Structural aspects of thiamine, its derivatives and their metal complexes in relation to the enzymatic action of thiamine enzymes

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CONTENTS

ABSTRACT

A review is given of the mechanism of the enzymatic action of thiamine (vitamin B_1) enzymes, based mainly on the metal complexes and the crystal structures of the ligand and its derivatives. It consists of three parts. Part 1 is a general introduction. Part 2 emphasizes the role of the pyrimidine and thiazole moities of thiamine and also of the pyrophosphate group and the bivalent metal ions in the enzymatic action, covered in four subsections referred to each of the above labels, the last of which describes the crystal structures of thiamine, its derivatives and their metal complexes. It attempts a correlation of the crystallographic data with the proposed mechanisms of the enzymatic action of thiamine. Part 3 is the

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conclusion, pointing to the stage of intervention of the metal ions and expanding the importance of the S conformation of the ligand during the enzymatic action of thiamine enzymes.

1. INTRODUCTION

1.1. Purpose and scope

Vitamin B_1 (thiamine) is the coenzyme of many enzymes catalyzing, among others, the decarboxylation of α-keto acids. Although it is known that the mechanism of the decarboxylation involves the formation of the "active aldehyde" intermediates, as proposed by Breslow [1], the detailed role played in the mechanism of each part of the molecule (pyrimidine, thiazole, etc.) is not known. Especially the role of the bivalent metal ions also required for its enzymatic action is not known. This paper mainly reviews the work performed on metal complexes of bivalent metal ions with thiamine and its derivatives, although other relevant work that constitutes an important background is also cited. The review tries to correlate the various crystal structures and other studies on metal complexes of thiamine and its derivatives with the mechanism of action of thiamine enzymes and the role played by the bivalent metal ions in it. No such review exists, though other relevant reviews on related subjects have been published [2].

2. CATALYSIS BY THIAMINE

2.1. Brief review

The pyrophosphate ester of thiamine (TPP) (Fig. 1) is also known as cocarboxylase, because it was first found as the coenzyme of carboxylase, an enzyme that catalyzes, among others, the decarboxylation of pyruvic acid. It is also the coenzyme of many other enzymes that catalyze the breaking of a C–C bond located near a carbonyl group [3]. The presence of bivalent metal ions is necessary for such reactions that have a common characteristic. They all lead to the formation of an acyl carbanion, RCO^-:

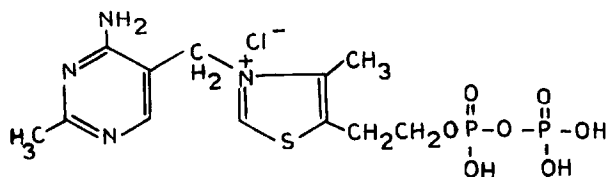$$RC(O)COO^- \rightarrow RCO^- + CO_2 \tag{1}$$

Fig. 1. Structure of the pyrophosphate ester of thiamine.

or of an equivalent intermediate [1,4]. This intermediate is called an "active alde-hyde". This anion reacts with a proton and forms an aldehyde (pyruvic decarboxyl-ase), with a second carboxyl group (α-acetolactic synthetase), with another aldehyde (pyruvic decarboxylase and transketolase) or with lipoic acid in a transketolase E_2, to be transferred to coenzyme A (pyruvic dehydrogenase), etc. [3].

The fact that thiamine alone, without the presence of enzymes, was able to catalyze reactions analogous to those catalyzed by pyruvic decarboxylase led to the conclusion that the mechanism of "enzymic and non-enzymic catalysis" should be common [2,5].

Based on model reactions, Breslow [1] proposed a mechanism for the enzy-matic action of thiamine which was generally approved and is still considered to be correct. This mechanism involves the ionization of a proton from the C(2) position of the thiazole ring and the formation of an ylid that could be added to the carbonyl group of pyruvic acid [1]. This intermediate product is the "active aldehyde" (Fig. 2) and its isolation proved its existence [6,7].

It should be noted, however, that despite the general acceptance of the mecha-nism of Breslow, there are several unanswered questions such as the role of the pyrimidine or thiazole moieties and the role played by the bivalent metal ions during the enzymatic action of thiamine.

Research work attempting to answer such questions is reviewed below.

2.2. Role of pyrimidine

Based on studies on model compounds, Schellenberger [8] concluded that the binding site of thiamine with the substrate is the N(1') atom of pyrimidine and this is achieved with the intervention of metal ions. It was also confirmed that the protonation of thiamine takes place at N(1') [9,10]. Schellenberger [8] also con-cluded that the 4' NH_2 group of pyrimidine acts as a proton acceptor (Lewis base), by accepting a proton from the intermediate of the "active aldehyde" which results in the liberation of aldehyde according to the mechanism shown in Scheme 1.

Jordan and Mariam [11] showed that the 4'-amino group of pyrimidine of a thiamine analog which bears a positive charge on N(1') can act as a weak acid (Fig. 3). They therefore proposed that such a system could take part in proton transfers, acting as a proton donor.
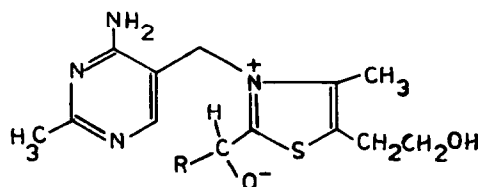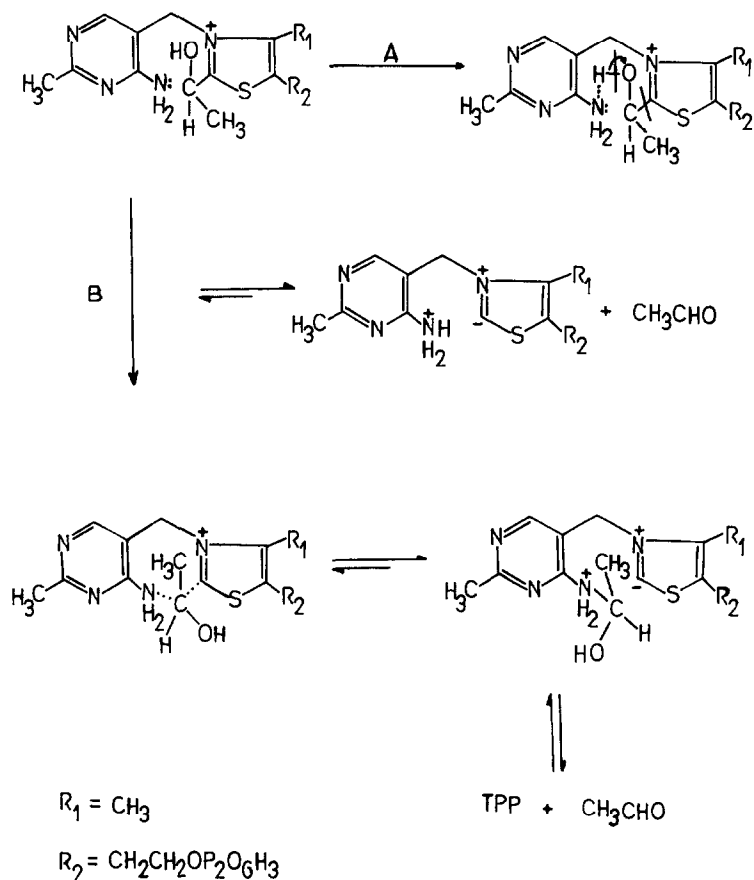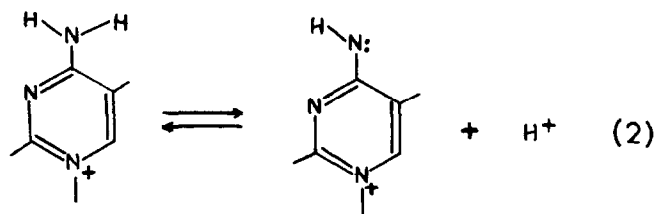

Fig. 2. Structure of the "active aldehyde" derivative.

$R_1 = CH_3$

$R_2 = CH_2CH_2OP_2O_6H_3$

Scheme 1.

Fig. 3. Action of the 4' α-amino group of pyrimidine as a weak acid.

