Physical Studies of Complexes of Co(II), Ni(II) and Zn(II) with Strong Field Thiolate Ligands as well as the Ni(II) Derivative of Azurin.
Evidence that the Protein Constrains the Stereochemistry about the Metal in Azurin

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*Schiff-base adducts of salicylaldehyde (*ortho*-hydroxybenzaldehyde) and* ortho*-mercaptobenzaldehyde with bulky amines t-butylamine or 2,5-diamino-2,5-dimethylhexane have been prepared and used to synthesize a series of Co(II), Ni(II) and Zn(II) complexes. In each case the salicylaldehyde complexes exhibit pseudotetrahedral coordination geometries, but the enhanced ligand-field stabilization energy associated with a thiolate donor strongly favors planar coordination in the case of nickel(II). However, even the nickel(II) complex is pseudotetrahedral when t-butylamine is involved as can be seen from the near infrared absorption spectrum ($\epsilon \cong 30$ M^{-1} cm^{-1} at 1300 nm) and the magnetic moment of the complex in solution (3.0 B.M.). The nickel(II) derivative of azurin has a similar near infrared absorption spectrum indicating that internal interactions of the polypeptide structure impose a pseudotetrahedral coordination geometry on the metal center.*

Introduction

For two decades researchers have been trying to determine the relationship between the electronic spectrum and the structure of the blue copper binding site in proteins. Consensus assignments for the observed electronic transitions are slowly emerging [1–3], now that crystallographic studies of plastocyanin [4, 5] and azurin [5–7] are available. Both plastocyanin and azurin bind a single copper ion in distorted tetrahedral geometry with a N_2SS^* donor set comprised of two imidazole nitrogens from histidine residues as well as a thiolate sulfur and a thioether sulfur from cysteine and methonine residues, respectively. Although pseudotetrahedral geometry is sometimes found in copper(II) chemistry, it is generally not regarded as the preferred geometry. Gray and co-workers have suggested that this geometry may be imposed on copper(II) in the proteins to reduce the Franck-Condon barrier to electron transfer and to confer relatively positive reduction potentials upon the copper sites [2, 3]. Work in our laboratory indicates that other divalent metals probably bind with a similar geometry, at least in the case of azurin [8].

Although advances have been made in modeling blue copper centers with small molecules [9–11], further studies are needed to complete our understanding of the blue copper bonding site. The redox problems associated with copper(II)/thiolate systems complicate their usefulness in model systems. We are attempting to elaborate suitable ligands for model studies using Ni(II), Co(II) and Zn(II) as metal centers. Such an approach is possible because we have been able to replace Cu(II) in blue copper proteins with these ions and to characterize the resulting derivatives [8]. Here we report studies of a series of complexes presenting N_2S_2 donor environments and embodying steric constraints which favor pseudotetrahedral coordination geometries. The most interesting results pertain to the nickel(II) systems where, with the model complexes, a strong preference for planar coordination is observed in the presence of thiolate donors. With this work as a guide, we have been able to locate for the first time near infrared transitions of the Ni(II) derivative of azurin, denoted Ni(II)Az, and to confirm that a non-planar coordination geometry occurs in the protein.

Experimental

The *ortho*-mercaptobenzaldehyde (tsal) was prepared from 2,2′-dithiodibenzoic acid as described elsewhere [12]. The concentration of the aldehyde in solution was determined by weighing the 2,4-

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TABLE I. Magnetic Moments.[a]

Compound[b]	μ_{eff} (B.M.)	Compound	μ_{eff} (B.M.)
Co(tsal$_2$dadmh)	4.84	Co(sal$_2$dadmh)	4.69
Ni(tsal$_2$dadmh)	<0.1[c]	Ni(sal$_2$dadmh)	4.00
Zn(tsal$_2$dadmh)	0.0		
Ni(tsaltbu)$_2$	3.02[d]		
Zn(tsaltbu)$_2$	0.0		

[a] The magnetic properties of all compounds were determined in a solution of 88% $CDCl_3$, 11.8% $CHCl_3$ and 0.2% TMS on the NT-200. The temperature was 23 °C unless otherwise noted. [b] In designating the Schiff-base adducts: tsal denotes *ortho*-mercaptobenzaldehyde, sal denotes salicylaldehyde, tbu denotes t-butylamine and dadmh denotes 2,5-diamino-2,5-dimethylhexane. [c] See discussion in the text. [d] Bertini *et al* [14] report a value of 3.15.

