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The Reaction of Dimethyltin(IV) Dichloride with Thiamine Diphosphate (H_2TDP): Synthesis and Structure of $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$, and Possibility of a Hitherto Unsuspected Role of the Metal Cofactor in the Mechanism of Vitamin-B_1-Dependent Enzymes

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The complex $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$, synthesized by reaction between dimethyltin(IV) dichloride and thiamine diphosphate hydrochloride (H_3TDPCl) in water, was characterized by X-ray diffractometry and IR and Raman spectroscopy in the solid state, and by electrospray mass spectrometry (ESMS) and NMR spectroscopy (^1H, ^{13}C, ^{31}P, ^{119}Sn and inverse-detection ^1H,^{15}N HMBC) in aqueous solution. In the solid state the $HTDP^-$ anion chelates the metal via one oxygen atom of each phosphate group [Sn–O = 2.062(3), 2.292(3) Å], and another oxygen atom belonging to the terminal phosphate links the $SnMe_2^{2+}$ cations into chains. The tin atom has distorted octahedral coordination involving the *trans* methyl groups, the above-mentioned diphosphate oxygen atoms, and the oxygen atom of the coordinated water molecule. The thiamine moiety has F conformation. NMR studies suggest that the interaction between the organometallic cation and the $HTDP^-$ ligand persists in D_2O solution, which is in keeping with the ESMS spectrum showing a peak corresponding to $[SnMe_2(HTDP)]$. Both in the solid state and in solution, the acidic $HTDP^-$ proton in the complex is located on the N(1′) atom of the pyrimidine ring. The enzymatic behavior of native pyruvate decarboxylase (EC 4.1.1.1, PDC), obtained from baker's yeast, was compared in a coupled assay with that shown by the "$SnMe_2$-holoenzyme" created by incubation of apoPDC with $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$. The $SnMe_2$-holoenzyme exhibited about 34% of the activity of the native enzyme (with a Michaelis–Menten constant of 2.7 μM, as against 6.4 μM for native PDC), so confirming the very low specificity of PDC regarding the identity of its metal ion cofactor. In view of the observed protonation of N(1′), it is suggested that the role of divalent cations in the mechanism of thiamine-diphosphate-dependent enzymes may be not only to anchor the cofactor in its binding site but also to shift the acidic proton of $HTDP^-$ from the diphosphate group to N(1′); protonation of N(1′) is widely believed to be important for enzyme function, but the origin of the proton has never been clarified.

1. Introduction

All thiamine-diphosphate-dependent enzymes also require divalent metal ions M(II) for their activity,[2] the complex formed by M(II) and the thiamine derivative being the true cofactor.[3] Recognition of this has stimulated steady interest in the coordination chemistry of thiamine diphosphate (H_2TDP when formulated as shown, as a double zwitterion).

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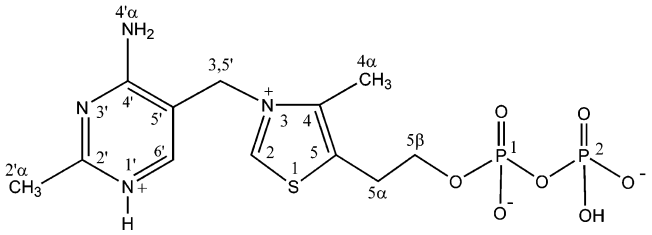

Studies have mainly addressed the formation equilibria of its complexes with metal cations,[4] and the structures of these complexes in solution:[5] NMR measurements and other data suggest that thiamine diphosphate binds to metal ions in solution via both the diphosphate group and also, except in a rhodium(III) complex, via the pyrimidine N(1′) atom.[5f] By contrast, very few complexes between thiamine diphosphate and metal ions have been isolated and characterized in the solid state,[6] and in the only two that have been studied by X-ray diffractometry,[6a,d] thiamine diphosphate is bound to the metal ion only via the diphosphate group. It is this latter binding mode that has been observed in all X-ray crystallographic studies of thiamine-diphosphate-dependent enzymes,[7] and it is on the basis of these studies that it is commonly assumed that the only role of the M(II) cation in the mechanism of the enzyme is to anchor the thiamine diphosphate molecule in the active site.

We describe here the synthesis of the new thiamine diphosphate derivative $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$, its structures in the solid state and in water, and the results of kinetic experiments showing that when $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$ is incubated with apoPDC (PDC = pyruvate decarboxylase, EC 4.1.1.1), the resulting "$SnMe_2$-holoenzyme" retains a significant proportion of the catalytic activity of the native enzyme despite the methyl groups on the metal atom. This evidence of the relative innocuity of the methyl groups, and the fact that apart from these groups the only ligands in the new complex are thiamine diphosphate and water, suggests that the structure of the complex may be more relevant to the native enzyme than those of the two previous crystallographically characterized complexes[6a,d] (one of these contained a phenanthroline ligand foreign to the native holoenzyme and the other was a trinuclear complex with oxo and hydroxo ligands). In particular, the finding that in the new complex N(1′) is protonated both in the solid state and in solution suggests that induction of the protonation of this nitrogen (a putatively essential but ill-clarified step in the catalytic process)[8] may constitute a second major role for M(II) cations in the mechanism of thiamine-diphosphate-dependent enzymes.

