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Synthesis and Characterization of $M^{II}(N_2S)(SR)$ Complexes (M = Co, Cu): Synthetic Approximations to the Active Site in Poplar Plastocyanin

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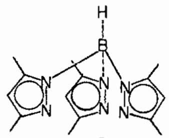
The blue (Type 1) copper binding site has been under intensive study recently because of its occurrence in a number of biologically important copper proteins and its unusual spectroscopic properties. An important development in the structural chemistry of these proteins has been the elucidation by single crystal X-ray diffraction methods of the structure of the Cu^{II} poplar (*Populus nigra Var. italica*) plastocyanin [1] and *Pseudomonas aeruginosa* azurin [2]. The copper ion in both cases has been shown to coordinate to two histidine nitrogen atoms, a cysteine sulfur atom, and a methionine sulfur atom with a distorted tetrahedral metal coordination sphere. Although there have been intensive efforts directed toward the synthesis of low molecular weight compounds with this $N_2S(SR)$ metal ligation, none has been reported to date. We recently reported the synthesis and characterization of a series of Cu^I, Cu^{II}, and Co^{II} complexes that have a tetrahedral $N_3(SR)$ metal coordination sphere as an approximation to a proposed active site configuration [3–5]. The three-nitrogen donor ligand was hydrotris(3,5-dimethyl-1-pyrazolyl)-borate, $HB(3,5\text{-}Me_2pz)_3^-$, I. Although the metal coordination does not match exactly that of the structurally characterized proteins, the spectral properties of the native and synthetic systems are very similar [3–5]. An important question remaining, however, is how the spectral properties respond to a change from an $N_3(SR)$ to an $N_2S(SR)$ donor set. To answer this question we have synthesized $N_2S(SR)$ analogues of the $N_3(SR)$ complexes previously reported. The $N_2S(SR)$ donor is the new tripod ligand hydrobis(3,5-dimethyl-1-pyrazolyl)(S-*p*-toluenethiolato)borate, $HB(3,5\text{-}Me_2pz)_2(SC_7H_7)^-$, II. We herein communicate our initial results on the synthesis and spectral properties of $Cu^{II}N_2S(SR)$ and $Co^{II}N_2S(SR)$ complexes.



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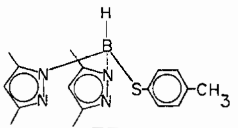

II

Experimental

All chemicals and solvents were handled as described previously [3–5]. Standard Schlenk ware and glove bag techniques were used in the manipulation of complexes described below. Elemental analyses and spectral studies were performed as previously described [3–5].

*Preparation of Potassium Hydrobis(3,5-dimethyl-1-pyrazolyl)(S-*p*-toluenethiolato)borate, $KHB(3,5\text{-}Me_2pz)_2(SC_7H_7)$, II*

Potassium dihydrobis(3,5-dimethyl-1-pyrazolyl)-borate, $KH_2B(3,5\text{-}Me_2pz)_2$, was prepared by a literature procedure [6]. The crude reaction product was dissolved in tetrahydrofuran (THF). Filtration and evaporation of solvent gave a white solid. Residual 3,5-dimethylpyrazole was then removed by sublimation at 80 °C under high vacuum. To 50 ml of THF was added $KH_2B(3,5\text{-}Me_2pz)_2$ (1.2 g, 5.0 mmol) and *p*-thiocresol (*p*-toluenethiol) (0.625 g, 5.0 mmol). The stirred reaction mixture was refluxed at approximately 80 °C for two days. The volume of the reaction mixture was reduced to 25 ml, and the resulting solution poured into 100 ml of hexane. Filtration and evaporation of solvent under reduced pressure yielded a faintly yellow solid, II, which was heated at 75 °C under high vacuum to remove residual *p*-thiocresol.

*Preparation of Thiolato{hydrobis(3,5-dimethyl-1-pyrazolyl)(S-*p*-toluenethiolato)borato}cobalt(II), $Co\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}(SR)$, III*

The $Co\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}(SR)$ complexes with SR = O-ethylcysteinate ($SCH_2CH(NH_2)(COOC_2H_5)$, IIIa, *p*-nitrobenzenethiolate ($p\text{-}SC_6H_4NO_2$, IIIb), and pentafluorophenylthiolate (SC_6F_5, IIIc) were prepared in a manner analogous to that used to synthesize the $Co\{HB(3,5\text{-}Me_2pz)_3\}(SR)$ complexes previously described [5].