Later, Schellenberger [12] proposed that the amino–pyrimidine system acts as an integrated part in the catalytic functions of thiamine and that the 4'-NH$_2$ group takes part in both the binding of the molecule to the substrate and in the liberation of the aldehyde through the formation of a hydrogen bond of the type NH···O (Scheme 2). Such a role for the 4'-NH$_2$ group, however, requires a suitable orientation
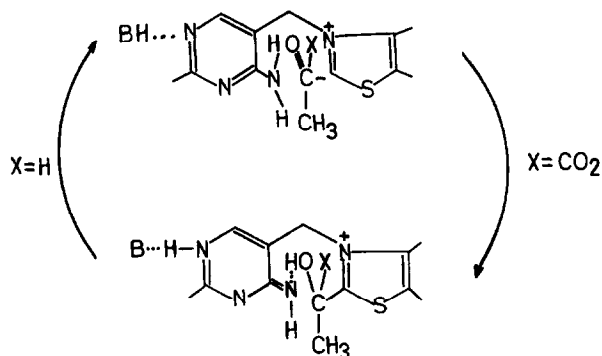
Scheme 2.

of the pyrimidine and the thiazole rings, which could be realized with the aid of the protein [12].

The relative orientations of the pyrimidine and thiazole rings in thiamine are determined by the torsional angles $\phi_P = $ N(3)–C(b)–C(5')–C(4') and $\phi_T = $ C(5')–C(b)–N(3)–C(2), according to Sax et al. [13], based on crystallographic studies (Fig. 4). These angles have positive values in the clockwise direction and according to their values there are three main configurations of thiamine and its derivatives. The S configuration with $\phi_T = \pm 100°$, $\phi_P = \pm 150°$, characteristic of the C(2)-substituted derivatives of thiamine, the F configuration with $\phi_T = 0°$ and $\phi_P = \pm 90°$, common in the non-C(2)-substituted derivatives, and the V configuration ($\phi_T = \pm 90°$, $\phi_P = \pm 90°$), found only rarely [14]. The mechanism of action of thiamine proposed by Schellenberger [8,12] requires the V configuration for the molecule, where the 4'-NH$_2$ group approaches the C(2) site of thiazole [15]. In the F configuration, H(2) is oriented over C(5') of pyrimidine, whereas in the S configuration H(6') is near N(3) of thiazole [15]. Until recently, only two examples of thiamine derivatives were known in the V configuration, however: oxythiamine [16,17] with the 4'-amino group substituted by a keto group and the pyrophosphate ester of thiaminethiazolone [18] with a keto group at the C(2) position of thiazole. Neither of those two derivatives catalyzes the thiamine reactions, although oxythiamine forms an "active aldehyde" intermediate. Even this last "active aldehyde" derivative,
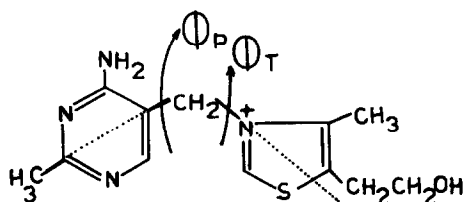
Fig. 4. Schematic presentation of torsional angles ϕ_P and ϕ_T.

2-(α-hydroxybenzyl)oxythiamine [19], was not found in the V but in the S configuration as in all the C(2)-substituted derivatives of thiamine. Recently, however, the three-dimensional structure of transketolase from *Saccharomyces cerevisiae*, a thiamine diphosphate-dependent enzyme, showed [20] that thiamine was in the V conformation.

On the other hand, Gallo and Sable [21] proposed a completely opposite role for the 4'-amino group of pyrimidine, which according to them does not take part in the ionization of the C(2) proton of TPP at all. The conclusions of Gallo and Sable [21] were supported by the kinetic studies of Washabaugh and Jencks [22].

Also, in order to elucidate the role played by the 2'-CH$_3$ group of pyrimidine, the enzymatic action of various model compounds, analogs of thiamine, bearing a hydroxy, methoxy or n-butyl group or a proton at the 2'-position was studied [23]. Although certain of them [23] showed a small relationship with the apoenzyme, none had a complete action [23]. The existence of a hydrophobic interaction between the 2'-CH$_3$ group of thiamine and the apoenzyme, probably playing an important role in the stabilization of the coenzyme–apoenzyme system, was proposed, based on these observations [23].

Finally, the lengthening of the bridging methylenic group with an additional CH$_2$ group, in thiamine, resulted in the complete enzymatic destruction of the enzymatic action of the latter, because it changed completely the geometry of the molecule [24]. Its catalytic action was also reduced in the non-enzymatic reactions, since these were similar to those of thiazolic salts, bearing an alkyl group at the 3-position of the thiazole ring, instead of a pyrimidine ring.

2.3. Role of thiazole and the pyrophosphate group

Concerning the role of thiazole, the formation of the "active aldehyde" through the ionized C(2) position of thiazole and the creation of an α-carbanion is a process already known. Breslow [1] proposed that the α-carbanion is stabilized through a resonance structure which does not bear a positive charge on the thiazole ring but instead has a double bond between the C(2) and C(2α) atoms (Fig. 5).

In 1982, however, Schellenberger [12] proposed that the α-carbanion (A) more successfully represents the intermediate of the various enzymatic mechanisms and not the enamine (B) or the enol (C) (Fig. 6).

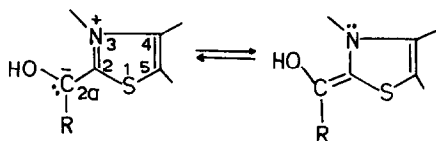Haake and co-workers [25–27] tried to understand the possible role played
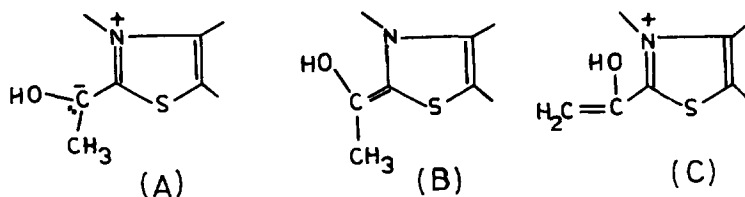

Fig. 5. Resonance structure of α-carbanion.

Fig. 6. Structures of (A) the α-carbanion, (B) enamine and (C) enol.

by the S atom of thiazole in the enzymatic reactions. They concluded that the thiazolium ion was a better catalyst than the imidazolium or the oxazolium ions in the enzymatic reactions, owing to the better stabilization of the ylid intermediate by the former, because of the 3d orbitals of sulfur taking part therein. More particularly, Hogg [28] proposed that the S atom of TPP uses the 3d orbitals to stabilize the negative charge on the oxygen of the carbonyl group of the transition state, which leads to the formation of the "active aldehyde" (Fig. 7).

The determination of the crystal structure of several derivatives and analogs of thiamine by X-ray diffraction showed a strong electrostatic interaction between the S atom of thiazole and the carbonyl oxygen of the substituent at the C(2α) position. For example, in the crystal structures of 2-(α-hydroxyethyl)-3,4-dimethylthiazole bromide [13], 2-(α-hydroxyethyl)thiamine chloride [13] and 2-(α-hydroxy-α-benzyl)thiamine chloride [29] there is an important interaction between the S atom and the oxygens of the hydroxyethyl and hydroxybenzyl groups attached at C(2α). The same is true also in the structure of 2-(α-hydroxybenzyl)oxythiamine chloride hydrochloride [19]. The theoretical calculations of Jordan [30] also predicted that these interactions play a crucial role in the configuration of the side-chain. The S···O electrostatic interactions are manifested from the relatively short distances of the two atoms, observed in the various crystal structures (see Table 4 and Fig. 8) and implies also a low value of $pK_a = 12$ for the hydroxylic proton [31]. Following the ionization of this proton, the S···O interaction becomes stronger and stabilizes the structure even more, owing to the creation of a negative charge on the O atom [28].

Oxythiamine, on the other hand, the pyrophosphate ester of which is a strong antagonist of TPP, forms an "active aldehyde" derivative but does not liberate the
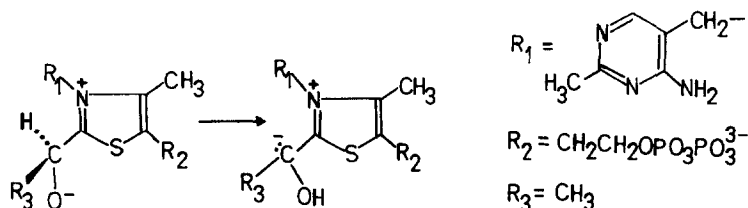

$$R_1 = $$

$$R_2 = CH_2CH_2OPO_3PO_3^{3-}$$

$$R_3 = CH_3$$

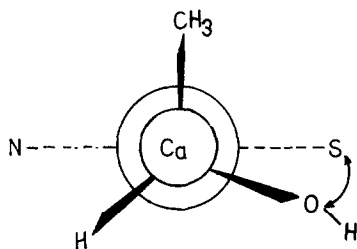Fig. 7. Formation of "active aldehyde" from the transition state.

