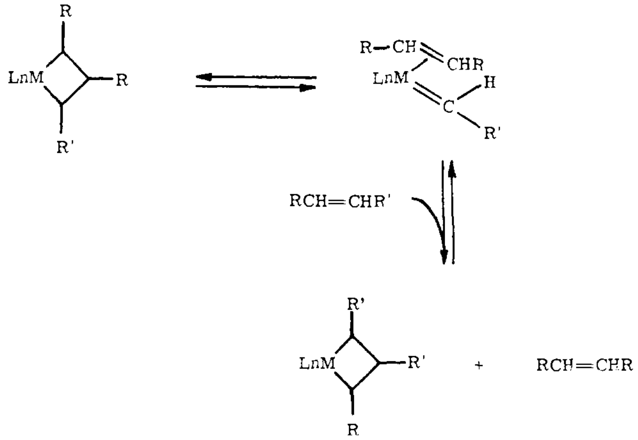
Scheme II



**4.** An attractive mechanism for metallacycle exchange with olefin or acetylene and therefore olefin metathesis is shown in Scheme II.

Whether the reaction proceeds through a diolefin–carbene or a free metal–carbene complex is not yet clear; this question will require further work. However, the former complex is formally a $20\ e^-$ species. Thus, there may be two classes of metathesis catalysts, those involving metal–carbene complexes[17] and those in which metallacyclobutanes are the chain-carrying species. Each class of catalyst would be expected to show different kinetic parameters and stereoselectivities.

Although previous work has provided evidence supporting a metal carbene mechanism, the above reactions give detailed structural information about the intermediates and provide evidence for two classes of catalysts. Reaction 3 is a model of the anticipated mechanism while 4 and 5 provide a new mechanistic pathway.

**Acknowledgment.** Support from the National Science Foundation (CHE-7904814) and helpful suggestions of F. Tebbe are gratefully acknowledged.

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(17) Wengrouins, J. H.; Schrock, R. R.; Churchill, M. R.; Wissert, J. R.; Youngs, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 4515.

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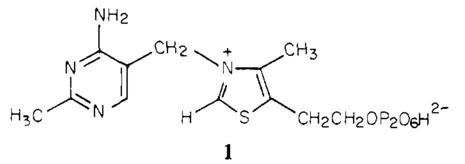
## A Model for Coenzyme–Metal Ion–Apoenzyme Interactions: Crystal Structure of the Ternary Complex [(Thiamine pyrophosphate)(1,10-phenanthroline)aquacopper]–Dinitrate–Water

*Sir:*



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Thiamine pyrophosphate (**1**) (the cofactor form of vitamin $B_1$) is a coenzyme for a number of enzymatic processes catalyzing the decarboxylation of α-keto acids and the transfer of aldehyde or acyl groups. There is strong evidence suggesting that coenzyme thiamine pyrophosphate binds to the apoenzyme in a way where the thiamine ring moiety of the thiamine pyrophosphate molecule stacks with the indole moiety of the tryptophan residue in the active center of the apoenzyme.[1] Additionally, it has also been recognized that divalent metal ions such as Mg(II) and Mn(II) ions are also required as cofactor for the holoenzyme formation as well as the catalytic activity,[2] but their detailed roles and binding modes in their interactions with the coenzyme and apoenzyme are not as well-known. In this regard, of special interest is the observation that the charge-transfer complex between the tryptophan residue in yeast apopyruvate decarboxylase and thiochrome chromophore in thiochrome pyrophosphate, a competitive inhibitor with thiamine pyrophosphate, could be formed only in the presence of Mg(II) ions.[3] Sigel and co-workers have extensively demonstrated that metal ions could stabilize the aromatic ring stacking between nucleotide bases and heterocyclic amines by forming a metal–phosphate bridge; otherwise, such charge-transfer interactions would usually be weak.[4] In the present enzyme system, metal ions could, therefore, be expected to play such a role since thiamine pyrophosphate involves both the phosphate and ring moieties, and a proposed hypothetical structure of such a ternary adduct is shown in Figure 1.[5] In order to substantiate the formation of such a metal ion bridged stacking adduct and to elucidate its stereochemistry, we have undertaken X-ray investigations of the ternary complexes containing thiamine pyrophosphate, divalent metal ions, and aromatic heterocyclic amines such as 1,10-phenanthroline (phen), 2,2′-bipyridyl, and 2,2′-dipyridylamine which are known as useful models for aromatic amino acids.[4] We have reported here the preparation and the structure of the ternary thiamine pyrophosphate–Cu(II)–phen complex, which involves a metal–pyrophosphate bridge that connects the two constituents, as a possible model for coenzyme–metal ion–apoenzyme interactions. The possibility of the structural change from the unstacked opened structure found in the present complex to the stacked folded structure is also shown in terms of the conformational change about the ethyl ester pyrophosphate (C5α–C5β–O5γ–P5δ) side chain of the thiamine pyrophosphate molecule.