TABLE II. Microanalytical Data.

	Carbon		Hydrogen		Nitrogen	
	calc.	found	calc.	found	calc.	found
Co(tsal$_2$dadmh)	59.8	59.8	5.95	6.05	6.35	6.50
Ni(tsal$_2$dadmh)	59.8	59.6	5.94	5.93	6.35	6.12
Zn(tsaltbu)$_2$	58.7	58.9	6.28	6.57	6.23	6.45
Ni(tsaltbu)$_2$	59.6	59.8	6.38	6.38	6.32	6.42

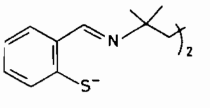
dinitrophenylhydrazone derivative. The Schiff-base ligands were obtained by adding the stoichiometric amount of amine (or polyamine) in absolute alcohol to a measured volume of the tsal solution in an ice bath. After 1 hour, the solution was concentrated under vacuum to remove the ether and then diluted with absolute ethanol to redissolve the ligand and give an approximately 0.1 *M* solution. Stoichiometric amounts of the metal acetate in absolute ethanol were added, and upon standing at 5 °C, crystals were obtained. The complexes were recrystallized in air from a mixture of chloroform and ethanol, except for the cobalt derivatives which were prepared and handled under nitrogen. The azurin and its derivatives were isolated and prepared as described previously [8].

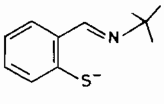
The UV–vis absorption spectra were obtained with a McPherson EU-700 spectrophotometer. All organic solvents used were Mallinckrodt Spect AR grade. The near-infrared spectra were obtained with a Cary-17 spectrophotometer, and the ^2H_2O used was purchased from MSD Isotopes, 99.7% d-atom. The magnetic susceptibilities were measured on a Nicolet Instrument Corporation NT-200 NMR spectrophotometer which is part of the Purdue University Biological Magnetic Resonance Laboratory (regional NIH facility), using the procedure outlined by Evans [13].

Results and Discussion

Characterization of the Complexes

The compounds studied and their respective magnetic moments are presented in Table I. The purity of key complexes from the series has been established by microanalysis (Table II); all others have been judged as pure on the basis of spectroscopic measurements. Ni(tsaltbu)$_2$ [14] and Ni(tsal$_2$dadmh) have been determined to be monomeric in solution by molecular weight measurements, and by analogy all other complexes are assumed to be monomeric.


tsal$_2$dadmh


tsaltbu

Models reveal that the chelate prepared from 2,5-diamino-2,5-dimethylhexane (dadmh) and (thio)-salicylaldehyde cannot easily form a planar complex about a central metal ion for steric reasons. Recently Elder and Hill have reported that Cu(sal$_2$dadmh) deviates appreciably from planar geometry and is best classified as pseudotetrahedral [15]. Consis-

TABLE III. Charge Transfer Transitions of Azurin Derivatives.

Protein	λ_{max}, nm	hν, μm^{-1}	Assignment
Co(II)Az	333	3.00	$\sigma S \rightarrow Co(II)$
	373	2.68	$\pi S \rightarrow Co(II)$
	408	2.45	$\pi S \rightarrow Co(II)$
Ni(II)Az	442	2.28	$\sigma S \rightarrow Ni(II)$
	500	2.00	$\pi S \rightarrow Ni(II)$
	562	1.78	$\pi S \rightarrow Ni(II)$