Fig. 8. Newman projection along the C(2α)–C(2) bond.

aldehyde. Schellenberger [8] proposed that this was due to its inability to form an intramolecular hydrogen bond, known to be formed between the 4'-NH$_2$ group and the hydroxylic proton attached to C(2α) in the "active aldehyde" derivatives of thiamine. Hogg [28], on the other hand, proposed rather, that the very strong S···O electrostatic interaction (S···O = 2.749 Å) observed in the crystal structure of 2-(α-hydroxybenzyl)oxythiamine [19] was responsible for the stabilization of the "active aldehyde" to such an extent that it did not allow the subsequent liberation of acetaldehyde. This behavior, together with other electronic and configurational differences, could be explained by the antagonistic properties of oxythiamine to TPP [28]. Hogg [28] also proposed a mechanism for the reaction of TPP with pyruvic acid, involving several intermediate steps, stabilized through the 3d orbitals of the S atom (Scheme 3).

The same role assigned to the 2'-CH$_3$ group, was also assigned to the 4-CH$_3$ group of thiazole [14,23]. These two groups constitute the second binding region of the coenzyme with the apoenzyme, achieved through hydrophobic interactions [14,23].

As far as the role of the pyrophosphate group is concerned, which is necessary for the enzymatic action [32], Wittorf and Gubler [23] believe that it is also used for the binding of the coenzyme to the apoenzyme. This binding [33] takes place through Mg(II) ions, between the pyrophosphate oxygens of TPP and polar groups of amino acids (perhaps carboxyls) of the apoenzyme and it is ionic. In fact, in the crystal structure (2.5 Å resolution) of transketolase [20] Ca^{2+} ions were bound through the pyrophosphate oxygens and the amino acids Asp 157, Asn 187 and Ile 189 of the protein, through their oxygen atoms, or amino groups.

2.4. Role of the bivalent metal ions

The enzymatic action of TPP *in vivo* also requires Mg(II) whereas other metal ions *in vitro* such as Co(II), Zn(II), Mn(II), Fe(II), Ni(II) and Cd(II) and others can replace it [32,34], although Mg(II) remains the most active. It was initially proposed [32,35] that the metal ions are used as bridges in the binding of the apoenzyme with the coenzyme.
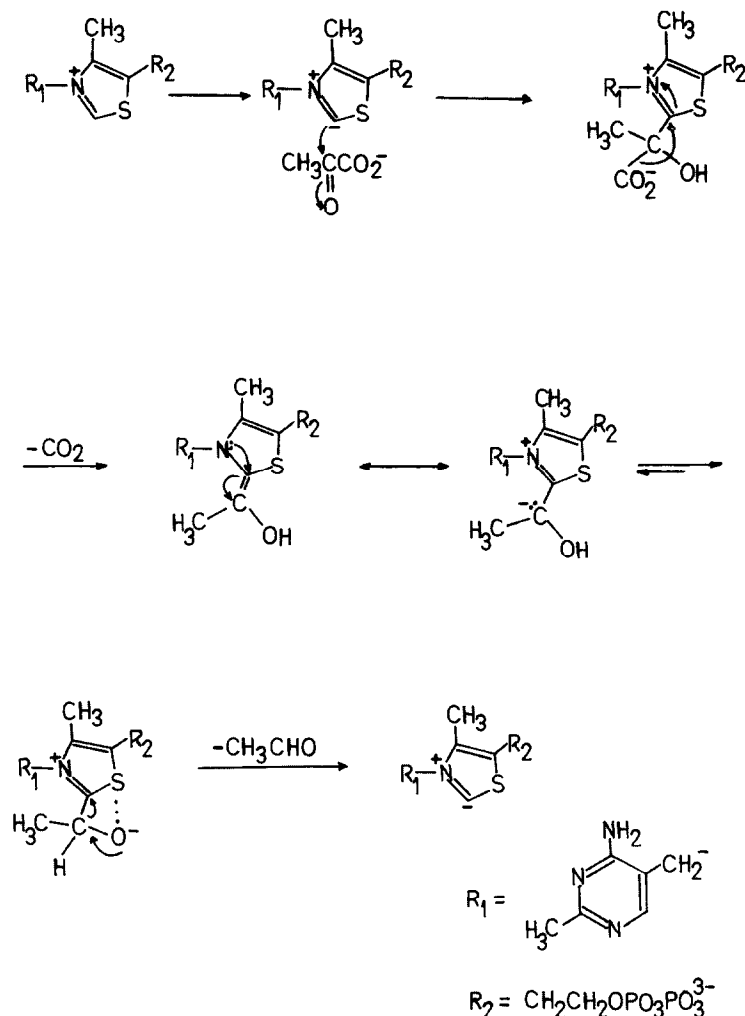
Scheme 3.

Later, Schellenberger [8], using kinetic methods, found that Mg(II) was indispensable for the stable binding of the apoenzyme with TPP and that the formation of the thermodynamically stable holoenzyme, being non-reversible, required the presence of all three constituents, since it was not possible to detect stable bonds between TPP and Mg(II) ions alone.

$$\text{apoenzyme} + \text{Mg}^{2+} + \text{coenzyme} \underset{K_{\text{back}}}{\overset{K}{\rightleftharpoons}} \text{holoenzyme} \tag{3}$$
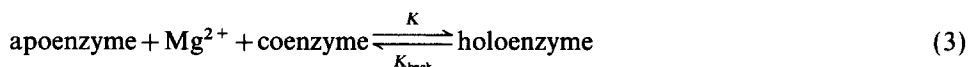
Schellenberger [8] also concluded that the binding of the metal ion with the

coenzyme must take place at the N(1') position of TPP, based on the fact that 4-aminopyrimidines are selectively protonated at the N(1') position.

Grande et al. [36], using NMR studies, proposed that Mn(II) binds with the pyrophosphate group of TPP. White and Drago [37], based on ^{1}H and ^{31}P NMR studies of interactions of TPP with Co(II) and Ni(II), also proposed that the metal ions are bound simultaneously with both the pyrophosphate group and the pyrimidine moiety (N(1')). This was confirmed by Gallo and co-workers [38,39], who proposed however, that Ni(II) binds through N(1') with the intervention of a water molecule and not directly.

Despite the strong indications for many years that the metallic ions bind the N(1') site of pyrimidine and directly or indirectly the pyrophosphate group of TPP all compounds of thiamine containing metal ions and isolated as solid adducts were ionic salts of the type $[MX_4]^{2-}[th]^{2+}$ or $[MX_4]^{2-}[th]_2^+$ or $[MX_3]_2^-[th]^{2+}$ [40–45], with M = Co(II), Ni(II), Cu(II), Zn(II), U(IV) and X = Cl, Br. This was due to the net positive charge at N(3) of thiazole and the pK_a value of N(1') of pyrimidine of thiamine [46] being about 5, together with the fact that the molecule is unstable at pH > 7. Consequently, at pH < 5 the molecule exists in solution as a dication, whereas at pH 5–7 it is a monocation.

Richardson et al. [44], based on the X-ray crystal structure determination of the ionic salt $[thH]^{2+}[CdCl_4]^{2-}\cdot H_2O$, concluded that a direct metal–ligand bonding with thiamine is not energetically favored. Only when the metal–ligand bonding energy is larger than the loss of resonance energy plus the loss in solvation energy of the metal ion will the existence of such a direct metal–ligand bond be possible [44].

The first examples, however, of metal complexes of thiamine and its phosphate derivatives displaying direct bonding of the metals with the N(1') of pyrimidine involved Pt(II) and Pd(II) and had zwitterionic structures [47] (Fig. 9).