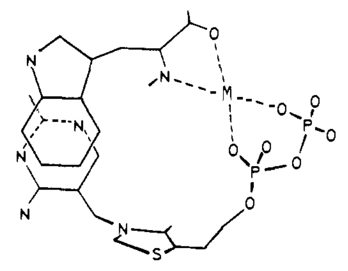

**Figure 1.** A proposed hypothetical structure of the M(thiaminine pyrophosphate)(tryptophan) ternary complex in the active center of the enzyme.

The complex was prepared from thiamine pyrophosphate, phen, and $Cu(NO_3)_2{\cdot}2H_2O$ in an equimolar (0.1 mmol) ratio. The mixture (pH ca. 3) was allowed to stand at room temperature. Pale blue thin plates formed after ca. 3 weeks. They were collected, washed with a little water, and air dried. Precession and Weissenberg photographs indicated that the crystals were monoclinic with the systematic absence of $h0l$ ($h + l = 2n + 1$) and $0k0$ ($k = 2n + 1$), consistent with space group $P2_1/n$. Cell constants of $a = 11.005$ (2), $b = 43.984$ (12), $c = 6.775$ (3) Å, and $\beta = 92.59$ (3)° were determined from 13 high-order reflections ($20° < 2\theta < 29°$) on a Rigaku four-circle automated diffractometer ($T = 23$ °C, $\lambda = 0.71069$ Å). The unit-cell volume is

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(1) Kochetov, G. A.; Usmanov, R. A.; Mevkh, A. T. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 1619–1626. Kochetov, G. A.; Usmanov, R. A. *Ibid.* **1970**, *41*, 1134–1140. Heinrich, C. P.; Moack, K.; Wiss, O. *Ibid.* **1971**, *44*, 275–279.

(2) Gallo, A. A.; Hansen, I. L.; Sable, H. Z.; Swift, T. J. *J. Biol. Chem.* **1972**, *247*, 5913–5920.

(3) Wittorf, J. H.; Gubler, C. J. *Eur. J. Biochem.* **1970**, *14*, 53–60.

(4) See, for example: Chaudhuri, P.; Sigel, H. *J. Am. Chem. Soc.* **1977**, *99*, 3142–3150.

(5) This model can also be shown to be compatible with a similar complex with thiochrome pyrophosphate by the use of space-filling models (CPK atomic models by Ealing Corp.), although thiochrome has a planar tricyclic structure.

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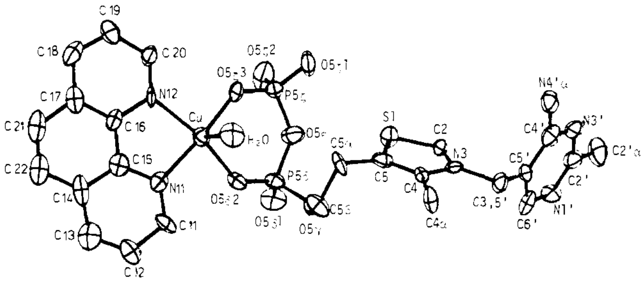

**Figure 2.** The molecular structure of the [(thiamine pyrophosphate)-(1,10-phenanthroline)aquacopper]$^{2+}$ unit, showing the metal ion bridge formation between the pyrophosphate group of the thiamine pyrophosphate molecule and the phen ligand and also showing the "extended opened" structure of the ternary complex.

