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Structural Behaviour of Vitamin B_1 in Bonding to Hydrogen and Metal Ions

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Abstract

Vitamin B_1 binds H^+ through the N1′ donor atom of the pyrimidine ring as observed by X-ray method, and no example of H^+ bonded to the N3′ donor atom is experimentally known. *Ab initio* calculations strengthen the view that the N1′ donor is the preferred binding site to H^+; in fact, the N1′–H isomer is 28.89 kJ mol^{-1} more stable than the N3′–H isomer. Upon protonation at N1′ site the pyrimidine ring undergoes few but experimentally well-defined changes towards the quinonic form; these variations appear by *ab initio* calculations to be due partially to σ and much more to π bond reorganization.

The *ab initio* method applied to the adduct obtained by binding $[ZnCl_3]^-$ anion at N1′ site of the pyrimidine ring indicates that the metal ion causes lower structural changes than H^+ ion and that the $C-NH_2$ and the two C–N1′ bonds involved with the nitrogen donor are the most affected by metal coordination.

Introduction

The biological role of vitamin B_1 (thiamine hydrochloride, T·HCl) as a necessary dietary constituent is well known [1]. Its pyrophosphate ester (TPP) is the coenzyme cocarboxylase which catalyzes the decarboxylation of α-ketoacids to corresponding aldehydes in the presence of divalent metal ions such as Mg(II), Mn(II), and Co(II) [2, 3] (Fig. 1).

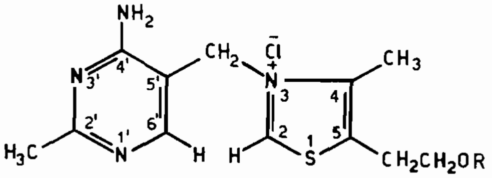

Fig. 1. Structural formula for thiamine derivatives. The common numbering scheme is used in the present paper. R = H (thiamine), $P_2O_7^{3-}$ (thiamine pyrophosphate, TPP).

A general mechanism of action of thiamine pyrophosphate in metal ion catalyzed reactions has been proposed [4, 5], but there are still uncertainties on the role of metal ions and the nature of binding sites [6, 7]. Hadjiliadis *et al.* [6], on the basis of ^1H and ^{13}C NMR studies in DMSO-d_6 of the interaction of thiamine and its phosphate esters with Pt(II) and Pd(II), has shown that the $M(thiamine)Cl_3$ complex is formed and concluded that the role of metal ions is to coordinate pyrimidine at the N1′ donor. Moreover, the X-ray structure of $Cd(thiamine)Cl_3$ [8] showed that the N1′ donor is bound to the metal ion. Gary and Adeyemo [7], through NMR measurements, attempted to show that Zn(II), Cd(II) and Hg(II) are bound to the N3′ donor of the pyrimidine ring, but further investigations persuaded these authors to formulate the complexes as $(thiamine)MCl_4$ [M = Zn(II), Cd(II), Hg(II)] in which no direct bonding of metal ion to thiamine occurs with metal present in the anionic species MCl_4^{2-} [9]. The latter complexes in the presence of Pt(II) and Pd(II) give the already mentioned complexes [6] $M(thiamine)Cl_3$ [M = Pt(II), Pd(II)] where the metal ion is coordinated to N1′ donor [10].

The interaction of dioxouranium(VI) with thiamine and cocarboxylase has been extensively studied by us in methanol solution [11–16]. Physicochemical studies, including X-ray analysis, showed that anionic species such as $[UO_2Cl_4]^{2-}$ or polymeric $[UO_2Cl_3]^-$ are preferentially formed, proving the high tendency of uranyl ion to coordinate chloride ions rather than other electron donors of the thiamine molecule [11, 12, 14].

