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(21) Diazomethane cycloadditions are HO(diazomethane)–LU(olefin) controlled,[22] whereas the other 1,3-dipolar cycloadditions of Table II receive contributions from both HO–LU interactions (Sustmann's type II).[23] The Diels–Alder reaction of the tetrazine dicarboxylate **7** is LU(diene)–HO(dienophile) controlled. The ionization potentials (cyclohexene, 9.18; **1**, 8.97; **2**, 9.05; **4**, 8.63 eV) do not provide a key to the understanding of the rate sequences.
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Spectroscopic Studies on Cobalt(II) Metallothionein: Evidence for Pseudotetrahedral Metal Coordination

Sir:

The elucidation of the spatial structure of the metal binding sites in metallothionein, a widely occurring metal- and sulfur-rich protein, is indispensable for the understanding of the proposed involvement of this protein in metabolism, homeostasis and detoxification of zinc and other metals.[1] The most characteristic features of mammalian metallothioneins are the presence of seven atoms of Zn and/or Cd and of 20 cysteinyl residues (Cys) per chain weight of 6100. The position of these residues in the amino acid sequence is extremely preserved in evolution.[2] In contrast to most other metal thiolate proteins for which Cys-X-Y-Cys metal-binding (X, Y = amino acid residues other than Cys) sequences are typical,[3] mammalian metallothioneins contain seven Cys-X-Cys sequences.[2]

There is unambiguous evidence that all 20 cysteinyl residues participate in metal thiolate coordination.[4a,5] From the Cys/metal ratio and from complexometric titration studies and charge measurements, it has been inferred that the minimum metal binding unit of metallothionein contains as a rule three thiolate ligands and one or possibly more as yet unidentified nonprotogenic ligands. Based on sequence information and arguments of stoichiometry, each zinc and other bivalent metal ion is thought to be bound to the protein through mercaptide bonds with a Cys-X-Cys chelating structure and, in all but one case, also with an additional cysteinyl residue brought into position by appropriate tertiary structure folding.[4b] Recent ^{1}H NMR studies have established the existence of a compact and well-defined structure of metallothionein.[6]

In the present work, the coordination geometry of the metal binding sites of metallothionein is explored by means of optical and magnetooptical spectroscopy using the cobalt(II)-substituted protein. Replacement of the metal by cobalt(II) has been commonly employed in the study of zinc metalloenzymes and metalloproteins.[7] Because of their similarity, such a substitution does not measurably alter the overall protein conformation and often preserves the catalytic function of the enzyme. Cobalt(II) is particularly suitable as an environmental probe owing to its paramagnetism and the sensitivity of its d–d transitions to changes in coordination geometry.[7]