tent with these results, Ni(sal$_2$dadmh) and Co(sal$_2$dadmh) exhibit magnetic moments in keeping with pseudotetrahedral geometries (Table I). Although the sulfur-analogue Co(tsal$_2$dadmh) is also pseudotetrahedral, this cannot be the case for Ni(tsal$_2$dadmh) which is effectively diamagnetic in solution. Since the latter complex is a monomer, the donor atoms must be configured in a planar fashion about nickel(II) despite the steric constraints. It is well known that strong field ligands tend to induce planar geometry about nickel(II), hence in the case of Ni(tsal$_2$dadmh) the increased ligand field stabilization energy associated with the planar geometry apparently overcomes the steric forces. The relative energies of the planar and pseudotetrahedral forms are not known, but solutions of Ni(tsal$_2$dadmh) exhibit weak paramagnetism at ~40 °C which might be explained by formation of small amounts of pseudotetrahedral forms.

In order to obtain a pseudotetrahedral complex of nickel(II) with an N_2S_2 donor set we prepared Ni(tsaltbu)$_2$. In agreement with Bertini and coworkers [14] we find this complex has a magnetic moment consistent with a pseudotetrahedral coordination geometry (Table I).

Electronic Spectra

At the outset we were interested in correlating the S → M(II) charge-transfer transitions of the N_2S_2 complexes with analogous transitions in the spectra of metal-replaced derivatives of azurin, denoted M(II)Az. The relevant bands from the protein spectra are presented in Table III [8]. (The σ and π designations in Table III refer to the type of overlap between the metal-acceptor orbitals and the donor orbitals of the cysteine sulfur.) The near-UV spectra of the Zn(II), Co(II) and Ni(II) complexes of the tsaltbu and (tsal)$_2$dadmh ligands are presented in Figs. 1 and 2. We had hoped that difference spectra would enable us to resolve intraligand and charge-transfer transitions since the latter should be displaced to much higher energies in the case of the Zn(II) complexes. However, no clear separation has been achieved. Thus neither Co(tsaltbu)$_2$ nor Ni(tsalbu)$_2$ exhibit a maximum at 405 nm where an intense intraligand transition of the zinc analogue occurs. In fact, difference spectra suggest that at least some of the intraligand band intensity has shifted to ~450 nm in the spectra of the Co(II) and Ni(II) complexes.

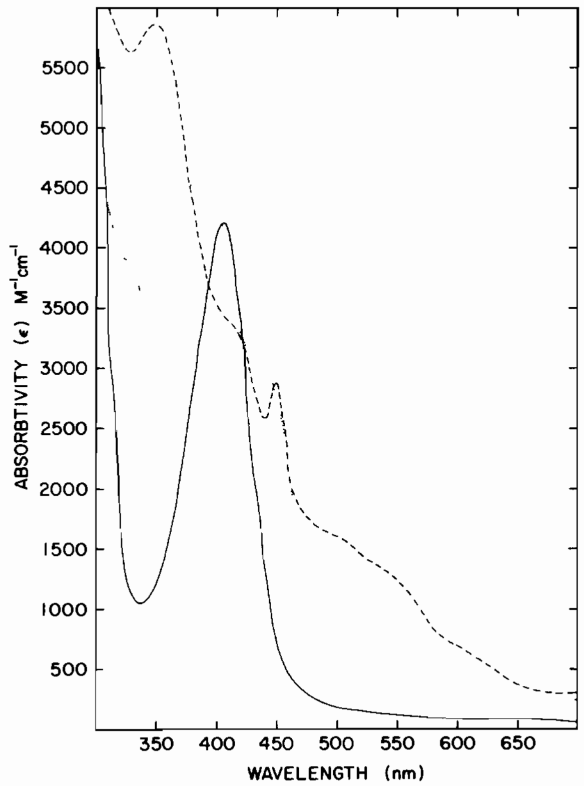


Fig. 1. The near UV and visible spectra of tsaltbu complexes: Zn(tsaltbu)$_2$ (———), Ni(tsaltbu)$_2$ (------) and Co(tsaltbu)$_2$ (·······) in CHCl$_3$ at 22 °C.