On the other hand, when unprotonated thiamine nitrate reacts with $UO_2(NO_3)_2·6H_2O$, pyrimidine nitrogen donors of thiamine become favoured with respect to NO_3^-, and the $[UO_2(NO_3)_3]^-$ complex,

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TABLE I. *Ab Initio* Bond Order Values and Distances in Pyrimidine Ring of Thiamine Bound to H^+ and Metal Ions[a]

Linkage	Bond order				Bond distances (Å)					
	Free base	H^+ at N1′	H^+ at N3′	$[Zn(py)Cl_3]^-$	Free base	H^+ at N1′	$CdTCl_3$ (−166 °C)	$(TH)_2UO_2Cl_4$	$(TH)_2CuCl_4$	$(TH)_2CdCl_4$
C4′–NH_2	0.3728σ 0.0461π	0.3718σ 0.0796π	0.3760σ 0.0720π	0.3717σ 0.0512π	1.334	1.315	1.346	1.32	1.323	1.323
N3′–C4′	0.3429σ 0.0960π	0.3389σ 0.0822π	0.3598σ 0.0723π	0.3400σ 0.0928π	1.350	1.358	1.355	1.36	1.360	1.364
C2′–N3′	0.3369σ 0.1024π	0.3462σ 0.1161π	0.3333σ 0.0725π	0.3363σ 0.1058π	1.332	1.308	1.329	1.30	1.313	1.312
N1′–C2′	0.3335σ 0.1035π	0.3357σ 0.0803π	0.3413σ 0.1190π	0.3437σ 0.0960π	1.335	1.345	1.353	1.35	1.349	1.337
N1′–C6′	0.3398σ 0.0991π	0.3492σ 0.0681π	0.3345σ 0.0866π	0.3526σ 0.0922π	1.342	1.352	1.353	1.40	1.342	1.356
C4′–C5′	0.3958σ 0.0978π	0.3910σ 0.0788π	0.3959σ 0.0958π	0.3945σ 0.0960π	1.416	1.428	1.417	1.44	1.430	1.422
C5′–C6′	0.3959σ 0.1145π	0.4028σ 0.1345π	0.3928σ 0.1178π	0.3948σ 0.1171π	1.369	1.349	1.369	1.35	1.369	1.343

[a]Abbreviations: T = Thiamine; TH = Thiamine protonated at N1′ site; py = pyrimidine. Values in columns V, VII and X are taken from Ibers *et al.*, ref. 8.

endowed with stereo-electronic hindrance [13], is not formed.

Furthermore, when UO_2–TPP chelate complexes are formed, the pyrophosphate side chain is mainly involved since uranyl ion has a high affinity for the pyrophosphate group [15].

In order to clarify the stereochemical behaviour of the pyrimidine ring of thiamine, we have performed *ab initio* calculations on such a system and its metal derivatives.

Calculations

Ab initio LCAO–SCF MO-calculations were performed using the GAUSSIAN 80 (QCPE 446) set of programs [17] running on a VAX 11/780 computer. The basic set of contracted Gaussian functions used was the usual STO-3G for H, C, N, while for Zn the contracted set of Gaussian functions (4333/43/5) $+P(\zeta_1, \zeta_2)$, P = polarization function, recently published by Tomonari *et al.* [18] was used.

Since the interaction of thiamine with H^+ and metal ions occurs through the pyrimidine ring and to save computation time, we have performed the theoretical studies using only the pyrimidine ring in the same conformation found in the crystal by Kraut and Reed [19], and the thiamine bridging $-CH_2-$ group was substituted by a $-CH_3$ group. The distances in the hexatomic ring were taken equal to: C–N = 1.342 Å; C–C = 1.390 Å; and in the extra-anular ring: C–N = 1.316 Å; C–H = 1.075 Å; the two C–CH_3 distances equal to 1.496 Å and 1.492 Å as found in the crystal. For the $[Zn(pyrimidine)Cl_3]^-$ complex the distances were taken as equal to $Zn-N_{aromatic} = 2.065$ Å and Zn–Cl = 2.215 Å, very near to the values found in refs. 20 and 21 and corrected for the lack of a hydrogen bond. The conformation imposed on the $ZnCl_3^-$ group was that of $CdCl_3^-$ found in $Cd(thiamine)Cl_3$ complex [8].

