeneous bridging N-donor ligands.

The hexadentate ligand H_2L was synthesised by a simple Schiff base condensation reaction with 2-hydroxyacetophenone and triethylenetetramine in 2:1 molar ratio shown in the following Scheme 1.

The resulting Schiff base is a yellow oil and was not isolated as a solid but was treated with the metal salt *in situ*. The reaction of copper(II) perchlorate hexahydrate with this hitherto unknown ligand in 2:1 molar ratio in alcoholic media and at room temperature and in presence of different exogenous bridging ligands affords dark green $[\text{Cu}^{\text{II}}_2(\mu\text{-X})(\text{L})](\text{ClO}_4)_2 \cdot 2\text{H}_2\text{O}$ (**1a** and **2a**) ($\text{X} = \text{Pz}, \text{N}_3$) complexes. These represent a new family of pyrazolato- and azido- bridged dicopper(II/II) complexes. Both the complexes are insoluble in water and separate immediately from the reaction medium. The complexes are soluble in DMF and DMSO. The molar conductivity measurements of both the complexes in DMF showed values of 70 to $80 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ (28 °C) and were consistent with a 1:1 electrolyte, *i.e.*, a monocationic metal complex formulation.⁵



Scheme 1



A geometric optimisation of the synthesized ligand and its complexes was made by the method of molecular mechanics (MM2)⁶ to establish the preferred molecular geometries of the complexes and the nature of exogenous bridging. The object of molecular mechanics is to predict the energy associated with a given conformation of a molecule. Bond lengths, bond angles and torsion angles are all altered using potential energy functions during a process known as optimisation. The data for the most stable conformations of the ligand and the complexes are listed in Table 1. These three energy minimised conformations are shown in Fig. 1. The total energy for the stable conformation of ligand is calculated to be -10.537 kcal/mol and the total energies for the corresponding stable conformations of two complexes

Table 1 Energies of the most stable conformations of the ligand and the complexes from the MM2 force field calculations (energies in kcal/mol)

Molecule	Total	Stretch bend	Angle	Torsional (dihedral)	van der Waals	Stretch	Electrostatic
Ligand	-10.548	0.289	10.046	-40.179	12.388	1.023	5.885
Complex 1a	77.355	0.574	61.032	-2.419	24.052	4.600	-10.484
Complex 2a	46.675	0.635	40.659	-11.171	22.276	3.718	-9.442

* Correspondence. E-mail: dray@chem.iitkgp.ernet.in

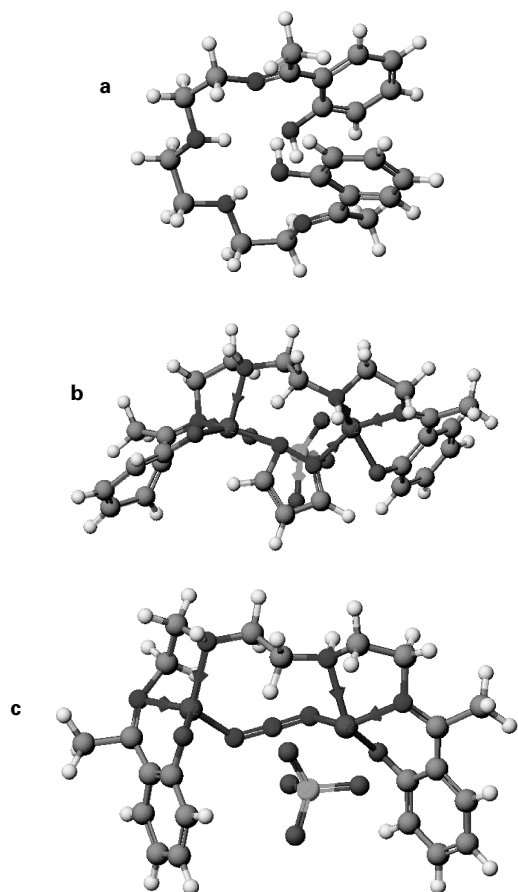


Fig. 1 (a) Calculated (MM2) conformation for the ligand H_2L . (Total strain energy is -10.537 kcal/mol). (b) Calculated (MM2) conformation for the compound $[Cu^{II}_2(\mu-Pz)(L)](ClO_4) \cdot 2H_2O$. (Total strain energy is 77.355 kcal/mol). (c) Calculated (MM2) conformation for the compound $[Cu^{II}_2(\mu-N_3)(L)](ClO_4) \cdot 2H_2O$. (Total strain energy is 46.675 kcal/mol).