3266.2 Å$^3$, yielding a calculated density of 1.684 g cm$^{-3}$ for $M_r$ = 828.097, $[\mathrm{Cu(C_{12}H_{18}N_4O_7P_2S)(C_{12}H_8N_2)(H_2O)]\cdot(NO_3)_2\cdot H_2O}$, and $Z = 4$. The density determined by flotation in a methyl iodide/chloroform mixture was 1.67 g cm$^{-3}$. The crystal with six faces of the form {100}, {010}, and {101} was mounted such that the $c$ axis was nearly parallel to the $\phi$ axis of a Rigaku four-circle diffractometer. The distances between parallel faces were 0.0154, 0.0068, and 0.0375 cm, respectively. Integrated intensities were collected by the $2\theta-\omega$ scan method with graphite-monochromated Mo K$\alpha$ radiation.[6] A scan rate of 2 deg min$^{-1}$ was employed. Stationary background counts of 10 s were taken at the lower and upper limits of each scan. Three standard reflections were monitored before every 50 measurements, but no deterioration was observed. The diffraction pattern was in general quite weak and did not extend much beyond $2\theta = 35°$, mostly due to the small crystal size. A total of 4192 independent intensities in the range $2\theta < 45°$ were measured. The 1834 reflections for which $F_o > 3\sigma(F_o)$ were used in the solution and refinement of the structure. Intensities were corrected for Lorentz and polarization effects. The linear absorption coefficient of this compound for Mo K$\alpha$ radiation is 9.4 cm$^{-1}$, and calculated transmission factors varied from 0.98 to 1.0,[7] and then no absorption correction was made. The structure was solved by Patterson and Fourier methods and refined by the block-diagonal least-squares method. A total of 46 atoms out of the 51 nonhydrogen atoms were assigned anisotropic thermal parameters, and one nitrate atom and one crystallization water atom[8] were assigned isotropic ones. The function $\Sigma w(F_o - |F_c|)^2$ was minimized with the weighting factor ($w$) equal to $1/\sigma(F_o)^2$. The current discrepancy indices $R_F$[9] and $R_{wF}$ are 0.092 and 0.073, respectively, and the standard deviation of one observation of unit weight is 2.3 for 1834 reflections ($m$) and 435 variables ($n$), from which $m/n = 4.2$. No unusual trend was observed in an analysis of $\Sigma w(F_o - |F_c|)^2$ as a function of either $\sin\theta/\lambda$ or $F_o$.[10] Neutral atomic scattering factors[11] were used with Cu, S, and P corrected for anomalous dispersion.[12] Current positional and thermal parameters, bond distances and angles, and observed and calculated structure factor amplitudes are found in the Supplementary Material.[13]

The complex is composed of the discrete [(thiamine pyrophosphate)(1,10-phenanthroline)aquacopper]$^{2+}$ cation, two nitrate anions, and a crystallization water molecule. Figure 2 shows the molecular structure of the [(thiamine pyrophosphate)(1,10-phenanthroline)aquacopper]$^{2+}$ unit, where the thiamine pyrophosphate is a neutral zwitterion with the pyrimidine ring protonated at N1′[14] and the pyrophosphate group doubly ionized.[15] The Cu atom is in a distorted square-pyramidal environment, with the basal plane defined by two oxygens of the pyrophosphate moiety [$\mathrm{Cu-O_{av}}$ = 1.92 (3) Å] and the bidentate phen ligand [$\mathrm{Cu-N_{av}}$ = 2.01 (1) Å], and the axial position occupied by a water molecule [$\mathrm{Cu-H_2O}$ = 2.31 (1) Å]; the Cu atom is displaced by 0.22 Å out of this basal plane toward the axial water ligand.