Preparation of p*-Nitrobenzenethiolato{hydrobis(3,5-dimethyl-1-pyrazolyl)(S-*p*-toluenthiolato)borato}copper(II), $Cu\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}(p\text{-}SC_6H_4NO_2)$, IV*

The intensely blue complex $Cu\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}(p\text{-}SC_6H_4NO_2)$ was synthesized in a manner analogous to that for the preparation of $Cu\{HB(3,5\text{-}Me_2pz)_3\}(p\text{-}SC_6H_4NO_2)$ described previously [3, 4].

TABLE I. Visible Spectral Data for Co$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}$(SR) and Co$\{HB(3,5\text{-}Me_2pz)_3\}$(SR) Complexes and Several Cobalt(II)–Substituted Proteins.

Compounds	λ, nm (ϵ_m, M^{-1} cm^{-1})	Ref.
Co$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}$ $\{SCH_2CH(NH_2)(COOC_2H_5)\}$, (IIIa)	620 (~300)[a]	
Co$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}$ (p-$SC_6H_4NO_2$), (IIIb)	593 (370)	
	630 (390)	
	655 (330)	
Co$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}$($SC_6F_5$), (IIIc)	590 (310)	
	620 (330)	
	642 (270)	
Co$\{HB(3,5\text{-}Me_2pz)_3\}$ $\{SCH_2CH(NH_2)(COOC_2H_5)\}$	595 (560)	5
	630 (800)	
	650 (735)	
Co$\{HB(3,5\text{-}Me_2pz)_3\}$ (p-$SC_6H_4NO_2$)	586 (246)	5
	655 (670)	
	675 (550)	
Co$\{HB(3,5\text{-}Me_2pz)_3\}$($SC_6F_5$)	570 (270)	5
	605 (350)	
	660 (95)	
Proteins		
Phasedus Vulgaris plastocyanin	500 (~400)	b
	665 (~450)	
Rhus vernicifera stellacyanin	525 (250)	b
	640 (~450)	

[a] The spectrum of IIIa was poorly resolved and complicated by the overlap with the spectrum of Co$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}_2$ (λ = 520 nm, ϵ = 180). [b] D. R. McMillin, R. C. Rosenberg and H. B. Gray, *Proc. Nat Acad. Sci. USA., 71,* 4760 (1974).

Results and Discussion

Synthesis of Ligand and Complexes

The synthesis of $HB(3,5\text{-}Me_2pz)_2(SC_7H_7)^-$, II, is shown in eqn. (1) and is analogous to that for $HB(3,5\text{-}Me_2pz)_3^-$, I, eqn. (2) [6].

$$KH_2B(3,5\text{-}Me_2pz)_2 + HSC_7H_7 \rightarrow KHB(3,5\text{-}Me_2pz)_2(SC_7H_7) + H_2 \qquad (1)$$

$$KH_2B(3,5\text{-}Me_2pz)_2 + H(3,5\text{-}Me_2pz) \rightarrow KHB(3,5\text{-}Me_2pz)_3 + H_2 \qquad (2)$$

The ligand II was designed to have as many properties of I as possible: negative charge, bulky substituents, and the ability to coordinate in a stereospecific manner. With the use of II we are able to synthesize N_2S(SR) complexes without significant changes in geometry or charge from those of the N_3(SR) analogues. Thus, we are able to study the effects on spectral properties of substitution of an S atom for an N atom in the metal coordination sphere, while holding other variables essentially constant.

The $Co^{II}N_2S$(SR) complexes were synthesized by a procedure that is entirely analogous to that for the corresponding N_3(SR) complexes [5]. These N_2-S(SR) complexes have the same colors as the N_3(SR) derivatives: IIIa and IIIb are green, whereas IIIc is blue. Similarly, the $Cu^{II}N_2S$(SR) complex IV was synthesized in a manner analogous to that for the corresponding $Cu^{II}N_3$(SR) complex [3, 4]. At temperatures below –30 °C, IV has an intensely blue color; warming above this temperature yields a reddish-purple complex which is the Cu^I analogue of IV and is also obtained by the reaction of II with Cu^I(p-$SC_6H_4NO_2$) [3, 4]. Warming of IV above ~–30 °C also generates a new Cu^{II} species, presumably Cu$\{HB(3,5\text{-}Me_2pz)_2(SC_7H_7)\}_2$, based on its epr parameters. In an identical manner, $Cu(HB(3,5\text{-}Me_2pz)_3)_2$ is obtained on warming the N_3(SR) complex to ambient temperatures [3, 4].

TABLE II. Visible Absorption Data for Cu{HB(3,5-$Me_2pz)_2(SC_7H_7)$}(p-$SC_6H_4NO_2$) (IV), Cu{HB(3,5-$Me_2pz)_3$}(p-SC_6H_4-NO_2), and Selected Blue Copper Proteins.