2. Experimental Section

Methods and Materials. Elemental analyses, IR and Raman spectra, and ^1H, ^{13}C, ^{31}P and ^{119}Sn NMR spectra were obtained as described elsewhere.[6d] Inverse detection 2D $^1H,^{15}N$ HMBC spectra were obtained with ^{15}N in natural abundance at 300 K on a Bruker AMX 500 NMR spectrometer using an inverse broadband probe and operating at 500.14 MHz (^1H) and 50.69 MHz (^{15}N), with ^1H signals referred to DSS and ^{15}N signals to external neat CH_3NO_2 (δ $^{15}N = 0$); samples were run in D_2O solution in 5 mm o.d. tubes.

Electrospray mass spectra (ESMS) were obtained using a Hewlett-Packard HP1100 mass spectrometer in positive ion mode, with a quadrupole as analyzer. A 5×10^{-3} M solution of the sample in 5:5:1 v/v/v methanol/water/acetic acid was automatically injected into the spectrometer with a flow rate of 0.2 mL min^{-1}. Nitrogen with a flow rate of 7 L min^{-1} was employed both as drying gas (temperature 325 °C) and nebulizing gas (nebulizer pressure 30 psi). The capillary voltage was 4.0 kV. Collision-induced dissociation (CID) voltages were typically varied from 10 to 200 V. The relative molecular masses of the metal-containing species were calculated assuming tin to be ^{120}Sn. Theoretical isotope patterns were calculated using the program ISOPRO 3.0 [MS/MS Software, Sunnyvale, CA]; agreement between observed and calculated isotope distribution patterns was excellent for all the major peaks.

Thiamine diphosphate hydrochloride (H_3TDPCl) (Sigma) and dimethyltin(IV) dichloride (Aldrich) were used as supplied.

Synthesis of $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$. A solution of H_3TDPCl (0.10 g, 0.22 mmol) in 3 mL of water was brought to pH 5.6 (the optimal pH for PDC activity) by addition of 0.5 M aqueous NaOH, and it was then stirred for 1 h and added to a solution of dimethyltin dichloride (0.06 g, 0.22 mmol) in 3 mL of water. The new solution (pH 1.5) was brought to pH 5.6 with 0.5 M aqueous NaOH and stirred for 4 h at room temperature. The resulting clear solution was kept in the refrigerator for 5 days, giving white crystals suitable for X-ray diffractometry. Yield: ca. 34%. Mp > 280 °C. Anal. Calcd for $C_{14}ClH_{27}N_4O_9P_2SSn$: C, 26.1; N, 8.7; H, 4.2; S 5.0%. Found: C, 26.1; N, 8.8; H, 4.3; S, 5.0%. ESMS: $[SnMe_2(HTDP)]^+$, 573 *m/z* (100%); $[SnMe_2(H_2TDP)]^{2+}$, 287 *m/z* (72%); $[SnMe_2(C_6H_{15}NO_7P_2S)]^+$, 452 *m/z* (42%); $[H_3TDP]^+$, 425 (25%). IR and Raman (in parentheses): 3501m, 3396m, ν(OH); 3205w, 3096m, ν(NH); 1657s, 1627m, 1600sh (1655w, 1625w, 1600w), $\delta(NH_2)$ + ν(ring); 1544m, ν(ring); 1171vs, $\nu_{asym}[P(1)O_2]$; 1095s, 1072s (1105w, 1065w), $\nu_{asym}[P(2)O_3]$ + $\nu_{sym}[P(1)O_2]$; 943s, $\nu_{asym}[P(1)OP(2)]$; (929w), $\nu_{sym}[P(2)O_3]$; 757w (751m), $\nu_{sym}[P(1)OP(2)]$; 544m, ν(COP) (includes ligand vibration); (546w), ν_{asym}[C−Sn−C]; 530sh (531s), ν_{sym}[C−Sn−C]. ^1H NMR: δ[C(2)H] = 9.38 (this signal vanishes in a few