The relatively easy formation of these complexes was attributed to the high bond strength of Pd–N and Pt–N [47,48]. As a result, Pd^{2+} and Pt^{2+} compete favorably for the H$^+$ of the N(1') site at pH 3.5 and form these products [47,48]. The proposed structure (Fig. 9), based on ^{1}H and ^{13}C NMR and IR spectrometry, was later confirmed [47] by an X-ray crystal structure determination of the corresponding Pt^{2+} complex of formula Pt(th)Cl$_3\cdot$H$_2$O [49]. Prior to this, the crystal
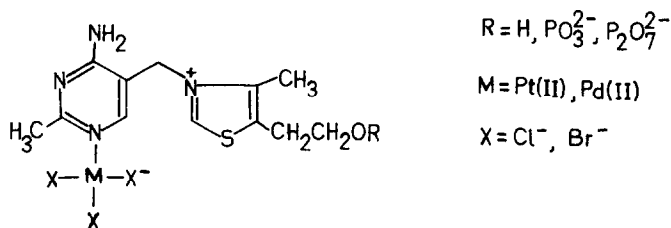

Fig. 9. Structure of the complexes of platinum(II) and palladium(II) with thiamine, thiamine monophosphate and thiamine pyrophosphate.

structure of the complex Cd(th)Cl$_3$·H$_2$O, with a direct Cd–N(1′) bond, was solved by X-ray determination by Cramer et al. [50] in 1981. The structures of the complexes Cu(th)Cl$_2$ [51], Rh$_2$(CH$_3$COO)$_4$(TMP)$_2$·1.5H$_2$O [52], Zn(th)Cl$_3$·0·4H$_2$O [53], Cu(th)Br$_2$ [54], Hg(HBT)Cl$_3$·H$_2$O [55], Co(th)Cl$_3$·0·4H$_2$O [56], Zn(th)Br$_3$·0.2H$_2$O [56] and Zn(th) (SCN)$_3$ [57], containing direct metal–ligand bonds, were subsequently determined. In all these cases the metals were bound to N(1′) of pyrimidine, owing to the high basicity of this site [52]. Exceptions were the Cu(II) complex, (Fig. 10), with the metal bound to the pyrophosphate group of TPP [58], the complex Cd(th) (SCN)$_3$ [57] with Cd(II) bound to O(5γ) of the side hydroxyethyl group and the complex [Mn(th)Cl$_2$(H$_2$O)]$_2$[th]$_2$Cl$_4$·2H$_2$O [59], where Mn(II) binds the N(1′) of pyrimidine of one thiamine molecule and the O(5γ) atom of the side hydroxyethyl group of a second thiamine molecule. Another exception was the transketolase enzymatic system itself [20], where Ca^{2+} was bound to the pyrophosphate oxygens.

In none of the crystal structures of thiamine metal complexes was the V configuration ever found for the ligand, except of course the recent transketolase structure [20]. If true, this is not in favor of the proposal of Schellenberger [8,12] for an approach of the NH$_2$ group to the C(2) position of thiazole, and its subsequent action as a Lewis base or acid. On the contrary, the thiamine configurations found by X-ray measurements were either the S or the most common F configuration, characteristic of its derivatives unsubstituted at C(2) [14]. In these F and S thiamine configurations, however, in all the metal complexes, there is a strong electrostatic interaction between the S atom of thiazole and the O atom of the side-chain at C(5). This is obviously due to the localization of a partial positive charge on the S atom, in agreement also with the theoretical predictions of Jordan [30]. This type of S$^+$···O$^-$ interaction seems to influence several steps in the decarboxylation of pyruvic acid, as Hogg had also proposed [28].

This strong electrostatic S···O(2a) interaction results in the delocalization of positive charge on the N(3) atom of thiazole to the whole ring and possibly facilitates the proton ionization from O(2α) in the "active aldehyde" intermediates of thiamine.
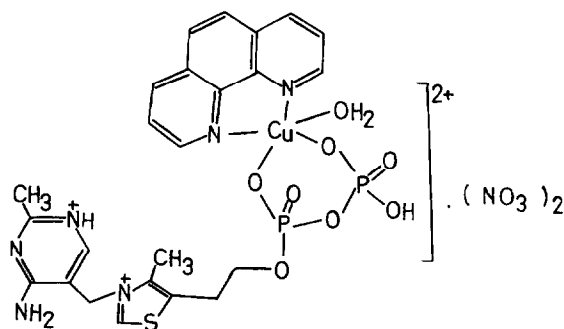

Fig. 10. Structure of the complex [TPP–Cu^{2+}–phen] (NO$_3$)$_2$.

This observation led us to the hypothesis of the easier formation of complexes of bivalent metal ions with these intermediates with a direct metal–ligand bond [55,60–63], in contrast to the ionic salts that were very easily obtained with thiamine itself [40–45]. This was proved to be true (see below).

Therefore, the role of metal ions, as also proposed by Schellenberger [8], could be to connect the coenzyme with the catalytic center of the enzyme acting as a bridge between the two. The metal most probably binds through N(1') and/or the pyrophosphate group, although the ethyl pyrophosphate group at C(5) is extremely flexible [52]. In addition, Aoki and Yamazaki [52] with the hypothesis that TPP keeps the stable F configuration when it binds the pyruvate anion, assigned one more role to the metal ion. They influence the electronic density of the ligand by attracting electrons and this results in favorable interactions of pyrimidine with the substrate. These hypotheses, however, contradict the following facts. First, in the crystal structure of an analog of 2-(α-lactyl)thiamine [64], the initial intermediate formed in the decarboxylation of pyruvic acid, and also of 2-(α-hydroxyethyl)thiamine [13] and 2-(α-hydroxybenzyl)thiamine [29], both "active aldehyde" intermediates, were found in the S configuration. Second, complexes of two "active aldehyde" compounds, namely of 2-(α-hydroxybenzyl)thiamine [55] with Hg^{2+} and of 2-(α-hydroxy-α-cyclohexyl)thiamine [60] with Zn^{2+} having a direct metal–ligand bond at the N(1') site, were also found to have the S configuration. Third, such complexes bound at N(1') are very easily formed [55,61] owing to the electrostatic interaction of the sulfur atom with the oxygen atom of the hydroxyl at C(2) $(S^+ \cdots O^-)$, due to the partial delocalization of the positive charge at N(3) of thiazole. Fourth, it has been concluded, based on spectroscopic (NMR, IR, Raman) studies [62,63], that in all metal complexes of "active aldehyde" derivatives of thiamine, the ligands correspond to an S configuration in the solid state which is also retained in solution. All the above facts emphasize the crucial role that may be played by the sulfur atom of thiazole, substantiated in the strong $S \cdots O(2\alpha)$ interaction, and suggest that the intervention of the metal ions should follow the formation of the "active aldehyde" intermediates in the enzymatic action of thiamine [55,60–63]. Certainly, the recently solved X-ray crystal structure of transketolase emphasizes again the importance of the V conformation for thiamine as at least one of the possible conformations that the molecule may take during the enzymatic action. The importance of the S conformation cannot be minimized, however, besides the V conformation [20]. An X-ray structure of a real enzymatic system containing an active aldehyde intermediate of thiamine could prove it unambiguously.

2.5. Discussion of crystallographic data

2.5.1. Pyrimidine

It is known that the protonation site of thiamine and its derivatives is the N(1') site of the pyrimidine ring. The same site is also the preferred metallation site

in metal complexes of thiamine [49–57], although some exceptions have been observed [57–59]. The high basicity of this N(1′) site rather than stereochemical reasons is probably responsible for its binding with metal ions [52]. In the case of the complexes Cd(Th) (SCN)₃ [57] and TPP–Cu²⁺–phen [58] and Ca²⁺ transketolase [20], the metals bind to O(5γ) in the first and to the pyrophosphate group in the other two, whereas in the case of [Mn(th)Cl₂(H₂O)]₂[th]₂Cl₄·2H₂O [59], Mn²⁺ binds to N(1′) of one thiamine molecule and to O(5γ) of the side hydroxyethyl chain of a second thiamine molecule. Mn²⁺ being a "hard acid" can explain its binding to O(5γ), being a "hard base" [59]. In the case of the Cd²⁺ complex, however, the metal binding to O(5γ) cannot be explained with the theory of binding of a "hard base" to a "hard acid" or of a "soft base" to a "soft acid", Cd²⁺ being a soft acid [57]. In this case the polymeric nature of the complex may contribute to its stability [57].

The role of the 4′-NH₂ group of thiamine depends on its basicity and its subsequent participation in hydrogen bonding and this is a function of the C(4′)–N(4′α) bond length. This is because, according to Kraut and Reed [9], pyrimidine in its resonance form III caused by protonation or metallation at N(1′) should also influence the C(4′)–N(4′α) bond length (Fig. 11).