Compounds	λ, nm (ϵ_m, M^{-1} cm^{-1})	Ref.
Cu{HB(3,5-$Me_2pz)_2(SC_7H_7)$}(p-$SC_6H_4NO_2$), (IV)	595 (4100)	
Cu{HB(3,5-$Me_2pz)_3$}(p-$SC_6H_4NO_2$)	588 (3900)	3, 4
Proteins		
Spinach Plastocyanin	597 (4900)	a
Pseudomonas aeruginosa azurin	625 (3500)	a
	625 (3500)	b
	625 (5700)	c
Rhus vernicifera stellacyanin	605 (4050)	a

[a] J. A. Fee, *Struct. Bonding, 23,* 1 (1975). [b] A. S. Brill, G. F. Bryce and H. J. Maria, *Biochim. Biophys. Acta, 154,* 342 (1968). [c] P. Rosen and I. Pecht, *Biochem., 15,* 775 (1976).

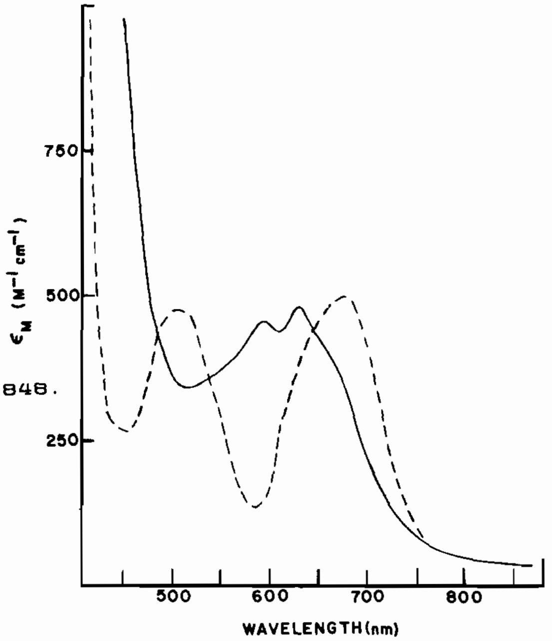

Fig. 1. Optical spectra of Co{HB(3,5-$Me_3pz)_2(SC_7H_7)$}(p-$SC_6H_4NO_2$), IIIb (———), and cobalt(II)–substituted *Phaseolus vulgaris* plastocyanin (– – –) from reference b of Table I.

Spectral Properties of $M^{II}N_2S(SR)$ Complexes

We have shown previously that $Co^{II}N_3$(SR) complexes have electronic spectral properties that in terms of band positions and intensities are unequivocally characteristic of tetrahedral Co^{II} complexes [5]. The splitting of the highest d–d transitions suggested a distorted tetrahedral geometry, which was confirmed in the crystal structure determination of the complex with SR = SC_6F_5 [5]. The close similarity of band positions, shapes, and intensities in the absorption spectra of IIIa–IIIc to those of the N_3-(SR) analogues is excellent evidence for formation of N_2S(SR) complexes with trigonally distorted tetrahedral geometries. Table I lists the band positions and intensities for IIIa–IIIc as well as values for the N_3(SR) analogues; the spectrum of IIIb (SR = p-$SC_6H_4NO_2$) is shown in Fig. 1. Also listed in Table I are data for several Co^{II}–substituted proteins; a typical spectrum is shown in Fig. 1. Comparison of spectral data for the Co^{II}–substituted proteins with those for $Co^{II}N_2S$(SR) and $Co^{II}N_3$(SR) complexes shows a strong similarity of band shapes, positions, and intensities, suggesting again a distorted tetrahedral coordination sphere about the Co^{II} ion in the substituted proteins.

Perhaps the most striking feature of the $Cu^{II}N_2$-S(SR) complex IV is its intense blue color. Table II lists band positions and intensities for IV, the N_3(SR) analogue, and several blue copper proteins. We have shown previously that the intense blue color of the $Cu^{II}N_3$(SR) complexes [3, 4] results largely from $\sigma S \rightarrow Cu$ charge transfer. This assignment has also been made for the ~600 nm absorption of the oxidized native systems [3–5]. A mercaptide $\sigma S \rightarrow Cu$ charge transfer can also be assigned to the 595 nm absorption of IV, based on band position and intensity. Indeed, the close similarity of spectra of IV and the N_3(SR) analogue is surprising in light of the differences in hard–soft character of a pyrazole nitrogen atom and a thioether-like sulfur atom. *A priori,* a soft ligand would favor the Cu^I oxidation state, and a shift to lower energy of the charge transfer band might be expected with a change in X from N to S in an N_2X(SR) coordination sphere. However, it seems that the $\sigma S \rightarrow Cu$ charge transfer band energy is not particularly sensitive to the nature of X in this particular coordination environment. Owing to the breadth of the 600 nm absorption and absorption by the HB(3,5-$Me_2pz)_2$ fragment at wavelengths shorter than ca. 475 nm, it has not been possible to identify

TABLE III. EPR Data for Models and Proteins.