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Table 1. Crystal and Structure Refinement Data for $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$

formula	$C_{14}H_{27}ClN_4O_9P_2SSn$
fw	643.54
T	296(2)
radiation	Mo Kα ($\lambda = 0.71073$ Å)
cryst syst, space group	orthorhombic, *Pbcn* (No. 60)
cell params	$a = 22.2209(4)$ Å
	$b = 8.8241(2)$ Å
	$c = 24.1119(6)$ Å
	$\alpha = \beta = \gamma = 90°$
V	4727.85(18) Å^3
Z, D_{calcd}	8, 1.808 mg/m^3
abs coeff	1.470 mm^{-1}
F(000)	2592
cryst size	$0.14 \times 0.05 \times 0.02$ mm^3
θ range	3.07−25.00°.
hkl collected	$0 \le h \le 26$
	$-10 \le k \le 10$
	$-28 \le l \le 28$
no. obsd reflns/unique	50196/4166 [R(int) = 0.1052]
GOF on F^2	1.024
final R indices [$I > 2\sigma(I)$]	R1 = 0.0355, wR2 = 0.0812
R indices (all data)	R1 = 0.0652, wR2 = 0.0975
largest diff peak and hole	0.779 and −0.720 e Å^{-3}

minutes); δ[C(6′)H] = 7.88s (1); δ[C(3,5′)H_2] = 5.53s (2); δ[C(5β)-H_2] = 4.22c (2), $^3J(^1H-^{31}P) = 5.9$; δ[C(5α)H_2] = 3.33t (2), $^3J(^1H-^{31}P) = 5.3$; δ[C(2′α)H_3] = 2.58s (3); δ[C(4α)H_3] = 2.52s (3); δ[Sn−CH_3] = 0.92s (6), $^2J(^1H-^{119}Sn) = 100.40, 96.86$. ^{13}C NMR: δ[C(2′)] = 164.0; δ[C(4′)] = 163.5; δ[C(6′)] = 145.2; δ[C(4)] = 144.1; δ[C(5)] = 136.1; δ[C(5′)] = 106.9; δ[C(5β)] = 66.0, $^2J(^{13}C-^{31}P) = 5.2$; δ[C(3,5′)] = 50.6; δ[C(5α)] = 27.8, $^3J(^{13}C-^{31}P) = 7.9$; δ[C(2′α)] = 21.7; δ[C(4α)] = 11.8; δ[Sn−CH_3] = 10.1, $^1J(^{13}C-Sn) = 906/849$. ^{31}P NMR: δ[P(1)] = −11.1dt; δ[P(2)] = −9.6d, $^2J[^{31}P(1)-^{31}P(2)] = 19.7$, $^3J[^{31}P(1)-^1H] = 6.4$. ^{119}Sn NMR: δ(Sn) = −277brs.

X-ray Crystallography. Table 1 lists crystal and refinement data. The intensities of reflections (Mo Kα radiation, $\lambda = 0.71073$ Å) were measured in $\omega/2\theta$ scan mode on a Siemens SMART system equipped with a CCD detector, and Lorentz and polarization corrections were applied. An absorption correction was performed using SADABS.[9] The structure was solved as indicated elsewhere[6d] using SHELXL97[10] with atomic scattering factors from *International Tables for X-ray Crystallography*.[11] All hydrogen atoms were located on a difference map [in particular, N(1′)−H], but except for those of the water molecules, their actual coordinates were calculated on stereochemical grounds and refined with the riding model. The water hydrogens were left free to refine with the O−H lengths restrained to the target value 0.88(2) Å. The usual programs were employed for plotting molecular and crystal structures.[12] Since the space group is centrosymmetric (see Table 1), the crystal contains both enantiomers of the complex.

Purification of Enzyme. Crude PDC was isolated from baker's yeast by a modified[13] Ullrich procedure.[14] The fresh yeast was stored at −30 °C for 24 h, the frozen cells were then lysed by fast freezing in liquid N_2 followed by mechanical shearing in a steel blender, and the fine powder so obtained was added at room temperature to the glycerol/EDTA/ammonium sulfate-buffer solution described by Ullrich.[14]

ApoPDC was also prepared by published methods.[14] It was normally used immediately, but sometimes after storage at −30 °C for a few days (which does not cause significant loss of reconstitution capacity).

Enzyme Assay. PDC activity was assayed as the rate of production of acetaldehyde from pyruvate at 30 °C, which was measured by following the oxidation of NADH to NAD^+ in a coupled assay.[15] Alcohol dehydrogenase, NADH, and pyruvate in 0.3 M citrate buffer were added to the PDC solution, and activity was followed at 340 nm in a Pye Unicam PU-8732 spectrophotometer.

Holoenzyme was reconstituted from apoPDC 10 min before assay by adding either the native cofactors or the diorganotin(IV) complex in 0.05 M MES buffer (pH 6.2) at 30 °C. No activity was observed in assays of apoPDC alone, apoPDC plus added Mg^{2+} (without added H_2TDP), or apoPDC plus added H_2TDP (without added dication), showing that the purified apoPDC was not contaminated by H_2TDP or Mg^{2+}.

Kinetic parameters were calculated using the *Grafit* program (Erithacus Software, Staines, U.K.). Results are given as means ± SEMs (standard errors of means) of four or five determinations. All kinetic plots had correlation coefficients of 0.97−0.99.