Results and Discussion

Structural results both in solid state and solution indicate that the pyrimidine ring of vitamin B_1 coordinates H^+ ions at the N1′ position. Upon protonation the pyrimidine ring undergoes a structural change in bonds and angles. In particular C4′–NH_2, C2′–N3′ and C5′–C6′ bonds are remarkably shortened by ≅0.020 Å, whereas N3′–C4′, N1′–C2′, N1′–C6′ and C4′–C5′ bonds are lengthened by ≅0.010 Å (see Table I). Kraut and Reed [19], using qualitative resonance theory, showed that the quinonic form of the pyrimidine ring gives the highest partial contribution upon protonation. Protonation at the N1′ site is maintained in the $(TH)_2XCl_4$ complexes ($X = UO_2^{2+}$, Cu^{2+}, Cd^{2+}) where the metal is bound to four chloride atoms as anionic species and not to the pyrimidine ring.

The *ab initio* method shows that the calculated total energy of the pyrimidine ring protonated at the N1′ site is = −391.31163 a.u., while when the N3′ site is protonated, the total energy is = −391.30063 a.u. Thus the N1′ isomer is 28.89 kJ mol^{-1} more stable than the N3′ isomer, indicating that N1′ binding is preferred over the N3′, in agreement with experimental results. Moreover, for a better understanding of the bonding situation in this system, we have defined, following Mulliken [22], a π-bond order (hereafter B_π) as due only to the $2p_z$ orbitals (*i.e.*, the 2p orbitals perpendicular to the pyrimidine ring) and a σ-bond order (hereafter B_σ) as due to the interaction of $1s-2s-2p_x-2p_y$ orbitals on each atom. Table I reports B_σ and B_π values of the pyrimidine ring for the free base (column I), the protonated form at N1′ (column II), the protonated form at N3′ (column III) and the $[Zn(pyrimidine)Cl_3]^-$ complex (column IV, discussed later).

The variation of the bond length (Δl) upon protonation may be ascribed to the variation of B_σ (ΔB_σ) and B_π (ΔB_π) bond orders according to one linear equation for each bond.

$$\Delta l = a\Delta B_\sigma + b\Delta B_\pi \qquad (1)$$

Consequently, from the resulting seven equations the two unknowns a and b are determined by the least-squares method. The values obtained for a = 1.103 Å and b = 0.587 Å indicate that to observe the same Δl variation ΔB_π must assume twice the ΔB_σ value. Nevertheless the variations of bond distances are mainly due to variations in π bond orders, since the ΔB_σ values are much lower than the ΔB_π values. The individual contribution of σ and π to the bond length variation is shown in Table II.

The Cd^{2+} coordination at N1′ (column VII, Table I) causes, as noted by Ibers *et al.* [8], a recovery of the pyrimidine ring towards that of the free form, as indicated by the increase of C4′–NH_2 (0.03 Å), C2′–N3′ (0.02 Å) and C5′–C6′ (0.02 Å) bond lengths. However, the structural effects of complexation of Cd^{2+} at the N1′ site go in opposite directions for the N1′–C6′ and N1′–C2′ bonds, being closer to the protonated N1′ form rather than the thiamine free base. Thus, the metal coordination causes a structural change only in bonds near the coordination site.

In order to gain a general view of the N1′-pyrimidine metal bonding we have carried out *ab initio* calculations on $[Zn(pyrimidine)Cl_3]^-$ complex. This metal complex was chosen in order to limit the computing time without loss of chemical significance. The bond order values, reported in column IV,

TABLE II. Variation of Bond Lengths (Å × 10^3) and Individual Contribution of σ and π Bonds in the Pyrimidine Ring

Linkage	Δl^a	$\Delta l_\sigma{}^b$	$\Delta l_\pi{}^b$	$\Delta l_1{}^c$
C4'–NH$_2$	19	−1	20	−12
N3'–C4'	−8	−4	−8	−5
C2'–N3'	24	10	8	3
N1'–C2'	−10	2	−14	−18
N1'–C6'	−10	10	−18	−11
C4'–C5'	−12	−5	−11	−1
C5'–C6'	20	8	12	0

[a] Δl represent the experimental differences in bond lengths between the free base and the protonated N1' form. The sign is assumed positive for a shortening and negative for a lengthening. [b] Δl_σ and Δl_π are the values calculated from eqn. (1). [c] Δl_1 represent the Δl values between the free base and the $[Zn(py)Cl_3]^-$ complex.