The conformation of thiamine pyrophosphate is important for several aspects of the catalytic mechanism.[16] The thiamine moiety exhibits the characteristic F conformation,[17] which is usually observed for thiamines with no substituent at C2,[18] with respect to the C3,5′ methylene bridge atom: $\phi_T$[17] = C5′–C3,5′–N3–C2 = −1° and $\phi_P$ = N3–C3,5′–C5′–C4′ = 95°. The dihedral angle between the pyrimidinium and thiazolium ring planes is 86°. The C5 ethyl ester pyrophosphate side chain assumes the partly folded, but as a whole rather extended, conformation;[19] the bridge oxygen O5$\epsilon$ makes close contact with the thiazolium moiety, O5$\epsilon$...S1 = 3.17 (2) Å (sum of van der Waals radii[20] = 3.25 Å) and 0.74-Å deviation out of the thiazolium plane, whereas the terminal phosphate extends away from the thiazolium moiety, apparently due to steric effects between them. The pyrophosphate group is in the nearly perfect eclipsed conformation of the oxygen atoms[21] and forms a fairly planar six-membered chelate ring involving the Cu atom with the largest deviation of 0.07 Å for the O5$\epsilon$ atom. The ethyl ester pyrophosphate conformation in the current structure differs from the extensively folded form with the staggered conformation found in thiamine pyrophosphate hydrochloride,[22] and also from the extended form with the staggered conformation in the neutral zwitterions;[23,24] the primary difference occurs at $\phi_{5\gamma}$, whereas $\phi_{5\delta}$ was the first major difference for the change between the folded and extended conformations.[24] This is consistent with the conformational variability of the C5 side chain as a function of its environment, as pointed out by Pletcher and Sax.[23]

The crystal packing is mainly dominated by an extensive hydrogen-bonding system involving nitrate ions and water molecules and by the stacking arrangement of the phen ligands (Figure 3).

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(6) For each reflection, the intensity, $I$, and its standard deviation, $\sigma(I)$, were calculated from the equations $I = I_p - (T_p/T_B)(B_1 + B_2)/2$ and $\sigma(I) = Q[I_p + (T_p/T_B)^2(B_1 + B_2)/4]^{1/2}$, where $I_p$ is the total integrated count, $T_p$ is the counting time for the scan, $T_B$ is the counting time for each background, $B_1$ and $B_2$ are the background counts on each side of the scan, and $Q$ is the reliability factor and was set equal to 1.0 in this case.

(7) Transmission factors were calculated by the absorption-correction program ABS10 (by Ito, T.) and used the Gaussian integration (grid 10 × 10 × 10) method of Busing and Levy. The following programs were used during the course of the structural analyses: READ80, by Kobayashi, K.; Sakurai, T.; data reduction on a FACOM 230-75 computer, FANDFR by Iitaka, Y.; structure factor calculation and Fourier synthesis, HBLS by Ashida, T.; structure factor calculation and block-diagonal least-squares refinement, BOND by Iitaka, Y.; bond distances, angles, and least-squares planes, ORTEP by Johnson, C. K.; thermal ellipsoid drawings.

(8) Large values of thermal parameters of these atoms show that they are probably disordered; thus they were assigned isotropic thermal paramters.

(9) $R_F = \Sigma|F_o - |F_c||/\Sigma F_o$, $R_{wF} = (\Sigma w|F_o - |F_c||^2/\Sigma w F_o^2)^{1/2}$, and the standard deviation of an observation of unit weight = $[\Sigma w|F_o - |F_c||^2/(m - n)]^{1/2}$.

(10) A difference Fourier map computed at the end of the refinement showed no other features with an absolute value greater than 0.8 e Å$^{-3}$ near the disordered nitrate and the crystallization water atoms; however, no further analysis and no attempt to locate hydrogen atoms have been made at the present time.