3. Results and Discussion

Synthesis of the Complex. The reaction between thiamine diphosphate hydrochloride and $SnMe_2Cl_2$ in water potentially involves two pH-dependent processes: the deprotonation of H_3TDPCl[4e] and the hydrolysis of dichlorodimethyltin(IV).[16] The H_3TDP^+ monocation has three acidic protons, one on each phosphate group and one on the pyrimidine ring.[4e] When pH rises in the absence of metal ions, H_3TDP^+ evolves first to the zwitterion H_2TDP by loss of the proton on P(1)−O, and then, by deprotonation of N(1′), to $HTDP^-$, which is the major species between pH 5 and 6.[4e] At the pH at which the complex was synthesized (pH 5.6, the value at which PDC activity peaks),[17] thiamine diphosphate is thus mainly monoanionic.

Although the hydrolysis of $SnMe_2Cl_2$ can lead to the formation of hydroxo and/or oxohydroxo Sn(IV) derivatives with or without chloride anions,[16] under the working conditions used ($SnMe_2Cl_2$ concentration 0.036 M, pH 5.6) the presence of $HTDP^-$ prevents hydrolysis and ensures coordination of the $SnMe_2^{2+}$ cation in what seems to be a straightforward reaction:

$$SnMe_2Cl_2(aq) + H_3TDPCl(aq) + 2NaOH(aq) \rightarrow [SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O(s) + 2Na^+(aq) + 2Cl^-(aq)$$

The product clearly differs from the dimethyltin(IV) complex prepared by Fiore et al.[6b] by refluxing a methanolic suspension of $H_2TDP\cdot 4H_2O$ and $SnMe_2O$, which was studied by IR and Mössbauer spectroscopy.

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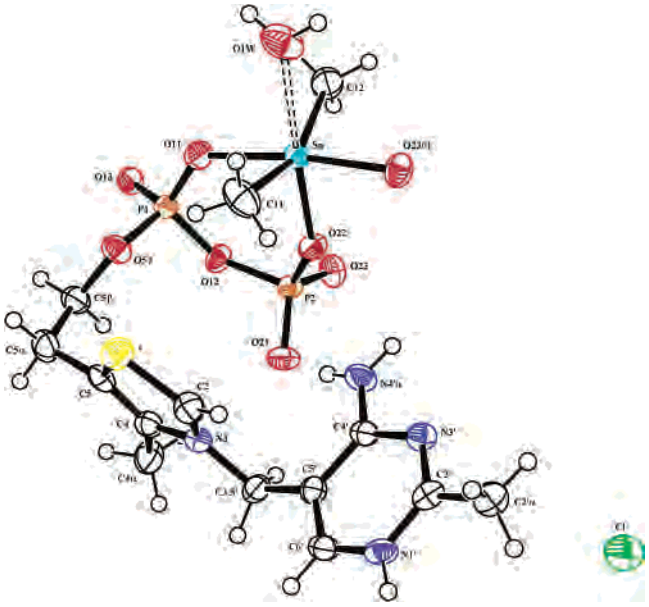

Figure 1. ORTEP view of the cation $[SnMe_2(HTDP)(H_2O)]^+$.

Table 2. Selected Bond Lengths [Å] and Angles [deg] in $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$[a]

Sn−O(22)	2.062(3)	C(12)−Sn−O(1W)	80.51(17)
Sn−C(12)	2.093(5)	C(11)−Sn−O(1W)	79.67(18)
Sn−O(23)#1	2.112(3)	O(22)−Sn−O(23)#1	85.88(12)
Sn−C(11)	2.096(5)	C(12)−Sn−O(23)#1	94.46(16)
Sn−O(11)	2.292(3)	O(22)−Sn−C(11)	97.03(18)
Sn−O(1W)	2.586(4)	C(12)−Sn−C(11)	157.4(2)
P(2)−O(21)	1.486(3)	C(12)−Sn−O(11)	83.76(16)
P(2)−O(22)	1.512(3)	O(23)#1−Sn−O(11)	168.57(11)
P(2)−O(23)	1.517(3)	O(23)#1−Sn−C(11)	96.09(18)
P(2)−O(12)	1.623(3)	O(22)−Sn−O(11)	83.58(11)
P(1)−O(11)	1.489(3)	O(11)−Sn−O(1W)	101.31(12)
P(1)−O(13)	1.492(3)	C(11)−Sn-O(11)	89.60(18)
P(1)−O(12)	1.589(3)	O(22)−Sn−C(12)	103.62(17)
O(22)−Sn−O(1W)	174.01(12)	O(23)#1−Sn−O(1W)	89.49(13)

[a] Symmetry transformations used to generate equivalent atoms: (#1) $-x + {}^1/_2, y + {}^1/_2, z$; (#2) $-x + {}^1/_2, y - {}^1/_2, z$.