$[Cu^{II}_2(\mu-Pz)(L)](ClO_4) \cdot 2H_2O$ (**1a**) and $[Cu^{II}_2(\mu-N_3)(L)](ClO_4) \cdot 2H_2O$ (**2a**) are calculated to be 77.355 kcal/mol and 46.675 kcal/mol respectively. The analysis of the total energies for all the conformations have shown that the energies are primarily due to especially the torsional strain and van der Waals interactions.

The IR spectra of the complex **1a** show the strong C=N stretching frequency of the terminal imine functions at 1605 cm^{-1} indicating the presence of C=N functionalities and the N-N stretching frequency at 1984 cm^{-1} indicating the bridging pyrazolate. From the IR spectra of the complex **2a** it is clear that the C=N stretching frequency of the terminal imine function appears at 1609 cm^{-1} and the N-N stretching frequency of the

single *cis*- μ -1,3-bridging (end-to-end) azido- group appears at 2050 cm^{-1} .^{7, 8} The band at 2050 cm^{-1} is attributable to the ν_{as} stretching frequency and supports the azido-bridging mode through the two terminal nitrogen atoms as suggested from MM2 calculations (*vide infra*). The absence of a band near 1300 cm^{-1} due to ν_s stretching mode for end-on (μ -1,1) bridging eliminates the alternative possibility. IR spectra of the two complexes are similar and the stretching mode of C=O at 1700 cm^{-1} for the acetophenone disappeared during Schiff base formation. Also a broad band around 3426 cm^{-1} suggests the presence of lattice water molecules only. The strong unsplit band ($\nu_{ClO_4^-}$) in both the complexes at around 1094 cm^{-1} suggests no coordination of perchlorate ions.⁹

The UV-vis spectroscopic properties of both the compounds were studied to gain insight into the co-ordination geometry of the compounds. The spectra of the complexes were recorded in DMF in the range $200 - 800$ nm. The electronic absorption spectra of the dicopper (II/II) complexes in DMF solution show several intense absorption bands in the visible region which are probably due to the allowed d-d and charge transfer transitions. The complexes **1a** and **2a** exhibit broad bands at 606 nm and 602 nm with molar extinction coefficients of 325 and 440 $M^{-1}cm^{-1}$ respectively, indicating d-d transitions. The higher intensity charge transfer transitions for complex **1a** are exhibited at 366 and 273 nm with molar extinction coefficients of 9115 and 23745 $M^{-1}cm^{-1}$ respectively and similar bands for complex **2a** are exhibited at 367 and 273 nm with molar extinction coefficients of 7480 and 22565 $M^{-1}cm^{-1}$ respectively. One of the representative spectra for complex **1a** is shown in Fig 2. When $[Cu(dien)]^{2+}$ binds one azide in the equatorial plane, the azide-to-copper CT occurs at 345 nm with an ϵ ¹⁰ of 2600 $M^{-1}cm^{-1}$. The equatorial binding of N_3^- is therefore assigned for the present complexes by comparing the bands at 366 and 367 nm. These therefore support the singly bridged, square-planar geometry around copper for both the complexes.

The room temperature magnetic susceptibilities of the complexes were measured with a Gouy balance using powdered samples. A diamagnetic correction of 278.43×10^{-6} cgsu per complex, as calculated from the Pascal's constants, was made on the susceptibility values.¹¹ At room temperature, the effective magnetic moment value of complex **1a** in the powdered state is $2.40 \mu_B$ ($1.69 \mu_B/Cu$). The $\chi_M T$ product at room temperature, 0.71 $cm^3 mol^{-1} K$, is slightly lower than the spin-only value of 0.75 $cm^3 mol^{-1} K$ expected for two isolated copper(II) ions ($S = 1/2$) assuming $g = 2.00$. The singly-bridging pyrazolate anion was not involved in strong antiferromagnetic interaction as seen from the room temperature magnetic moment value (which is $0.98 \mu_B$ for alkoxide-pyrazolate double bridging).¹² The effective magnetic moment value of complex **2a** in the powdered state is $2.48 \mu_B$ ($1.75 \mu_B/Cu$) at room temperature which is also slightly higher than the spin only value. The $\chi_M T$ product at room temperature, 0.76 $cm^3 mol^{-1} K$, is slightly larger than the