According to crystallographic data, protonation or complexation at the N(1′) site of pyrimidine influences the C(4′)–N(4′α) bond length of the exocyclic amino group in a different fashion. More particularly, protonation at this site decreases the bond length, whereas complexation increases it almost to the length of the free base (Table 1).

In conclusion, complexation at N(1′) destabilizes structure III and increases the C(4′)–N(4′α) bond length, which corresponds to a single rather than a double bond. Structure III therefore is more favored from protonation rather than complexation, which increases the C(4′)–N(4′α) bond length and the basic character of the amino group at N(4′α) reducing its ability to act as a proton donor.

The increase of the C(4′)–N(4′α) bond length in complexes with a direct metal–N(1′) bond compared to the protonated ligand is independent of the ligand conformation (F or S) and of the nature of the coordinating group at N(1′), e.g.
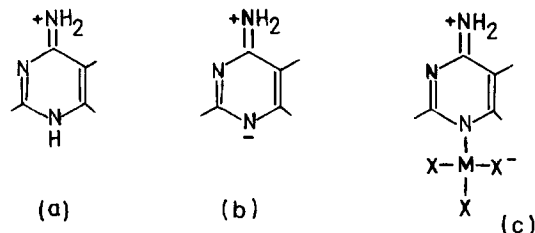

Fig. 11. Resonance form III of (a) protonated pyrimidine, (b) free base and (c) its metal complex at N(1′).

TABLE 1

Comparison of the C(4')–N(4'α) bond length in structures of thiamines, protonated thiamines and their metal complexes

	Configuration	C(4')–N(4'α) bond length (Å)	Ref.
Protonated thiamine			
(thH)(CdCl$_4$)	S	1.323	44
(thH)(CoCl$_4$)	S	1.327	66
(thH)(PtCl$_4$)	F	1.340	49
(thH)$_2$(PtCl$_4$)Cl$_2$	F	1.321	49
(thH)(CuCl$_4$)	F	1.323	42
TMP(PF$_6$)	F	1.321	68
HBT·HCl	S	1.310	29
Thiamine (free base)			
thCl	F	1.338	65
thCl	F	1.338	65
thCl	F	1.338	65
th	F	1.338	65
Metal complex			
Cd(th)Cl$_3$	S	1.346	50
Co(th)Cl$_3$	S	1.347	56
Pt(th)Cl$_3$	F	1.340	49
Cu(th)Cl$_2$	F	1.339	51
Rh$_2$(CH$_3$COO)$_4$(TMP)$_2$	F	1.340	52
Hg(HBT)Cl$_3$	S	1.370	55

neutral Rh$_2$(CH$_3$COO)$_4$ or anionic [CdCl$_3$]$^-$, [CuCl$_2$]$^-$ [PtCl$_3$]$^-$, [CoCl$_3$]$^-$ and [HgCl$_3$]$^-$ (Table 1).

The size of the C(2')–N(1')–C(6') angle is also influenced by protonation or complexation at N(1'). Table 2 shows that protonation increases this angle more than complexation.

It was suggested [51] that the C(2')–N(1')–C(6') angle values, in addition to the C(4')–N(4'α) bond lengths, may depend linearly on the Lewis acid strength of the metallic ions. Such a trend does not seem to be true, however, according to the values given in Tables 1 and 2, but there are not yet available enough experimental data on isostructural thiamine–metal complexes with M–N(1') bonding. We can only note the systematic increase of the C(2')–N(1')–C(6') angle in the series Zn^{2+} < Cu$^+$ < Cd^{2+} < Hg^{2+}, even though the structure with Hg^{2+} involves HBT and not thiamine as in the case of Zn^{2+} and Cd^{2+}, as well as CuthCl$_2$ in the case of Cu$^+$. It should also be noted that the C(4')–N(4'α) bond length increases in the series Cu$^+$ < Zn^{2+} < Cd^{2+} < Hg^{2+}.

In many cases the C(2')–C(2'α) bond length of thiamine is significantly shorter

TABLE 2

Comparison of the C(2')–N(1')–C(6') angle in structures of thiamines, protonated thiamines and their metal complexes

	Configuration	C(2')–N(1')–C(6') angle (°)	Ref.
Protonated thiamine			
(thH)(CdCl$_4$)	S	120.2	44
(thH)(CoCl$_4$)	S	120.7	66
(thH)(PtCl$_4$)	F	118.0	49
(thH)$_2$(PtCl$_4$)Cl$_2$	F	119.9	49
(thH)(CuCl$_4$)	F	122.1	42
TMP(PF$_6$)	F	119.7	68
HBT·HCl	S	121.1	29
Thiamine (free base)			
thCl	F	115.0	65
thCl	F	115.0	65
thCl	F	115.0	65
th	F	115.0	65
Metal complex			
Cd(th)Cl$_3$	S	116.5	50
Co(th)Cl$_3$	S	115.7	56
Pt(th)Cl$_3$	F	115.0	49
Cu(th)Cl$_2$	F	115.6	51
Rh$_2$(CH$_3$COO)$_4$(TMP)$_2$	F	115.0	52
Hg(HBT)Cl$_3$	S	117.0	55

than that expected for a simple sp^2–sp^3 bond. It was proposed [10] that when the C(2'α)-methyl group of thiamine is near anionic ions, or species of electronegative atoms, the corresponding C(2')–C(2'α) bond length decreases, possibly owing to stabilization through hyperconjugate resonance structures (Fig. 12).

Although the length of this bond is <1.6 Å in all known thiamine structures and its derivatives (Table 3), this is obvious only whenever full data are available, as for example 1.477 Å in HBT·HCl [29], with Cl$^-$ and O of H$_2$O approaching the CH$_3$ group, 1.466 Å in HBOT.HCl [19], with the same atoms approaching CH$_3$,
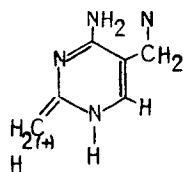
Fig. 12. Hyperconjugate resonance structure of pyrimidine of thiamine.

1.463 Å in oxythiamine [16] (Cl⁻ approach), 1.477 Å in TPP·HCl [10] (O approach), 1.485 Å in Zn(th)Cl₃ [53] (Cl⁻ approach) and 1.48 Å in Zn(th)Br₃ [56] (Br⁻ approach).

Table 3 summarizes the main crystallographic characteristics of all the crystal structures of thiamine, its derivatives and their metal complexes currently known.

2.5.2. Thiazole and the pyrophosphate group

The relative orientations of the hydroxyethyl group and the pyrophosphate side-chain of thiazole in thiamine or TPP, respectively, are determined by the torsional angles $\phi_{5\alpha} = S(1)-C(5)-C(5\alpha)-C(5\beta)$, $\phi_{5\beta} = C(5)-C(5\alpha)-C(5\beta)-O(5\gamma)$, $\phi_{5\gamma} = C(5\alpha)-C(5\beta)-O(5\gamma)-P(5\delta)$, $\phi_{5\delta} = C(5\beta)-O(5\gamma)-P(5\delta)-(5\varepsilon)$ and $\phi_{5\varepsilon} = O(5\gamma)-P(5\delta)-O(5\varepsilon)-P(5z)$ [58].

When the angle $\phi_{5\beta}$ has the characteristic value of approximately $\pm 60°$, a strong $S(1)\cdots O(5\gamma)$ electrostatic interaction takes place. This interaction has been assigned to the existence of resonance structures (Fig. 13) which concentrate a positive charge on the S atom, as quantum mechanical calculations also indicate [9,13,30].

Complexation at N(1′) of pyrimidine by metal ions creates a strong $S(1)\cdots O(5\gamma)$ interaction. The $S(1)\cdots O(5\gamma)$ distance in the thiamine complexes is usually smaller than in thiamine derivatives, including the distance observed in TPP, where the pyrophosphate group bears a net negative charge (Table 4). This strong intramolecular interaction in thiamine metal complexes may be due to transfer of an additional, small positive charge on the S(1) atom on complex formation at N(1′) [50].

In the Cd(th)(SCN)₃ structure, on the other hand, the $S(1)\cdots O(5\gamma)$ interaction is little influenced by the complexation of the metal at O(5γ) and this emphasizes the importance of such an electrostatic interaction [57]. Further, the longer $S(1)\cdots O(5\gamma)$ distance observed in the structure of the $[Mn(th)Cl_2(H_2O)]_2(th)_2Cl_4$ complex (Table 4) is probably not due to the Mn(II) binding to O(5γ), but rather to an additional interaction of the S(1) atom with a Cl⁻ ion.