Structure of the Complex. a. Solid-state Studies. The complex consists of $[SnMe_2(HTDP)(H_2O)]^+$ cations, the Cl^- anion, and one molecule of water of crystallization. In the cation, the $HTDP^-$ ligand chelates the metal center via one oxygen atom of each phosphate group (Figure 1), as has been observed in X-ray studies of thiamine-diphosphate-dependent enzymes.[7] Via O(23), the terminal phosphate group is also linked to another tin atom, so that O(22)−P(2)−O(23) bridges between metal centers create zigzag chains. The tin−oxygen distances in the bridge [2.062(3) Å for Sn−O(22) and 2.112(3) Å for Sn−O(23)#1; see Table 2] are close to but shorter than those of the almost regular octahedral coordination polyhedron of $(SnMe_2)_3(PO_4)_2\cdot 8H_2O$, 2.17(2) and 2.18(2) Å.[18] The Sn−O(11) distance, 2.292(3) Å, is longer than those of the terminal phosphate, but still short enough to indicate a significant bond (sum of the covalent radii = 2.13 Å; sum of the van der Waals radii = 3.7 Å[19]). The interaction with the water molecule is weaker [Sn−O(1W) = 2.586(4) Å], but again, the interatomic distance is in the normal range for Sn−O bonding.[20] These four Sn−O bonds, together with the two Sn−Me bonds, give the tin atom a distorted octahedral coordination polyhedron somewhat similar to that of Mg^{2+} in PDCs,[21] in which the magnesium atom is coordinated by one O atom of each thiamine diphosphate phosphate group, one molecule of water, and three apoenzyme amino acids (Gly, Asp, and Asn).[21]

Table 3. Main Hydrogen Bonds in $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$[a]

D−H···A	d(D−H) Å	d(H···A) Å	d(D···A) Å	∠(DHA) (deg)
N(1′)−H(1′)···O(13)#3	0.8600	1.8200	2.682(5)	177.3
N(4′α)−H(4′1)···O(21)	0.8600	2.1000	2.908(5)	157.0
N(4′α)−H(4′2)···O(21)#1	0.8600	2.0000	2.848(5)	169.7
O(1W)−H(1W)···O(13)#6	0.8700(2)	1.9200(2)	2.791(5)	176.0(7)
O(1W)−H(1W′)···Cl#5	0.8800(2)	2.3100(2)	3.186(4)	175.0(5)
O(2W)−H(2W)···Cl#4	0.8800(2)	2.3000(2)	3.177(7)	173.0(8)
O(2W)−H(2W′)···Cl#5	0.8800(2)	2.5500(3)	3.426(8)	173.0(10)

[a] Symmetry transformations used to generate equivalent atoms: (#1) $-x + {}^1/_2, y + {}^1/_2, z$; (#2) $-x + {}^1/_2, y - {}^1/_2, z$; (#3) $x, -y, z + {}^1/_2$; (#4) $x - {}^1/_2, y + {}^1/_2, -z + {}^1/_2$; (#5) $-x + {}^1/_2, -y + {}^1/_2, z - {}^1/_2$; (#6) $x, y + 1, z$.

The P−O bond lengths in the chelate ring reflect the differences between the Sn−O(11) and Sn−O(22) distances, the oxygen with the longer phosphorus−oxygen bond [O(22); P(2)−O(22) = 1.512(3) Å] having the shorter metal−oxygen bond [Sn−O(22)]. The P(2)−O(23) bond is also long [1.517(3) Å], in correspondence with the short Sn−O(23)# bond length. The relative orientation of the pyrimidine and thiazolium rings, defined by the torsion angles ϕ_T [= C(5′)−C(3,5′)−N(3)−C(2) = −11.0(6)°] and ϕ_P [= N(3)−C(3,5′)−C(5′)−C(4′) = −75.2(5)°] is of F type,[22−24] and the ethyl diphosphate side chain adopts a folded arrangement [$\phi_{5\alpha}$ = S−C(5)−C(5α)−C(5β) = 106.8(4)°, $\phi_{5\beta}$ = C(5)−C(α)−C(5β)−O(5γ) = −57.1(5)°, $\phi_{5\gamma}$ = C(5α)−C(5β)−O(5γ)−P(1) = 165.8(3)°, $\phi_{P(1)}$ = C(5β)−O(5γ)−P(1)−O(12) = −65.3(3)°, $\phi_{P(2)}$ = O(5γ)−P(1)−O(12)−P(2) = −90.7(3)°] that places the O(21) atom of the terminal phosphate near the pyrimidine $-NH_2$ group.

Although in aqueous solution, in the absence of a metal cation, the remaining acidic proton of $HTDP^-$ is probably located on the terminal phosphate group,[4e] this is not the case in the crystalline complex, in which it was found crystallographically to be located on N(1′). In fact, N(1′) acts as a donor in an intermolecular hydrogen bond (see Table 3). There is also, as in the case of the monomethyltin(IV) derivative,[6d] strong indirect evidence of its being located at this position. First, neither the P(2)−O(21) distance nor the fact that O(21) acts as the acceptor in an intramolecular hydrogen bond (vide infra) suggest protonation of this atom. Second, the C(2′)−N(1′)−C(6′) bond angle, 120.5(4)°, is