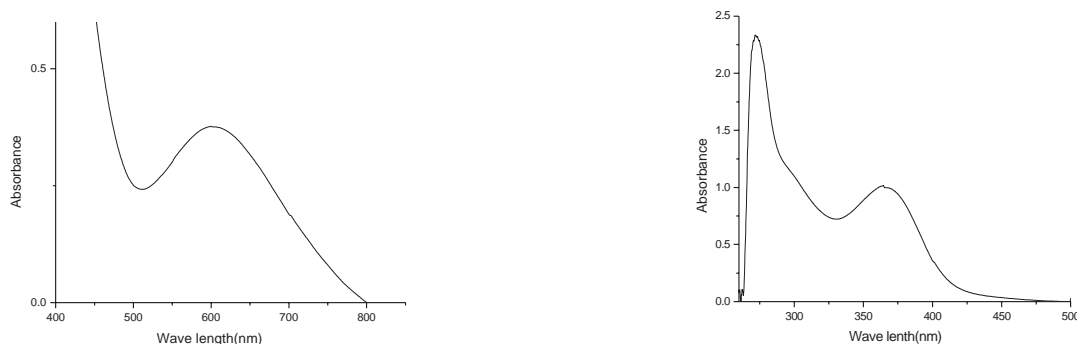


Fig. 2 Electronic spectra for complex $[Cu^{II}_2(\mu-Pz)(L)](ClO_4) \cdot 2H_2O$ (**1a**) in DMF at 298 K.

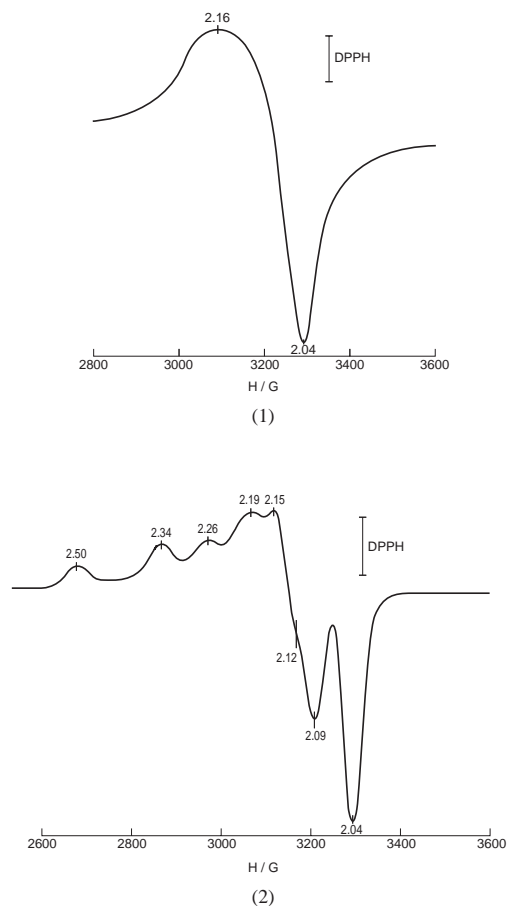


Fig. 3 X-band EPR spectra for complex $[\text{Cu}_2(\text{L})(\mu\text{-N}_3)](\text{ClO}_4)\cdot 2\text{H}_2\text{O}$ (**2a**). (1): solid powder spectrum at room temperature; (2): frozen DMF solution spectrum at 77 K.

spin-only value of $0.75 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$. Azide ion can bridge two copper(II) ions either in an end-on (EO; *cis*- $\mu_{1,1}$) fashion or in an end-to-end (EE; *cis*- $\mu_{1,3}$) manner. In the equatorial-equatorial (ee) configuration, where the azide group coordinates via the equatorial bonds to both the copper atoms in square (four coordinate) geometry, strong antiferromagnetic¹³ coupling can occur since the σ -orbitals on the azido group couple directly with the unpaired electron density in the $d_{x^2-y^2}$ orbitals. Weak antiferromagnetic interactions (-3.1 to -11.1 cm^{-1}) is observable in doubly $\mu_{1,3}$ -azide bridged complexes.¹⁴ Five coordinate and four coordinate dicopper complexes with end-to-end bridging behave differently. In square-pyramidal coordination environment $\mu_{1,3}$ - N_3 bridging leads to ferromagnetic interactions.¹⁵ All these data suggest that in the solid state some sort of antiferromagnetic spin exchange interaction is operative between the two metal centers through the bridging pyrazolato and bridging azido ligands in these complexes.

The polycrystalline X-band EPR spectrum of $[\text{Cu}_2(\text{L})(\mu\text{-N}_3)](\text{ClO}_4)\cdot 2\text{H}_2\text{O}$ at 77 K shows a quasi-isotropic signal around $g = 2$ region. The g values of the X-band spectrum in a DMF-glass are shown in Fig. 3. The characteristic "half-field" signal in the $g \sim 4$ region (diagnostic of triplet state and $\Delta M_S = 2$ transition) was very weak and could not be observed at the present spectrometer gain of 10×10^1 with microwave power 5 mW and obscured by the intense signal around $g = 2$. The latter signal is due to the dimer and similar to the Cu_A signal of N_2OR ¹⁴ in *Pseudomonas nautica* 617. The nature of the spectrum and the g values are typical of a variety of bridged copper(II) dimers. Here in the strongly coordinating solvent, dimethylformamide, the bridged structure is retained.