TABLE III. *Ab Initio* Total Atomic Charges (10^{-3} e) in Pyrimidine Ring of Thiamine Bound to H^+ and $ZnCl_3^-$ Ions

Atom or group	Free base	H^+ at N1'	H^+ at N3'	$[Zn(py)Cl_3]^-$
N amino	−468	−429	−437	−464
–NH$_2$	0	+130	+122	+8
C5'	−34	−3	−10	−40
C5'–CH$_3$	+7	+100	+91	+12
C6'	+46	+122	+112	+87
N1'	−295	−312	−240	−309
C2'	+215	+319	+310	+259
2'–CH$_3$	+50	+155	+153	+95
N3'	−311	−267	−319	−312
C4'	+245	+311	+337	+261
C6'–H	+77	+147	+143	+123
H^+–N1'		+306		
H^+–N3'			+302	
Zn				+472
Cl1				−553
Cl2[a]				−550
Cl3				−553

[a] The lower charge of Cl2 in respect to Cl1 and Cl3 atoms is likely due to the interaction with C6'–H proton (Cl2··· H–C6' = 2.931 Å), being Cl2 in the plane of pyrimidine ring.

Table I, indicate that the pyrimidine ring, upon metal ion coordination, assumes a geometry between the free base and the protonated form, but closer to the former. In fact, the B_σ and B_π bond order values for C4'–NH$_2$, N3'–C4', C2'–N3', C4'–C5' and C5'–C6' go towards the free base form. The values of the N1'–C2' and N1'–C6' B_π bond order, which are located within the protonated and unprotonated thiamine, are closer to the latter form, but a large variation of σ-bond order towards the N1' protonated form is observed for such linkages, thus precluding a quantitative correlation. In any case, the most important structural effect upon metal coordination, *i.e.*, the increase of the C4'–NH$_2$ bond length by about 0.03 Å, is also correctly predicted by theoretical computations. The experimental changes of bond distances reported above (0.01–0.03 Å), although small, are significant since the molecular structures have been accurately determined either by averaging on several measurements or by carrying out the diffraction experiment at low temperature (−166 °C). The good quality of the bond distances is also supported by their agreement with our theoretical calculations.

The total atomic charges of the pyrimidine derivatives are reported in Table III, where the charge of the whole group of atoms is given when an atom is bonded to two or more hydrogen atoms (e.g. $-CH_3$). The overall distribution of atomic charges is qualitatively that expected on the basis of the electronegativity principle. In fact, nitrogen atoms ($-NH_2$, N1' and N3') bear the largest negative charges, whereas carbon atoms have positive charges decreasing in the order: C4' ≅ C2' > C6'. Upon metal complexation the atomic charges move towards those of the free base, resembling the trend observed for bond order. The gross atomic orbital populations (GAOP) for (1s + 2s + 3s), (2p + 3p) and 3d orbitals of Zn are very near to 6, 12 and 10, respectively, indicating that 3p and 3d electrons do not participate appreciably in chemical bonding, whereas no neglectable values are found for 4s and *P* (4p) functions (0.56 and 0.95) showing the importance of the hybridization of the 4s and 4p functions.

In conclusion, the present *ab initio* calculations interpret the variation of bond lengths of the pyrimidine ring, observed by X-ray analysis, as due to σ but much more to π bonding reorganization and point out that the pyrimidine N1' donor of thiamine is the preferred binding site to H^+ and metal ions with respect to the N3' donor.

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