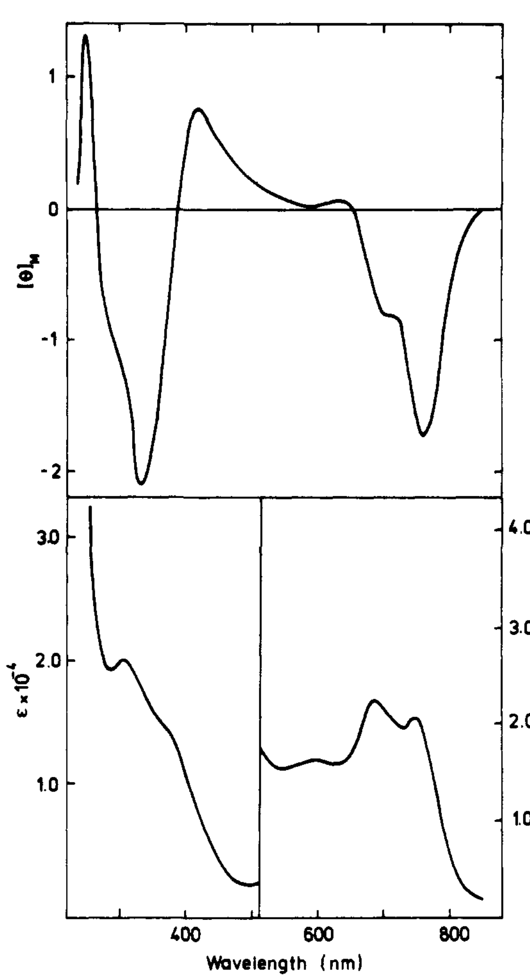

Figure 1. Magnetic circular dichroism (top) and absorption spectra (bottom) of cobalt(II) metallothionein. Protein concentration was 1.85 × 10^{-4} M in 0.1 M Tris-HCl. Measurements were performed using a path length of 1 cm or 1 mm. Molar absorptivity, ϵ, and magnetic ellipticity, $[\theta]_M$, are referred to the mol wt 6100 of the peptide chain of metallothionein. The natural ellipticity has been subtracted from the total ellipticity measured in the magnetic field before normalizing to unit field (magnetic field employed: 40 kG).

Metallothionein 1A was isolated from horse kidney according to Kojima et al.[4b] Cobalt(II) metallothionein containing 6 to 7 g-atoms of cobalt(II)/mol was prepared by the addition of 7 mmol of $CoSO_4$ to 0.7 mmol of metal-free protein (apometallothionein) obtained by dialyzing native metallothionein 1A against three changes of 0.1 M HCl and subsequent neutralization of the sample to pH 8 by Trizma base [tris(hydroxymethyl)aminomethane]. All preparative steps and measurements were carried out under nitrogen. The excess of cobalt(II) ions was removed by stirring with Chelex 100 followed by filtration. At neutral pH cobalt(II) metallothionein has a green color.

Figure 1 (bottom) shows the electronic absorption spectrum of cobalt(II) metallothionein. In the visible region broad bands with maxima at 600, 682, and 743 nm are discernible with molar absorptivities of ϵ 1560, 2220, and 2170 M^{-1} cm^{-1}, respectively. Similar spectra have also been reported for in-

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organic tetrathiolate complexes[8] and for cobalt derivatives of crystallographically defined metalloproteins where the $[Co(Cys^-)_4]^{2-}$ (Cys = cysteinyl residue) coordination is present, e.g., for cobalt(II) rubredoxin[9] and for horse liver alcohol dehydrogenase in which the zinc at the noncatalytic metal binding site is replaced.[10,11]

Evidence for tetrahedral symmetry comes also from the near-infrared spectrum of cobalt(II) metallothionein in D_2O (not shown) which exhibits a broad band with a maximum at 1230 nm (ϵ 720 M^{-1} cm^{-1}) and which is attributable to the spin-allowed ligand-field transition ν_2 $[^4A_2 \rightarrow {}^4T_1(F)]$.[22] However, both the position of the visible bands and their intensities suggest that the tetrahedral coordination of cobalt in metallothionein is distorted. The splitting of the spin allowed ν_3 $[^4A_2 \rightarrow {}^4T_1(P)]$ transition into three components with an energy separation of 2000 and 1200 cm^{-1} is larger than that to be expected from the spin-orbit coupling alone. This is most likely attributable to distortion from T_d symmetry which results in the removal of the degeneracy of the upper state $^4T_1(P)$. The molar absorptivity of ϵ 2220 M^{-1} cm^{-1} of the highest absorption maximum at 682 nm, i.e., ~300 M^{-1} cm^{-1}/cobalt(II), is also compatible with pseudotetrahedral symmetry (ϵ >250 M^{-1} cm^{-1}).[12]