Passing now to the conformation of the pyrophosphate side-chain in the crystal structure of TPP·HCl [10], we observe that the chain is folded over, whereas in the structure of neutral TPP, which is zwitterionic, it is extended far from the thiazole ring [67] (Fig. 14).

The so-formed extended and folded conformations of the pyrophosphate chain are presented by the torsional angles around the $O(5\gamma)-P(5\delta)$ and $P(5\delta)-O(5\varepsilon)$ bonds, represented by the Newman projections (Fig. 15).
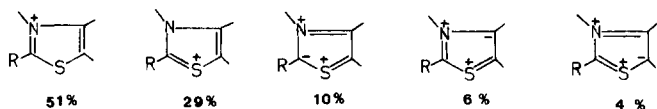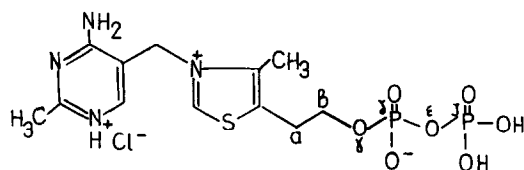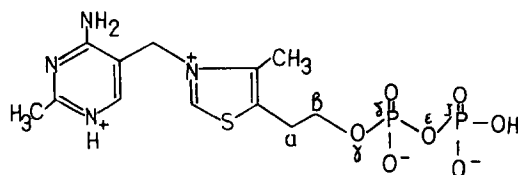

Fig. 13. Resonance structures of thiazole.

TPP.HCl

neutral of zwitterionic type

Fig. 14. Structure of TPP·HCl and neutral TPP.

It was suggested that the folded conformation in TPP·HCl is created because of the electrostatic intramolecular interactions, e.g. due to the tendency to minimize the distance between the two positively charged thiamine rings (thiazole, pyrimidine) and the negatively charged pyrophosphate ester [10]. The structure of TPP, however, where the pyrophosphate side-chain extends far from the thiazole ring, does not favor this suggestion since both rings of thiamine remain positively charged and the pyrophosphate ester now bears two negative charges [67]. In addition, in the complex [(TPP) (1,10-phenanthroline) (H$_2$O)Cu^{II}] the pyrophosphate group has an intermediate conformation with corresponding angle values $\phi_{5\delta} = 87°$ and $\phi_{5\varepsilon} = -130°$ (Fig. 16) [58]. This may be due, according to Pletcher et al. [67], to the fact that the conformation of the side-chain of C(5) can change easily.

The side-chain at C(5) and the amino group of pyrimidine can be oriented towards the same or opposite sides of the thiazole ring. In the first case the conformation is characterized as "syn" whereas in the second as "anti" [10] (Figs. 17, 18). The negative sign of the ϕ_p angle indicates that the chain at C(5) and the 4'α-NH$_2$ group are found in the "syn" conformation of the thiazole ring and the corresponding positive sign of ϕ_p that they are in the "anti" conformation (Table 4). There are no indications of whether or not this structural characteristic has any biological importance or plays any role at all in the mechanism of enzymatic action of thiamine.

The electrostatic interaction between the partially positively charged S(1) and O(2) observed in all the C(2)-substituted derivatives of thiamine strongly influences the side-chain conformation at C(2) (see Introduction). The corresponding torsional angle S(1)–C(2)–C(2α)–O(2α) is 20.6° in the structure of HET·HCl (Fig. 19), 8.4° in HBT·HCl (Fig. 19), 11.7° in HBOT·HCl and 10.5° in the complex Hg(HBT)Cl$_3$, such that the S(1)···O(2α) interactions are 2.901, 2.764, 2.749 and 2.79 Å, respectively.

TABLE 3

Relevant bond lengths and angles in crystal structures of thiamine, its derivatives and their metal complexes

Compound	Conformation	Dihedral angle between pyrimidine–thiazolium planes (°)	Bond length (Å)				Ref.
			N(1′)—C(6′)	N(1′)—C(2′)	C(4′)—N(4′α)	C(2′)—C(2′α)	
thCl·HCl	F	76.0	1.362	1.333	1.316	1.492	9
thCl·H₂O	F	77.2	1.343	1.330	1.338	1.496	65
thClO₄·H₂O	F	84.5	1.339	1.327	1.351	1.498	69
thBr·HBr·0.5H₂O	F		1.350	1.330	1.370	1.500	70
thI·HI	F		1.370	1.360	1.320	1.480	72
thNO₃·HNO₃	F		1.347	1.343	1.321	1.496	73
thNO₃	F	84.6	1.347	1.337	1.327	1.504	74
thCl·0.5SO₄·H₂O	F		1.343	1.345	1.328	1.485	75
[th(CH₃)]²⁺2I⁻	F	89.6	1.350	1.350	1.330	1.500	76
th·C₁₀H₆(SO₃)₂·H₂O	F	88.9	1.353	1.352	1.299	1.480	77
TMP·HPO₄·3H₂O	F	90.0	1.364	1.379	1.313	1.507	78
th·C₁₀H₇N₄O₅·2H₂O	F	77.5	1.340	1.340	1.331	1.482	14
th·PF₆·H₂O	F		1.349	1.336	1.342	1.500	68
th(ClO₄)₂	F		1.345	1.346	1.319	1.490	68
th(PF₆)₂·4H₂O	F		1.351	1.351	1.326	1.489	68
TMP(PF₆)·2H₂O	F		1.357	1.339	1.321	1.488	68
TPP·HCl	F	83.3	1.352	1.345	1.315	1.477	10
TPP	F		1.352	1.343	1.314	1.492	67
TPP·4.5H₂O	F		1.352	1.354	1.313	1.485	80
HET·HCl	S		1.350	1.340	1.315	1.481	13
HBT·HCl	S	84.9	1.345	1.335	1.310	1.477	29
PLT·Cl·3H₂O	S		1.320	1.330	1.290	1.460	64
HBOT·Cl·HCl·3H₂O	S	96.9	1.371	1.323	1.206	1.466	19
thBr·1.5H₂O⁹	F		1.340	1.320, 1.330	1.350	1.570, 1.490	71

Oxythiamine	V	91.7	1.382	1.321	1.213	1.463	16
TT	V	60.0	1.351	1.328	1.335	1.510	18
$(thH)_2(UOCl_4)$	F	74.4					41
$(thH)(CuCl_4)$	F	89.3	1.342	1.349	1.323	1.480	42
$(thH)(CdCl_4) \cdot H_2O$	S		1.356	1.337	1.323	1.496	44
$(thH)Cl \cdot HCl \cdot 0.5Mg(H_2O)_6$	F		1.344	1.355	1.311	1.476	81
$[Cu(thH)(1,10\text{-phen})(H_2O)]$							
$[(NO_3)_2] \cdot H_2O$	F	86.0					58
$Cd(th)Cl_3$	S		1.353	1.353	1.346	1.500	50
$(thH)(CoCl_4) \cdot H_2O$	S		1.354	1.338	1.327	1.489	66
$Cu(th)Cl_2$	F		1.356	1.354	1.339	1.494	51
$[Rh(CH_3COO)_2(TMP)] \cdot 2H_2O$	F	90.0	1.350	1.360	1.340	1.510	52
$Zn(th)Cl_3$	S	82.2	1.353	1.357	1.336	1.483	53
$(thH)(PtCl_4)$	F		1.360	1.340	1.340	1.520	49
$(thH)_2(PtCl_4)Cl_2$	F		1.359	1.341	1.321	1.511	49
$Pt(th)Cl_3$	F		1.320	1.390	1.340	1.460	49
$Cu(th)Br_2$	F	104.7	1.340	1.340	1.330	1.480	54
$Hg(HBT)Cl_3$	S	87.9	1.350	1.350	1.370	1.510	55
$Co(th)Cl_3 \cdot 0.4H_2O$	S	86.8	1.357	1.350	1.347	1.495	56
$Zn(th)Br_3 \cdot 0.2H_2O$	S	88.5	1.350	1.360	1.350	1.480	56
$Zn(th)(SCN)_3$	F	96.0	1.352	1.356	1.336	1.501	57
$Cd(th)(SCN)_3$	F	100.7	1.360	1.330	1.340	1.500	57
$[Mn(th)Cl_2(H_2O)]_2$							
$[(th)_2Cl_4] \cdot 2H_2O$	F	91.3	1.360	1.342	1.333	1.491	59
$(thH)(HgCl_4)$	S		1.358	1.310	1.347	1.510	82

TABLE 3 (*continued*)