In view of the complications which can arise from interactions between chromophores – hypochromic effects, *etc.* – a complete analysis will not be attempted, but a few comments can be made regarding the nickel complexes. Moderately intense bands occur as shoulders in the spectra of Ni(tsaltbu)$_2$ and Ni(tsal$_2$dadmh) at ~525 and ~475 nm, respectively. These bands are well below the intraligand bands of the Co(II) and Zn(II) analogues and are plausibly assigned as S → Ni(II) charge-transfer bands, although the intensities appear to be rather weak for a $\sigma S \rightarrow Ni(II)$ assignment. Evidently, arylthiolate ligands of the type employed here complicate even the most rudimentary analysis of the charge transfer spectra.

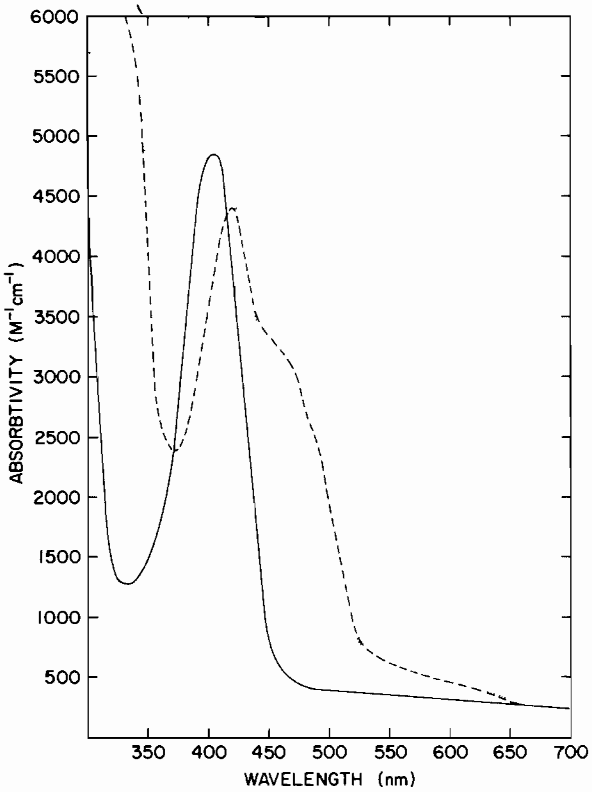


Fig. 2. The near UV and visible spectra of the tsaldmdah complexes: Zn(tsaldmdah) (————), Ni(tsaldmdah) (------) and Co(tsaldmdah) (·······) in $CHCl_3$ at 22 °C.

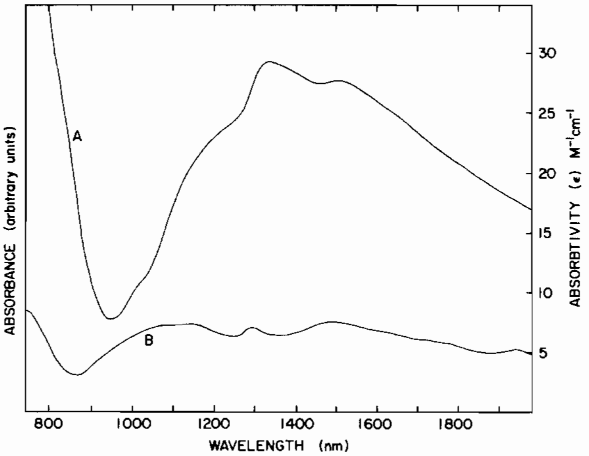


Fig. 3. The near infrared absorption spectra of Ni(tsaltbu)$_2$ in CS_2 solution (A) and the solid state (B).