(11) "International Tables for X-ray Crystallography", Vol. III; Kynoch Press: Birmingham, England, 1962; Table 3.3.1A.

(12) Cromer, D. T.; Liberman, D. *J. Chem. Phys.* **1970**, *53*, 1891–1898.

(13) See paragraph at end of paper regarding supplementary material.

(14) The C2′–N1′–C6′ angle of 122 (2)° shows the probable protonation at N1′; in contrast, the angle about the nonprotonated N3′ atom is 120 (2)°. N1′ protonation is further supported by the hydrogen-bonding system.

(15) The P5ζ–O5ζ2 bond length of 1.56 (2) Å shows the protonation on O5ζ2, and other bond lengths of P5δ–O5δ1, P5δ–O5δ2, P5ζ–O5ζ1, and P5ζ–O5ζ3 bonds of 1.49 (2), 1.48 (1), 1.46 (2), and 1.48 (1) Å, respectively, show their double-bond character. The hydrogen-bonding system further supports the O5ζ2 protonation.

(16) Schellenberger, A. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1024–1035.

(17) For a definition of these conformational terms, see: Pletcher, J.; Sax, M.; Blank, G.; Wood, M. *J. Am. Chem. Soc.* **1977**, *99*, 1396–1403.

(18) Shin, W.; Pletcher, J.; Blank, G.; Sax, M. *J. Am. Chem. Soc.* **1977**, *99*, 3491–3499.

(19) Torsion angles (deg) are $\phi_{5\alpha}$(S1–C5–C5$\alpha$–C5$\beta$) = 87, $\phi_{5\beta}$(C5–C5$\alpha$–C5$\beta$–O5$\gamma$) = −63, $\phi_{5\gamma}$(C5$\alpha$–C5$\beta$–O5$\gamma$–P5$\delta$) = −81, $\phi_{5\delta}$(C5$\beta$–O5$\gamma$–P5$\delta$–O5$\epsilon$) = 87, and $\phi_{5\epsilon}$(O5$\gamma$–P5$\delta$–O5$\epsilon$–P5$\zeta$) = −130°.

(20) Pauling, L. "The Nature of the Chemical Bond", 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 260.

(21) Pseudotorsion angles (deg) are O5δ2–P5δ–P5ζ–O5ζ3 = 0, O5δ1–P5δ–P5ζ–O5ζ2 = 10, and O5γ–P5δ–P5ζ–O5ζ1 = 10°.

(22) Pletcher, J.; Sax, M. *Science* **1966**, *154*, 1331–1333; *J. Am. Chem. Soc.* **1972**, *94*, 3998–4005.

(23) Pletcher, J.; Wood, M.; Blank, G.; Shin, W.; Sax, M. *Acta Crystallogr., Sect. B* **1977**, *B33*, 3349–3359.

(24) Pletcher, J.; Blank, G.; Wood, M.; Sax, M. *Acta Crystallogr., Sect. B* **1979**, *B35*, 1633–1637.