Evidence for tetrahedral coordination is provided also by magnetic circular dichroism measurements (MCD) of cobalt(II) metallothionein (Figure 1, top). The low-energy region of the MCD spectrum shows a strong negative band at 762 nm with a shoulder at 687 and a small positive band at 615 nm, all lying in the region of the ν_3 $[^4A_2 \rightarrow {}^4T_1(P)]$ transition. The same overall pattern was also observed and theoretically predicted for a number of inorganic tetrahedral and pseudotetrahedral complexes[14] as well as in proteins possessing this coordination geometry.[15] The most direct evidence for cobalt–sulfur coordination is given by the high energy region of the spectra. The absorption spectrum (Figure 1, bottom) exhibits a shoulder at ~400 and a band at 310 nm with molar absorptivities of 14 600 and 19 800 M^{-1} cm^{-1}, respectively. The MCD spectrum (Figure 1, top) shows in the same region two oppositely signed MCD bands (at 330 and 420 nm) and also a shoulder at 280 and a narrow positive band at 253 nm. The position of the absorption band at 310 nm and its intensity [ϵ_{310} 980 M^{-1} cm^{-1} per Co(II)–thiolate bond] are diagnostic of a charge-transfer transition from thiolate ligands to the central cobalt(II) ion [S → Co(II)] and are in excellent agreement with those of the above-mentioned cobalt(II)-substituted proteins and model systems.[8a,10,11,13] Very probably, the 400-nm shoulder is also attributable to a S → Co(II) charge-transfer transition.[16] Since metallothionein lacks aromatic amino acids, it is in fact likely that all high-energy absorption of cobalt(II) metallothionein recorded in this study is of charge-transfer origin.

The virtual identity of the high energy MCD spectrum and of the CD spectrum (not shown) of cobalt(II) metallothionein with those of cobalt(II) rubredoxin, a protein in which by X-ray crystallographic analysis[17] the involvement of four cysteinyl mercapto groups in metal coordination is documented, implies that, in metallothionein too, all four ligands are made up of the same sulfur-containing side chains. A similar implication was made on the basis of ^{113}Cd NMR measurements.[18] Since the protein contains only 20 cysteinyl residues, it follows, however, that the seven metal ions can be bound to four sulfur ligands only if some of the cysteinyl thiolate groups are bound to two metal ions forming sulfur-bridged metal clusters. This inference fits well with the result of a recent ^{113}Cd NMR study in which ^{113}Cd–^{113}Cd coupling through cysteinyl sulfur was observed,[18a] as well as with evidence from dark-field electron microscopy of metallothionein revealing the existence of three metal-binding domains.[19] The existence of metal clusters is also suggested by the observation that at 3.9 K cobalt(II) metallothionein gives a much weaker electron spin resonance signal than would be expected from the amount of cobalt(II) bound to the protein,[20] although the position of the signal (*g* value, 5.9) is compatible with the presence of high-spin cobalt(II).[21] The low intensity could be caused by antiparallel spin coupling of neighboring cobalt(II) complexes. Moreover, more detailed studies on spin diluted samples and/or magnetic susceptibility measurements are required to obtain an unambiguous explanation of this phenomenon.

Recent ^{113}Cd NMR measurements have emphasized the differences between the seven metal-binding sites in metallothionein.[18b] The present study stresses rather the opposite. The existence of well-resolved absorption and MCD spectra in cobalt(II) metallothionein and their resemblance to those of model compounds document a remarkable similarity of the organization of these sites. Their structural alikeness must be viewed as yet another manifestation of the strong selectional pressure that has operated on metallothionein during evolution and hence of the functional importance of these metal thiolate complexes.

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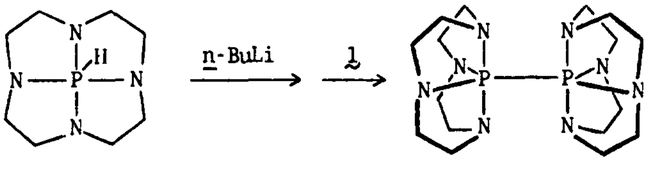
Synthesis and Structural Study of the First P(V)-P(V) Compound

Sir:

We report herein the synthesis and X-ray study of the first compound known to contain a P(V)-P(V) bond. Reaction of cyclen fluorophosphorane (**1**)[1,2] with the lithiated derivative of the related cyclen phosphorane (**2**)[3] resulted in the formation of the dicyclenphosphorane (**3**, $C_{16}H_{32}N_8P_2$) in 20-30% yield.[4]



2 3

The mass spectrum of **3** shows a molecular ion at *m/e* 398. The physical and spectroscopic properties of **3** are consistent with the polycyclic structure: mp >300 °C (sealed under N_2), sublimes at 150 °C (0.1 mm), soluble in warm hexane, ^{31}P chemical shift −36.8ppm (C_6D_6),[5] and a symmetrical AA′BB′ ^1H NMR spectrum. The structure was confirmed by single-crystal X-ray diffraction analysis of colorless crystals of **3** grown from warm hexane by cooling and slow evaporation at room temperature.