Compound	Conformation	Dihedral angle between pyrimidine– thiazolium planes (°)	Bond angle (Å)			Ref.
			C(6′)–N(1′)–C(2′)	C(2)–N(3)–C(b)	C(4)–N(3)–C(b)	
thCl·HCl	F	76.0	120.7	124.9	121.8	9
thCl·H₂O	F	77.2	115.0	123.4	122.5	65
thClO₄H₂O	F	84.5				69
thBr·HBr·0.5H₂O	F		121.0	124.0	115.0	70
thI·HI	F		119.0	122.1	123.2	72
thNO₃·HNO₃	F		120.6	123.6	121.5	72
thNO₃	F	84.6	114.8	123.5	122.1	74
thCl·0.5SO₄·H₂O	F		120.7	123.7	121.5	75
[th(CH₃)]²⁺2I⁻	F	89.6				76
th·C₁₀H₆(SO₃)₂·H₂O	F	88.9	120.2	123.6	121.6	77
TMP·HPO₄·3H₂O	F	90.0	118.7	123.5	122.4	78
th·C₁₀H₇N₄O₅·2H₂O	F	77.5	115.7	122.4	123.6	14
th·PF₆·H₂O	F		115.2	124.1	121.9	68
th(ClO₄)₂	F		121.1	123.5	122.5	68
th(PF₆)₂·4H₂O	F		120.9	124.6	122.1	68
TMP(PF₆)·2H₂O	F		119.7	124.1	122.6	68
TPP·HCl	F	83.3	120.2	124.3	121.9	10
TPP	F		120.3	124.4	121.8	67
TPP·4.5H₂O	F		120.0	124.0	121.6	80
HET·HCl	S		120.3	123.2	122.1	13
HBT·HCl	S	84.9	121.1	124.0	122.5	29
PLT·Cl·3H₂O	S		120.0	124.0	122.0	64
HBOT·Cl·HCl·3H₂O	S	96.9	122.6	122.8	123.1	19
thBr·1.5H₂Oᵃ	F		115.5, 115.2	122.9, 123.6	121.5, 122.7	71

Oxythiamine	V	91.7	122.2	122.0	125.1	16
TT	V	60.0	114.6	120.3	124.1	18
$(thH)_2(UOCl_4)$	F	74.4	118.4			41
$(thH)(CuCl_4)$	F	89.3	122.1	122.8	124.1	42
$(thH)(CdCl_4)\cdot H_2O$	S		120.2	120.8	120.8	44
$(thH)Cl\cdot HCl\cdot 0.5Mg(H_2O)_6$	F		120.9	124.5	121.4	81
$[Cu(thH)(1,10\text{-phen})(H_2O)]$						
$[(NO_3)_2]\cdot H_2O$	F	86.0				58
$Cd(th)Cl_3$	S		116.5	121.9	124.0	50
$(thH)(CoCl_4)\cdot H_2O$	S		120.7	121.1	124.2	66
$Cu(th)Cl_2$	F		115.6	124.2	121.3	51
$[Rh(CH_3COO)_2(TMP)]\cdot 2H_2O$	F	90.0	115.0	123.1	121.4	52
$Zn(th)Cl_3$	S	82.2	115.6	122.0	123.9	53
$(thH)(PtCl_4)$	F		118.0	125.0	120.0	49
$(thH)_2(PtCl_4)Cl_2$	F		119.9	124.9	120.2	49
$Pt(th)Cl_3$	F		115.0	124.0	122.0	49
$Cu(th)Br_2$	F	104.7	117.4	123.7	121.2	54
$Hg(HBT)Cl_3$	S	87.9	117.0	122.2	122.2	55
$Co(th)Cl_3\cdot 0.4H_2O$	S	86.8	115.7	121.9	123.5	56
$Zn(th)Br_3\cdot 0.2H_2O$	S	88.5	116.3	121.5	123.5	56
$Zn(th)(SCN)_3$	F	96.0	116.3	123.4	122.4	57
$Cd(th)(SCN)_3$	F	100.7	116.3	125.5	121.0	57
$[Mn(th)Cl_2(H_2O)]_2$						
$[(th)_2Cl_4]\cdot 2H_2O$	F	91.3	115.4	124.1	122.2	59
$(thH)(HgCl_4)$	S		119.6	118.8	127.7	82

[a]Two crystallographically independent molecules.

TABLE 4

Relevant sizes of the torsional angles (°) and distances of S···O interaction (Å) in structures of thiamine, its derivatives and their metal complexes

Compound	Conformation	ϕ_T	ϕ_P	$\phi_{5\alpha}$	$\phi_{5\beta}$	S···O(5γ)	S···O(2α)	$\phi_{5\gamma}$	S···O(5δ₁)	$\phi_{5\delta}$	$\phi_{5\varepsilon}$	Form
thCl·HCl	F	−9.4	−73.5	103.4	−53.8							
thCl·H₂O	F	−2.6	−76.0									
thClO₄H₂O	F	2.3	83.2	−17.8	54.3							
thBr·HBr·0.5H₂O	F	2.0	77.0	86.0	64.0							
thI·HI	F	−5.9	81.0	62.0	64.0	2.970						Folded
thNO₃·HNO₃	F											
thNO₃	F	5.9	83.1	−92.8	175.7	3.246						
thCl·0.5SO₄·H₂O	F	1.3	76.2	83.1	63.0	3.106						
[th(CH₃)]²⁺2I⁻	F	4.3	84.1			3.230						
th·C₁₀H₆(SO₃)₂·H₂O	F	−20.9	−79.0	−36.5	3.0							
TMP·HPO₄·3H₂O	F	−6.6	−85.4	105.1	−53.5							
th·C₁₀H₇N₄O₅·2H₂O	F	6.0	82.5	65.9	−68.2	3.060						
th·PF₆·H₂O	F	0.4	83.2	−20.0	55.7	2.882						
th(ClO₄)₂	F	−10.4	81.5	66.5	−62.6	3.001						
th(PF₆)₂·4H₂O	F	9.0	87.1	73.5	−62.5	3.096						
TMP(PF₆)·2H₂O	F	9.0	78.8	−66.1	57.3	2.892						
TPP·HCl	F	3.6	93.2					140.9		−78.1	139.9	Folded
TPP	F											
TPP·4.5H₂O	F	5.4	85.8	58.5	−66.1	2.952		185.9		58.0	78.4	Extended
HET·HCl	S	−100.3	−145.6				2.901					
HBT·HCl	S	92.7	−167.3	3.3	63.4	3.003	2.764					
PLT·Cl·3H₂O	S	−99.5	−173.6	80.0	−60.2	3.150						
HBOT·Cl·HCl·3H₂O	S	92.7	−167.3	91.8	−48.9		2.749					
Oxythiamine	V	105.5	−62.8	61.9	−66.8	3.070						
TT	V	104.1	−74.2	56.7	65.0							
(thH)₂(UOCl₄)	F											
(thH)(CuCl₄)	F	−14.1	−82.6	97.2	−60.6							
(thH)(CdCl₄)·H₂O	S	110.4	137.3	83.0	67.8							
(thH)Cl·HCl·0.5Mg(H₂O)₆	F	−2.0	−77.4									
[Cu(thH)(1,10-phen)(H₂O)]												
[(NO₃)₂]·H₂O	F	−1.0	95.0	87.0	−63.0			−81.0		87.0	−130.0	Folded
Cd(th)Cl₃	S	112.6	129.8	46.5	−68.8	2.879						
(thH)(CoCl₄)·H₂O	S	111.6	135.6			3.413						

Compound										Shape
Cu(th)Cl$_2$	F	−10.1	−83.8	14.2	−60.2	2.913				Folded
[Rh(CH$_3$COO)$_2$(TMP)]·2H$_2$O	F	−3.0	−81.0	76.0	63.0			−125.8	2.905	Folded
Zn(th)Cl$_3$	S	113.4	130.4	45.0	−67.6	2.880				
(thH)(PtCl$_4$)	F	9.0	76.7	−67.6	−56.3	3.020				
(thH)$_2$(PtCl$_4$)Cl$_2$	F	0.3	85.8	36.4	176.4					
Pt(th)Cl$_3$	F	−5.3	70.0	78.0	56.0	3.070				
Cu(th)Br$_2$	F	−12.0	82.0	13.0	−54.0	2.870				
Hg(HBT)Cl$_3$	S	−100.0	172.7	78.6	65.5	3.860	2.79			Folded
Co(th)Cl$_3$·0.4H$_2$O	S	128.9	111.8	47.3	−67.4	2.884				
Zn(th)Br$_3$·0.2H$_2$O	S	130.5	113.5	44.0	−70.0	2.870				
Zn(th)(SCN)$_3$	F	−10.2	−82.8	64.7	−66.6	2.915				
Cd(th)(SCN)$_3$	F	0.0	−80.0	−67.2	64.0	2.977				
[Mn(th)Cl$_2$(H$_2$O)]$_2$										
[(th)$_2$Cl$_4$]·2H$_2$O	F	10.0	85.6	79.8	−66.7	3.277				
(thH)(HgCl$_4$)	S	−103.0	179.0							