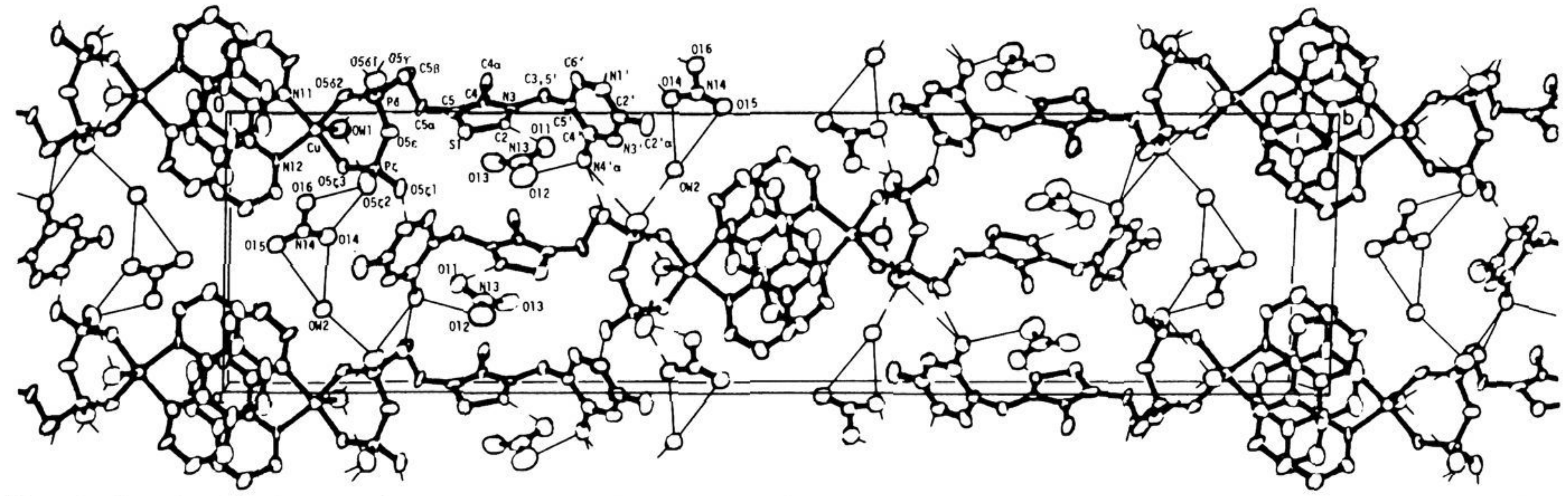


**Figure 3.** Crystal packing in [(thiamine pyrophosphate)(1,10-phenanthroline)aquacopper]–dinitrato–water viewed along the *c* axis. Note the self-stacking of the phen ligands. Thin lines show hydrogen bonds.

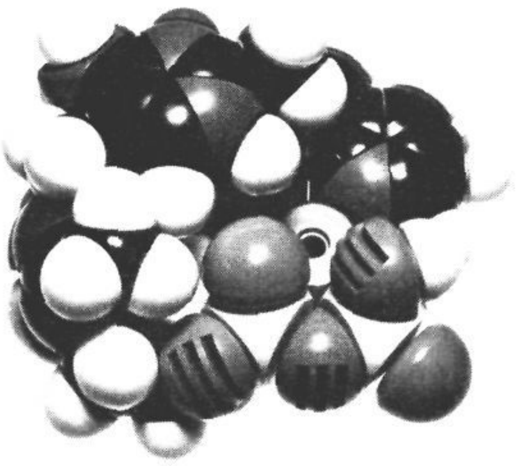
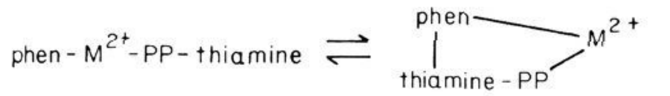


**Figure 4.** CPK model photograph.

The pyrimidine nitrogens N1′ and N4′α, and all pyrophosphate oxygens except for the bridge O5ε and the coordinating O5δ2 and O5ζ3 oxygens, are involved in the hydrogen-bonding system. The nitrate ions interact with the [(thiamine pyrophosphate)(1,10-phenanthroline)aquacopper]$^{2+}$ molecules in an interesting manner. One nitrate anion is connected with one thiazolium moiety by both the C2–H hydrogen bonding[25] [N–O···C2 = 3.20 (2)[27] Å] and the electrostatic interactions with the electropositive S1 atom [N–O···S1 = 2.90 (2) Å] and also connected with another thiazolium moiety by an intermolecular interaction involving the methyl C4α group [N–O···C4α = 2.93 (3) Å, sum of van der Waals radii[20] = 3.40 Å]. These are roughly coplanar. There is another type of interaction between this nitrate ion and the thiazolium moieties, electrostatic interactions with the negatively charged nitrate oxygens lying over or under the cationic quaternary nitrogen N3 atoms [3.19 (3) and 3.22 (2) Å]. Another nitrate ion, which is disordered, locates over the pyrimidine ring with the closest contact of 3.38 (4) Å between a nitrate oxygen and pyrimidine N3′ and participates in hydrogen bonding to a terminal phosphate oxygen and to a crystallization water, which is additionally hydrogen bonded to an inner phosphate oxygen. The water ligand further stabilizes the structure by forming hydrogen bonds with two pyrophosphate oxygens of the adjacent molecule. There is neither interbase pyrimidine stacking nor interbase hydrogen bonding.