Compound	$g_{\parallel}$	$A_{\parallel} \times 10^3$ cm^{-1}	Ref.
Cu{HB(3,5-Me$_2$pz)$_2$(SC$_7$H$_7$)}(*p*-SC$_6$H$_4$NO$_2$), (IV)	2.169	18.7	
Cu{HB(3,5-Me$_2$pz)$_3$}(*p*-SC$_6$H$_4$NO$_2$)	2.286	17.1	3, 4
Cu{HB(3,5-Me$_2$pz)$_3$}(*p*-OC$_6$H$_4$NO$_2$)	2.308	16.7	3, 4
Proteins			
Spinach plastocyanin	2.226	6.3	a
Rhus vernicifera stellacyanin	2.287	3.5	b, c, d
Pseudomonas aeruginosa azurin	2.260	6.0	e

[a] W. E. Blumberg and J. Peisach, *Biochim. Biophys. Acta, 126,* 269 (1966). [b] T. Vanngard, in 'Biological Applications of Electron Spin Resonance', eds. H. M. Swartz, J. R. Bolton and D. C. Borg, Wiley; New York, N.Y. (1972), Chap. 9. [c] G. H. Rist, J. S. Hyde and T. Vanngard, *Proc. Nat. Acad. Sci., U.S.A., 67,* 79 (1970). [d] B. G. Malmstrom, B. Reinhammer and T. Vanngard, *Biochim. Biophys. Acta, 205,* 48 (1970). [e] T. Stigbrand, B. G. Malmstrom and T. Vanngard, *FEBS Letters, 12,* 260 (1971).

unambiguously a transition involving the metal–thioether functionality.

The EPR parameters for IV are consistent with a distorted tetrahedral N$_2$S(SR) metal inner coordination sphere. Table III lists the $g_{\parallel}$ and $A_{\parallel}$ values of IV, N$_3$(SR), and N$_3$(OR) analogues, and several blue copper proteins. We have shown previously [3, 4], based on well-documented trends in the literature [4, 7–9], that the magnitude of $g_{\parallel}$ decreases along the series N$_3$O > N$_4$ > N$_3$S > N$_2$S$_2$ for copper complexes with a given geometry and charge. This trend mirrors the nephelauxetic effect [10]. On the other hand, the most important factor in determining the magnitude of $A_{\parallel}$ appears to be geometry, with the nature of the ligands and the charge on the complex having much smaller effects. Generally, substitution of an S ligand for an N or O ligand increases the magnitude of $A_{\parallel}$, providing that complex geometry and charge remain the same. The EPR parameters for IV fit these trends very well. Based on the nephelauxetic series [10], the value of $g_{\parallel}$ for IV should be and is smaller than those for N$_3$(SR) and N$_3$(OR) analogues. On the other hand, the magnitude of $A_{\parallel}$ for IV should be and is larger than for these complexes. Finally, the $g_{\parallel}$ values, but not the $A_{\parallel}$ values, of the N$_2$X(SR) complexes are similar to the native proteins, as expected from the aforementioned trends. The differences in both $g_{\parallel}$ and $A_{\parallel}$ most likely result from variations in the geometries of the metal coordination spheres [1–5].

Conclusions

This study extends our previous work on the synthesis and characterization of N$_2$X(SR) complexes that approximate proposed active sites of the blue copper proteins. The spectral properties for compounds with X = N and S reveal two properties of this unusual metal coordination sphere. Firstly, neither the absorption spectra of the Co^{II} complexes nor the σS → Cu charge transfer spectra of the Cu^{II} complexes is particularly sensitive to substitution of an S donor for an N donor. The controversy in the literature [11–15] over the nature of the ligands in the native systems can be understood at least in part by this lack of sensitivity to the nature of X. Secondly, the EPR parameters for Cu^{II} complexes of the same geometry and charge follow well-established trends, as outlined above. Although the magnitude of $g_{\parallel}$ appears to depend on the nature of the ligands, the $A_{\parallel}$ value does not vary greatly with a change in X from N or O to S; rather, the complex geometry appears to be the critical factor in determining the hyperfine constant.

Acknowledgments

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