The hyperfine splitting constant values are also small compared to a tetragonal spectrum. The spin-Hamiltonian parameters $g_z = 2.23$, $g_{x,y} = 2.04$, $A_z = 8.83 \text{ mT}$ for our complexes are similar to those of the dinuclear Cu_A centre of N_2O reductase¹ ($g_z = 2.172$, $g_{x,y} = 2.02$, $A_z = 3.54 \text{ mT}$).¹⁵⁻¹⁹ In the frozen solution EPR spectrum of complexes **1a** and **2a**, six of the seven hyperfine lines in the g_z peak are well-resolved. The nature of the EPR spectrum with this respect is very similar to the Cu_A center of N_2O reductase in *Pseudomonas nautica* 617.

We have synthesised a new family of binuclear copper(II/II) complexes of the new hexadentate amine phenol ligand formed from the Schiff base condensation of 2-hydroxy acetophenone and triethylenetetramine. Bis(tridentate) behaviour is shown by the ligand in association with exogenous bridging. The complexes were characterised by molecular modelling, spectral and magnetic studies to establish the proposed four-coordinated square-planar geometry. The crystals grown so far by any solvent diffusion techniques were not of X-ray diffraction quality.

Experimental

Synthesis of the complexes:

$[\text{Cu}_2(\text{L})(\mu\text{-Pz})](\text{ClO}_4)\cdot 2\text{H}_2\text{O}$ (**1a**): 2-Hydroxy acetophenone (0.3 ml, 2.2 mmol) and triethylenetetramine (0.2 ml, 1.3 mmol) were mixed in 30 ml methanol. The resulting light yellow solution was refluxed for 30 minutes. The colour of the solution became dark yellow. Then it was cooled to room temperature. To this methanolic solution of *in situ* generated ligand H_2L , was added the methanolic solution of $\text{Cu}(\text{ClO}_4)_2\cdot 6\text{H}_2\text{O}$ (0.97 g, 2.6 mmol). The colour of the solution immediately changed to bluish-violet. After 0.5 h. stirring, an aqueous solution of pyrazole (0.09 g, 1.3 mmol) was added to it followed by the addition of NEt_3 (0.54 ml, 3.9 mmol). The stirring was continued for total 1 h. Gradually the dark green compound separated out. The compound was isolated by filtration, and washed with water. Finally the isolated compound was dried *in vacuo* over P_4O_{10} . Yield: 0.81 g (82%).

Anal. Calc. for $\text{C}_{25}\text{H}_{35}\text{N}_6\text{O}_8\text{ClCu}_2$: C, 42.26; H, 5.0; N, 11.9; Cu, 17.9%. Found : C, 42.32; H, 4.7; N, 11.4, Cu, 17.5%. Infrared spectrum (cm^{-1} , KBr disk): $\nu = 3417(\text{b})$, 1984(s), 1605(vs), 1539(s), 1442(s), 1403(s), 1338(s), 1297(s), 1201(s), 1093(s), 899(s), 758(s). Molar conductance, Λ_M : (DMF solution) $75.24 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$. UV-vis spectra [λ_{max} , nm (ϵ , $\text{lmol}^{-1}\text{cm}^{-1}$): (DMF solution) 606 (327), 366 (9114), 273 (23746)]. μ_{eff} (tot.): $2.40 \mu_B$; $\mu_{\text{eff}} / \text{Cu}$: $1.69 \mu_B$.