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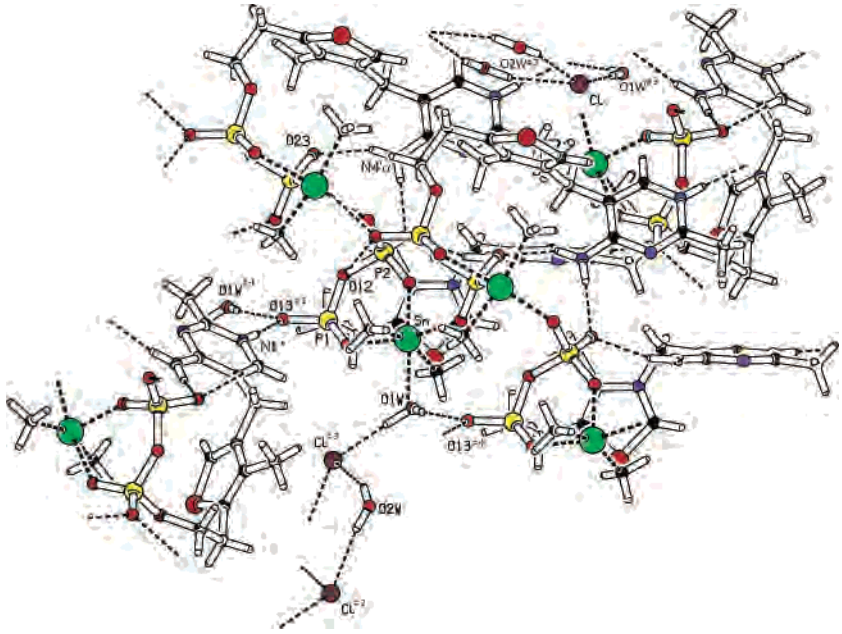

Figure 2. PLATON plot of $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$ showing the main hydrogen bond interactions contributing to the folding of the ethyl diphosphate side chain.

closer to the values reported for N(1′)-protonated thiamine diphosphate (119.3−120.3°)[22] than to that found in N(1′)-deprotonated thiamine (115.2°),[23] suggesting a protonation-induced contraction of the electron cloud of the N(1′) lone pair.

All the main hydrogen bond interactions are shown in Figure 2, and their parameters are listed in Table 3. The above-noted proximity between O(21) and the $-NH_2$ group, possible because of the folded conformation adopted by $HTDP^-$, leads to the intramolecular hydrogen bond N(4′α)−H(4′1)···O(21). The other H atom of the $-N(4'\alpha)H_2$ group is involved in an intermolecular hydrogen bond with O(21)[#1] ([#1] = $^1/_2 - x$, $^1/_2 + y$, z). Thus, O(21) forms both intramolecular and intermolecular hydrogen bonds with pyrimidine amino groups, both of them with unexceptional parameters.[25]

The shortest donor−acceptor distance among the hydrogen bonds listed in Table 3 is that of N(1′)−H(1′)···O(13)[#3] ([#3] = x, $-y$, $^1/_2 + z$), 2.682(5) Å, which is just slightly longer than a similar N(1′)−O hydrogen bond in the monomethyltin(IV) complex.[6d] The coordinated water molecule is involved in two hydrogen bonds, one with a chloride anion and another with O(13)[#6] ([#6] = x, $1 + y$, z). Thus, O(13) and O(21), the oxygen atoms of the diphosphate chain that are not involved in forming the chain or binding the metal, form two hydrogen bonds each, showing the capacity of these atoms to contribute to the anchoring of the coenzyme in the active site of PDCs. Finally, the chloride anions are linked in pairs by two molecules of water of crystallization which form a double bridge between the members of each pair.

The main IR and Raman bands (see Experimental Section) have been assigned on the basis of previous work and references therein.[6d] The band corresponding to ν_{asym}[P(1)-O_2] is shifted from 1226 cm^{-1} in $[\{SnMe(HTDP)(OH)\}_3O]$-$(OH)\cdot 21H_2O$[6d] to 1171 cm^{-1} in the dimethyltin(IV) complex, probably because one oxygen of this phosphate group is bound to the metal in the latter but not in the monomethyltin(IV) derivative. Also, ν_{asym}[P(1)OP(2)] lies at higher wavenumbers (943 cm^{-1}) than in the spectrum of the monomethyltin(IV) complex [931 (925) cm^{-1}], possibly because in the latter $HTDP^-$ coordinates through only one phosphate group, making the P−O−P fragment less rigid than in the dimethyltin(IV) complex.

b. Solution Studies. At CID voltages of 10−100 V, the base peak of the electrospray mass spectrum of $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$ is a signal due to the dehydrated cation, $[SnMe_2(HTDP)]^+$. The protonated species $[SnMe_2(H_2TDP)]^{2+}$ also appears with high intensity (72% of the base peak). These peaks show that the dimethyltin(IV) cation remains bound to thiamine diphosphate in water/methanol even in the presence of a small concentration of acetic acid (see Experimental Section). This conclusion is further supported by a signal at 452 *m*/*z* with a relative intensity of 42% that was identified as $[SnMe_2(C_6NSP_2O_7H_{15})]^+$, the result of $[SnMe_2(HTDP)]^+$ losing the fragment formed by the methylene bridge and the pyrimidine ring.