The most interesting feature in this structure is that the metal ion bridges between the bidentate phen ligand and the thiamine pyrophosphate molecule through the pyrophosphate group only. However, the complex exhibits neither intramolecular nor intermolecular stacking interactions between phen and pyrimidine rings. This is most probably due to the self-stacking of the phen ligands throughout the crystal (3.3 and 3.4 Å average spacings), thereby prohibiting the formation of intramolecular in addition to intermolecular stacking between them, and partly due to the existence of the nitrate ion which locates over the pyrimidine ring.

However, in dilute solution where the *inter*molecular forces are not so strong as in a crystal, the *intra*molecular aromatic ring stacking interactions may, we suggest, more readily occur, since the conformational flexibility of the C5 side chain and the connection of these two ligands by a metal-ion bridge may be favorable for the formation of such an *intra*molecular stacking structure. Further support for this idea can be obtained with the aid of space-filling models (Figure 4).[28] Therefore, we suggest the possibility of a conformational equibrium (eq 1) between the



$$\text{phen-M}^{2+}\text{-PP-thiamine} \rightleftharpoons \begin{matrix}\text{phen} \\ | \\ \text{thiamine-PP}\end{matrix}\!\!\!>\text{M}^{2+} \qquad (1)$$

unstacked opened and stacked folded structures within the ternary complex in dilute solution.[29] This is reminiscent of the structural change of the ternary complex found in the solid state[30] as well as in solution[4] for the nucleotide–metal ion–aromatic amine system, where the structural change between the unstacked and stacked structures is principally responsible for the conformational change of the sugar–phosphate backbone.

We are currently extending our X-ray studies on ternary complexes with other phosphate coenzymes such as nicotinamide adenine dinucleotide (or its phosphate), cytidine 5′-diphosphocholine, and uridine 5′-diphosphoglucose, which in many cases require activation by added divalent metal ion (typically Mg$^{2+}$ or Mn$^{2+}$), in the hope of finding additional examples of this interesting metal-binding mode.

**Supplementary Material Available:** Tables of positional and thermal parameters, bond distances and angles, and observed and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

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*Received February 8, 1980*

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(25) The corresponding C–H hydrogen bonding has been observed in many other thiamine structures.[26]

(26) Richardson, M. F.; Franklin, K.; Thompson, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3204–3209, and references cited therein.

(27) This interaction is probably very weak, but its direction is reasonable for a hydrogen bond: angles S1–C2···O = 109 (1)° and N3–C2···O = 138 (1)°.

(28) Many conformations with the general property of metal ion bridged aromatic ring stacking appear to be possible. For example, see the CPK model photograph (Figure 4), one of the possible models.

(29) No solution studies have yet been made for the current ternary system.

(30) Fischer, B. E.; Bau, R. *J. Chem. Soc., Chem. Commun.* **1977**, 272–273; *Inorg. Chem.* **1978**, *17*, 27–33. Aoki, K. *J. Am. Chem. Soc.* **1978**, *100*, 7106–7108. Wei, C. Y.; Fischer, B. E.; Bau, R. *J. Chem. Soc., Chem. Commun.* **1978**, 1053–1055. Aoki, K. *Ibid.* **1979**, 589–591. Gellert, R. W.; Fischer, B. E.; Bau, R. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 1443–1448.