Crystal Data. $C_{16}H_{32}N_8P_2$, orthorhombic, space group *Ccca* ($D2h^{22}$-No. 68),[6] with a = 14.777 (6), b = 14.549 (6), c = 8.664 (3) Å; z = 4; 1074 independent reflections measured on an Enraf-Nonius CAD4 automated diffractometer, using graphite-monochromated Mo $K\bar{\alpha}$ radiation and the θ-2θ scan mode, out to a maximum $2\theta_{\mathrm{Mo}\ K\bar{\alpha}}$ of 55°. The structure was solved using direct methods (MULTAN). Full-matrix least-squares refinement[7] (anisotropic for nonhydrogen atoms, isotropic for hydrogen atoms) has led to a conventional unweighted residual $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ of 0.038 for the 850 reflections having $I \geq 2\sigma_I$.

The molecule has crystallographic 222 symmetry, with the two phosphorus atoms lying on a 2-fold axis parallel to $\vec{a}$. Each half of the dimer has approximate *mm* symmetry, with the psuedo-mirror planes passing through a pair of nitrogen atoms and intersecting at the phosphorus atom. The P-P bond length of 2.264 (2) Å is 0.06 Å longer than twice the single bond covalent radius of phosphorus.[8a] Crowding between halves of the dimer is mitigated by a rotation about the P-P bond of 70.6° away from an eclipsed configuration. The distance between eclipsed axial N atoms would be 2.546 (4) Å (0.5 Å less than the van der Waals sum)[8b] in the absence of such a rotation. In the actual structure this distance is 3.301 (4) Å, and the only close contacts between halves of the dimer involve H atoms. With respect to the geometry about phosphorus, the structure is displaced 32.9% along the Berry coordinate (unit vectors) from the trigonal bipyramid toward the rectangular pyramidal configuration.[9,10] In keeping with trigonal-bipyramidal geometry, the P-N_{ax} bond is 0.088 (4) Å longer than the P-N_{eq} bond. (See Figure 1 caption.)

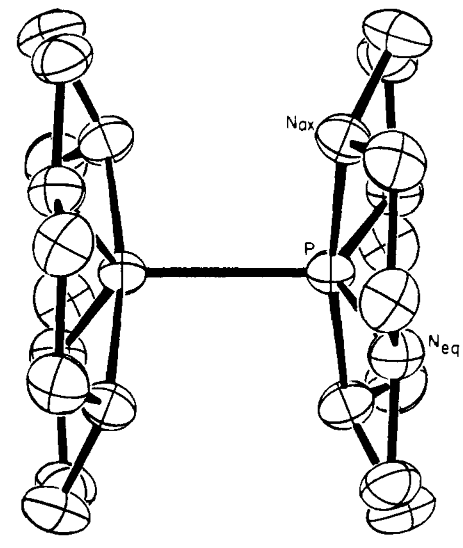


Figure 1. ORTEP plot of the cyclen phosphorane dimer viewed along the 2-fold axis parallel to $\vec{c}$, with thermal ellipsoids shown at the 50% probability level. Bond lengths: P-P = 2.264 (2), P-N_{ax} = 1.782 (2), P-N_{eq} = 1.694 (2) Å. Bond angles: N_{ax}-P-N_{ax} = 169.6 (1), N_{eq}-P-N_{eq} = 131.1 (1), N_{eq}-P-P = 114.4 (1), N_{ax}-P-P = 95.2 (1), N_{ax}-P-N_{eq} = 87.8 (1) and 87.9 (1)°.

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