The ^1H, ^{13}C, ^{15}N, ^{31}P, and ^{119}Sn NMR spectra of $[SnMe_2(HTDP)(H_2O)]Cl\cdot H_2O$ were recorded in D_2O solutions of concentrations ranging from ca. 10^{-1} (pH ≈ 3.7) to ca. 10^{-3} M (pH ≈ 4.3) that were obtained by direct solution of the solid complex. The absence of N(1′)H and N(4′α)H_2 signals from the proton spectrum seems likely to be due to rapid exchange with the solvent. The C(2)−H signal at 9.38 ppm appears in freshly prepared solutions but disappears even more quickly than that of free H_3TDPCl (9.48 ppm). The fact that the $^2J[^1H-^{117/119}Sn]$ coupling constants, 96.9/100.4 Hz, are smaller than those of dimethyltin(IV) perchlorate (104/108 Hz) supports the persistence of the interaction between the dimethyltin(IV) cation and $HTDP^-$ in solution,

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Table 4. ^{15}N NMR Chemical Shifts[a]

	N(1′)	N(3′)	N(3)
H_3TDPCl	−216	−141	−173
HTDP$^-$	−135	−144	−169
[$SnMe_2$(HTDP)(H_2O)]Cl·H_2O	−214	−141	−173

[a] In ppm relative to nitromethane.

as do the chemical shifts of the methyl groups linked to the tin atom (10.1 ppm in the complex, 11.8 ppm in the perchlorate) and the ^1J[^{13}C−$^{117/119}$Sn] coupling constants (849/906 Hz for the complex, 980.1/1024.8 for the perchlorate). The C−Sn−C angle of 156° calculated from the ^1J[^{13}C−^{119}Sn] values using the Lockhart−Manders[26] empirical equation is practically the same as that observed in the X-ray study of the solid state, 157.4(2)°.

Although no N(1′)H proton signal was observed, the chemical shifts of C(2′), C(2′α), and C(6′) were almost the same as for H_3TDPCl,[6d] in which N(1′) is protonated, and lie upfield from those of NaHTDP,[6d] in which it is not (according to Malandrinos et al.,[6c] it is usual for protonation or metalation to shield these carbons). In order to ascertain for sure the location of the acidic proton on HTDP$^-$ in the complex, ^{15}N NMR experiments were done using inverse detection ^{1}H,^{15}N HMBC (see Experimental Section). There appear to have been no previous studies of thiamine diphosphate using this technique. We recorded the spectra of the complex, of H_3TDPCl, and of a NaHTDP/Na_2TDP mixture prepared in situ by bringing a solution of H_3TDPCl in D_2O to ca. pH 7 by addition of NaOD. The N(4′α) signal was not located, but those of the other three nitrogen atoms were in all cases easily identified (Table 4). As expected, the resonances of H_3TDPCl are at positions very close to those of thiamine chloride hydrochloride[27] (note that in the spectrum of the latter compound the signals previously assigned to N(3) and N(3′) on the basis of a directly recorded spectrum[27,28] must probably be interchanged). The chemical shift of N(1′) in the complex is very similar to that of H_3TDPCl, in which N(1′) is protonated, but is almost 80 ppm upfield from that of the sodium salt, in which N(1′) is unprotonated. It may therefore be concluded that in aqueous solution the remaining acidic proton on HTDP$^-$ in the complex is located on N(1′) and not on the terminal phosphate, its position in aqueous solutions of free HTDP$^-$. This difference is probably made possible by the two dissociation constants of H_2TDP not being very different (pK = 5.05 and 6.40).[4e]

The proton-decoupled ^{31}P NMR data for [$SnMe_2$(HTDP)(H_2O)]Cl·H_2O (see the Experimental Section) show the expected[6d] two doublets for P(1) and P(2) at −11.1 and −9.6 ppm, respectively {^2J[P(1)−P(2)] = 19.7 Hz}. The chemical shift of P(1) is the same as for thiamine diphosphate hydrochloride,[6d] showing that metalation and protonation of the nonterminal phosphate have similar effects on this nuclide {δ[P(1)] for HTDP$^-$, recorded from solutions of NaHTDP, is −10.7 ppm}; while the chemical shift of P(2) is the same as for HTDP$^-$ [6d] instead of being deshielded as in complexes with Zn(II) and Cd(II),[6c] showing that interaction of the terminal phosphate group with the tin atom affects P(2) less than complexation with these other metals.

The ^{119}Sn chemical shift, −276.9 ppm, clearly differs from that of dimethyltin(IV) perchlorate (−336.7 Hz) and suggests that the tin atom has coordination number six,[29] once more corroborating the persistence of the interaction between the organometallic cation and the diphosphate group of HTDP$^-$.