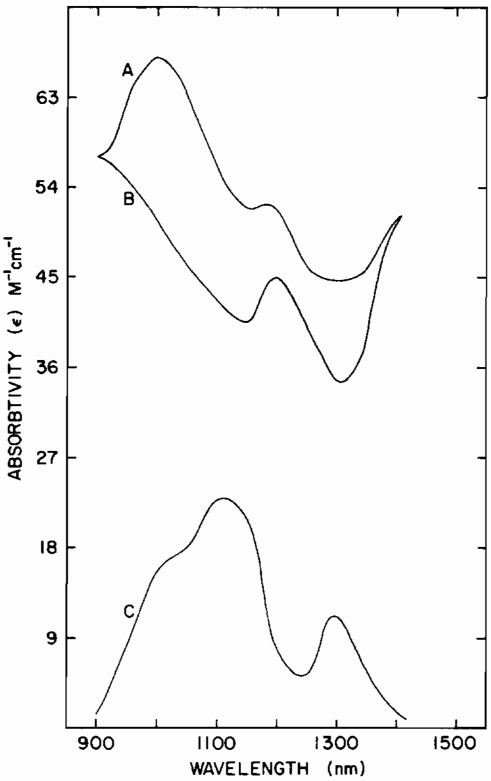


Fig. 4. The near infrared spectra of Ni(II)Az (A), Zn(II)Az (B) and a difference spectrum (C) in ^2H_2O (99.7% d-atom). The ε values in the difference spectrum are subject to an error of about 10%.

A more useful means of distinguishing tetrahedral and planar forms of Ni(II) is found in the d–d spectra. Tetrahedral complexes give a transition in the near infrared, labeled $\nu_2(^3T_1(F) \rightarrow {}^3A_2(F))$, whereas the d–d bands all occur at higher energies in planar complexes. In Fig. 3 we present the near infrared absorption spectra of Ni(tsaltbu)$_2$ in solution and the solid state (Ni(tsal$_2$dadmh) gives no absorption in this region). Three near-infrared transitions are apparent, each with relatively low molar absorptivities, the splitting being consistent with the distorted tetrahedral symmetry which is present. These results confirm the structural assignments based on the magnetic data. Moreover, they establish the conditions, especially the concentration requirement, necessary to detect analogous d–d transitions which might occur in the case of Ni(II)Az.

Near Infrared Spectrum of Ni(II)Az

Vibrational overtones complicate near-infrared spectra, especially in the case of protic solvents, so all protein spectra have been measured in ^2H_2O. In Fig. 4 the near infrared spectra of Ni(II)Az and Zn(II)Az are presented as well as the relevant difference spectrum. The difference spectrum exhibits a distinct maximum at *ca.* 1020 nm ($\epsilon \cong 23\ M^{-1}$ cm^{-1}) and another apparent maximum at *ca.* 1320 nm ($\epsilon \sim 13\ M^{-1}\ cm^{-1}$), although the latter occurs in the same region as a solvent peak and is less certainly established. The observation of near infrared absorption by Ni(II)Az proves that the nickel center in Ni(II)Az is not planar and indicates that a pseudotetrahedral geometry obtains as in the case of the naturally occurring copper analogue. A similar conclusion has been reached on the basis of ^{1}H-NMR studies of Ni(II)Az [16]. This result is significant because, as pointed out by Lum and Gray [17] and as discussed above, Ni(II) can be expected to prefer a planar geometry in the presence of a strong field thiolate donor. That a planar geometry is not achieved supports the notion that internal interactions of the protein confer a rather rigid geometry on the metal binding site of blue copper proteins.

Conclusions

The results show that four-coordinate complexes of Ni(II) involving thiolate ligands preferentially exhibit planar geometry unless severe steric constraints are introduced. Planar geometry is not, however, realized in the case of Ni(II)Az despite the presence of cysteine and methionine sulfur donors, suggesting that the secondary and tertiary structure of the polypeptide work to enforce a rather rigidly defined stereochemistry about the metal in blue copper proteins. Although a pseudotetrahedral coordination geometry is not generally considered ideal for copper(II), it is interesting to note that the proteins bind copper(II) very effectively and that they may even exhibit a kinetic selectivity for copper(II) over other divalent metal ions [8]. Whatever the strain that exists in the oxidized form of the blue copper site, the protein obviously has a strong affinity for copper(II).

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