$[\text{Cu}_2(\text{L})(\mu\text{-N}_3)](\text{ClO}_4)\cdot 2\text{H}_2\text{O}$ (**2a**): 2-Hydroxy acetophenone (0.3 ml, 2.2 mmol) and triethylenetetramine (0.2 ml, 1.3 mmol) were mixed in 30 ml methanol. The resulting light yellow solution was refluxed for 30 minutes. The colour of the solution became dark yellow. Then it was cooled to room temperature. To this methanolic solution of *in situ* generated ligand H_2L , was added a methanolic solution of $\text{Cu}(\text{ClO}_4)_2\cdot 6\text{H}_2\text{O}$ (0.97 g, 2.6 mmol). The colour of the solution immediately changed to bluish-violet. After 0.5 h. stirring, an aqueous solution of NaN_3 (0.09 g, 1.3 mmol) was added with constant stirring for total of 1 h. The dark green coloured compound gradually separated out and was filtered, washed with distilled water and dried over P_4O_{10} *in vacuo*. Yield 0.79 g (81%). Anal. Calc. for $\text{C}_{22}\text{H}_{22}\text{N}_7\text{O}_8\text{ClCu}_2$: C, 38.57; H, 4.71; N, 14.31; Cu, 18.54%. Found : C, 38.32; H, 4.86; N, 14.47, Cu, 18.49%. Infrared spectrum (cm^{-1} , KBr disk): $\nu = 3439(\text{b})$, 2050(s), 1609(vs), 1539(s), 1441(s), 1338(s), 1297(s), 1201(s), 1094(s), 889(s), 757(s). Molar conductance, Λ_M : (DMF solution) $70 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$. UV-vis spectra [λ_{max} , nm (ϵ , $\text{lmol}^{-1}\text{cm}^{-1}$): (DMF solution) 602 (439), 367 (7482), 273 (22564)]. μ_{eff} (tot.): $2.48 \mu_B$; $\mu_{\text{eff}} / \text{Cu}$: $1.75 \mu_B$.

CAUTION: Azide derivatives are potentially explosive, only a small amount of materials should be prepared, and they should be handled with proper care.

We thank the Council of Scientific and Industrial Research, New Delhi, for financial support.

Received 26 April 2004; accepted 29 June 2004
Paper 04/2488

References

- 1 M. Prudencio, A.S. Pereira, P. Tavares, S. Besson, I. Cabrito, K. Brown, B. Samyn, B. Devreese, J.V. Beeumen, F. Rusnak, G. Fauque, J.J.G. Moura, M. Tegoni, C. Cambillau and I. Moura, *Biochemistry*, 2000, **39**, 3899.
- 2 H. Oshio and U. Nagashima, *Inorg. Chem.*, 1990, **29**, 3321.
- 3 U. Mukhopadhyay, L. Govindasamy, K. Ravikumar, D. Velmurugan and D. Ray, *Inorg. Chem. Commun.*, 1998, **1**, 152.
- 4 U. Mukhopadhyay, L.R. Falvallo and D. Ray, *Eur. J. Inorg. Chem.*, 2001, 2823.
- 5 W.J. Geary, *Coord. Chem. Rev.*, 1971, **7**, 81.
- 6 N.L. Allinger, *J. Am. Chem. Soc.*, 1977, **99**, 8127.
- 7 S. Karabocek and N. Karabocek, *Polyhedron*, 1998, **17**, 319.
- 8 F. Tuczek and W. Bensch, *Inorg. Chem.*, 1995, **34**, 1482.
- 9 B.J. Hathaway, G. Wilkinson, R.G. Gillard and J.A. McCleverty, Eds. *Comprehensive Coordination Chemistry*, Pergamon, Oxford, 1987, vol. 2, p.413.
- 10 R.S. Himmelwright, N.C. Eickman, C.D. LuBien and E.I. Solomon, *J. Am. Chem. Soc.*, 1980, **102**, 5378.
- 11 R.L. Carlin, *Magnetochemistry*, 1986, (Springer-Verlag, New York).
- 12 T.N. Doman, D.E. Williams, J.F. Banks, R.M. Buchanan, H.R. Chang, R.J. Webb and D.N. Hendricks, *Inorg. Chem.*, 1990, **29**, 1058.
- 13 P. Comarmond, P. Plumere, J.M. Lehn, Y. Agnis, R. Louis, R. Weiss, O. Kahn, I. Morgenstern-Badarau, *J. Am. Chem. Soc.*, 1982, **104**, 6330.
14. Suharti, M.J.F. Strampraad, I. Schroder and S. de Vries, *Biochemistry*, 2001, **40**, 2632.
15. B.F. Van Gelder and H. Beinert, *Biochim. Biophys. Acta*, 1969, **189**, 1.
- 16 P.M. Kroneck, J. Riester, W.G. Zumft and W.E. Antholine, *Biol. Met.*, 1990, **3**, 103.
- 17 W.E. Antholine, D.H. Kastrau, G.C. Steffens, G. Buse, W.G. Zumft and P.M. Kroneck, *Eur. J. Biochem.*, 1992, **209**, 875.
18. P. Lappalainen, R. Aasa, B.G. Malmstrom and M. Saraste, *J. Biol. Chem.*, 1993, **268**, 26416.
- 19 C. von Wachenfeldt, S. de Vries and J. van der Oost, *FEBS Lett.*, 1994, **340**, 109.