Enzyme Studies. ApoPDC from baker's yeast was incubated with [$SnMe_2$(HTDP)(H_2O)]Cl·H_2O, and the catalytic activity of the resulting "$SnMe_2$-holoenzyme" was determined from saturation curves for pyruvate decarboxylation obtained in initial velocity kinetic experiments. The $SnMe_2$-holoenzyme exhibited Michaelis−Menten behavior, retaining 34% of the activity of the native enzyme under the same conditions; a Michaelis−Menten constant K_m of 2.7 μM was estimated using a Lineweaver−Burk double reciprocal plot, while the value for the native enzyme is 6.4 μM.[30] The addition of Mg^{2+} to the $SnMe_2$-holoenzyme did not increase its activity, suggesting that there was no free thiamine diphosphate in these solutions. These results confirm the low specificity of PDC regarding the identity of the metal ion cofactor,[2] showing that even the organometallic dication $SnMe_2^{2+}$ can enable significant enzymatic activity.

For *Zymomonas mobilis* PDC the possibility that the two native coenzymes bind to the apoenzyme simultaneously as a preformed HTDP−Mg(II) complex has been ruled out.[30a] In our experiments, on the other hand, the dimethyltin(IV)−HTDP complex does seem to have bound to the apoenzyme as a preformed unit. This difference may be due partly to the HTDP−$SnMe_2$ complex being more stable than Mg(II)−HTDP, and partly to the two methyl groups of $SnMe_2$ making the dimethyltin(IV) complex more hydrophobic than Mg(II)−HTDP.

Possible Relevance to Enzyme Mechanism. As noted in the Introduction, the role of the M(II) cofactor of PDCs and other TDP-dependent enzymes is usually assumed to be that of anchoring the diphosphate group to the protein.[21] The results described above and elsewhere[6d] suggest that the metal may also have another very significant role: displacement of the remaining acidic proton of the HTDP$^-$ coenzyme from the terminal phosphate to the N(1′) atom of the pyrimidine ring. This displacement would have at least two important effects. First, it would allow the deprotonated P(2)−O to interact more strongly with Ile472[21] (here we number amino acids as for *Z. mobilis* PDC), so that the diphosphate group is well anchored to the apoenzyme through all its terminal oxygens. Second, it would protonate N(1′): protonation of N(1′) is believed to be essential for proper enzyme function (it would promote transformation

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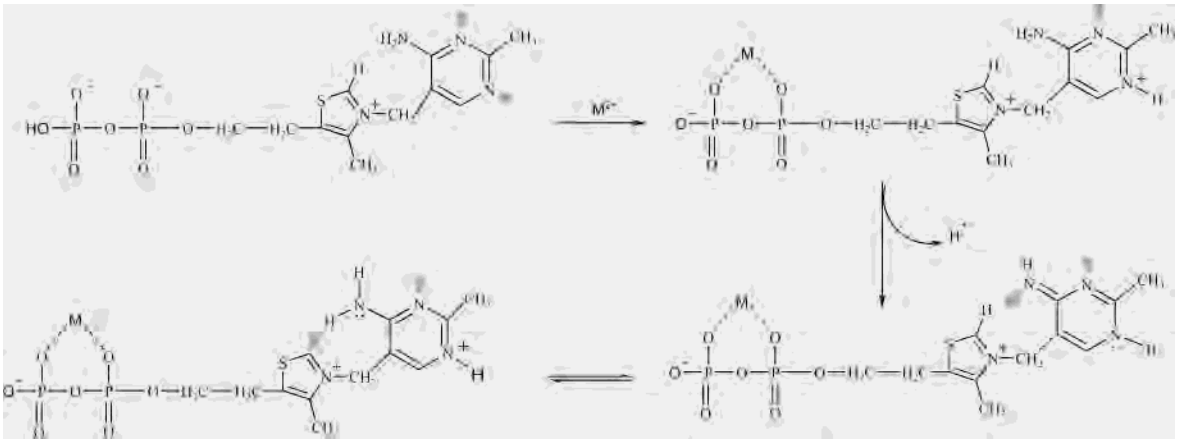
Reaction of Dimethyltin(IV) Dichloride with H_2TDP

Scheme 1



of the 4′-aminopyrimidine group into a 4′-iminopyrimidine capable of inducing the deprotonation of C(2)−H;[8] see Scheme 1), but the origin of the required proton has never been clarified (at the resolutions currently achieved in X-ray studies of thiamine diphosphate-dependent enzymes, hydrogen atoms are not located).[8]

To sum up, at the optimal pH for PDC activity (5.6),[17] thiamine diphosphate probably adopts the monoanionic form $HTDP^-$. In aqueous solution the remaining acidic proton of this ligand is located on the terminal phosphate group, but according to our hypothesis, complexation to the other cofactor, the M(II) cation, not only provides $HTDP^-$ with a means of anchoring to the protein matrix, but displaces its acidic proton from the diphosphate moiety to N(1′), so facilitating the formation of the ═N(4′α)H group and the consequent deprotonation of C(2), a key step in the enzyme mechanism.

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Supporting Information Available: Crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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