M. Louloudi and N. Hadjiliadis/Coord. Chem. Rev. 135/136 (1994) 429–468

451
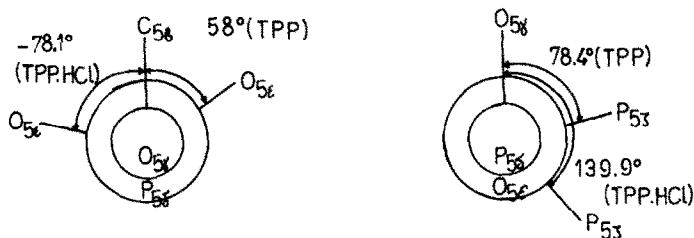
Fig. 15. Newman projections along the O(5γ)–P(5δ) and P(5δ)–O(5ε) bonds of TPP·HCl and neutral TPP.
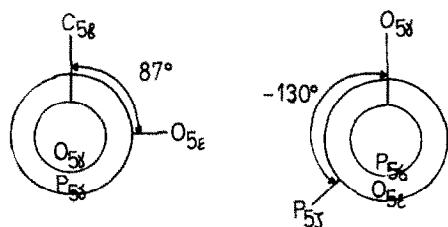
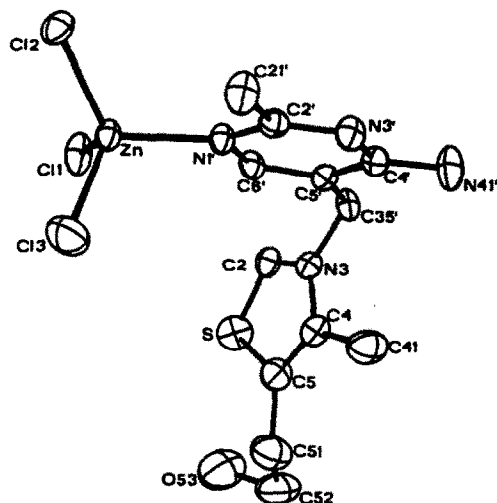Fig. 16. Newman projections along the O(5γ)–P(5δ) and P(5δ)–O(5ε) bonds of the complex [TPP–Cu²⁺–phen].

Fig. 17. Crystal structure of the complex Zn(th)Cl₃. In this structure the side-chain at C(5) and the amino group are oriented towards the opposite sides of the thiazole ring. This conformation is called "anti". Reproduced by permission from ref. 53.
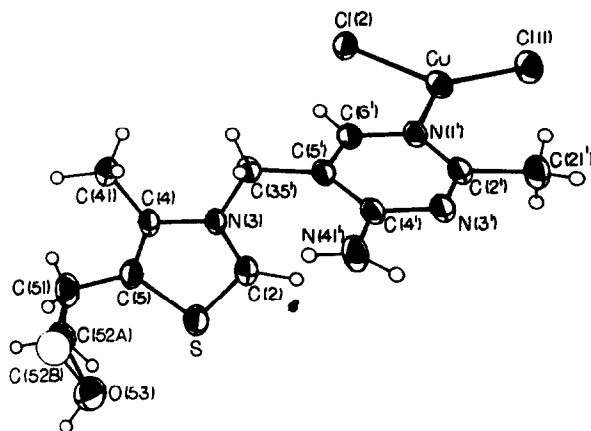
Fig. 18. Crystal structure of the complex Cu(th)Cl$_2$. In this structure the side-chain at C(5) and the amino group are oriented towards the same sides of the thiazole ring. This conformation is called "syn". Reproduced by permission from ref. 51.
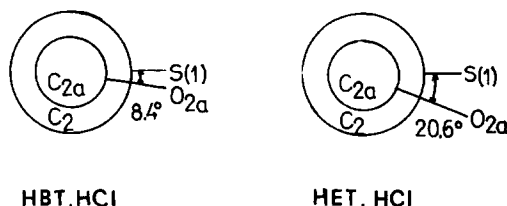

HBT.HCl   HET. HCl

Fig. 19. Newman projections along the C(2α)—C(2) bond of HBT·HCl and HET·HCl.

It was also suggested [13] that the S conformation found in all the C(2)-substituted derivatives of thiamine is stabilized by the strong S(1)···O(2α) electrostatic intramolecular interaction. The Breslow mechanism requires a proton liberation during the stage of the detachment of the final product from the coenzyme. Also, owing to the strong S(1)···O(2α) interaction, the pK_a of this proton is lowered (see Introduction) and proton liberation is facilitated. This enhances the strength of the S(1)···O(2α) interaction and increases the stability of the S conformation. Consequently, the same S(1)···O(2α) interaction may be responsible for the proton liberation of O(2α)H [29]. This is in favor of the mechanism of Hogg [28] and shows the important role of the sulfur atom of thiazole in enzymatic action.

The S(1)···O(2α) interaction stabilizes not only all the intermediates of the enzymatic action of thiamine which contain a hydroxyl group at C(2α), according to Hogg [28], but also facilitates the approach of metal cations, resulting in the facile formation of complexes with the "active aldehyde" intermediates with direct M–N(1') bonding [55,61]. The latter continue to retain the S conformation in both the solid state [55,60,62] and in solution [63].

An exception to the above suggestion [13] is shown by the crystal structure

of the methylacetylphosphonic derivative of thiamine at C(2), which was also found in the S conformation [64], but without an S(1)···O(2α) electrostatic interaction Figs. 20, 21).

In this structure, the conformation of the side-chain at C(2) is such that O(2α) does not interact electrostatically with S(1) and the phosphonic group is directed perpendicular to the thiazole ring [64] (Fig. 22).

This orientation of the C(2α)–P(2β) bond was assumed to be similar to that of the C(2α)–C bond in the "active acetaldehyde" intermediate of lactylthiamine (LT) containing pyruvic acid (Fig. 23) which decarboxylates to acetaldehyde [64].
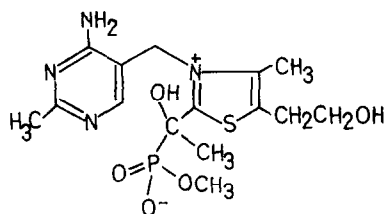

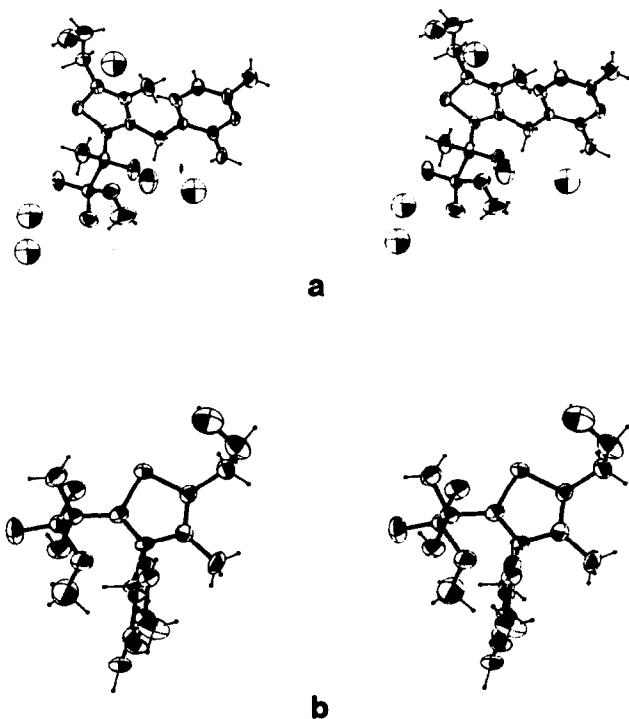Fig. 20. Structure of phosphalactylthiamine (PLT).

a

b

Fig. 21. Crystal structure of PLT·HCl·3H₂O. Reproduced by permission from ref. 64.
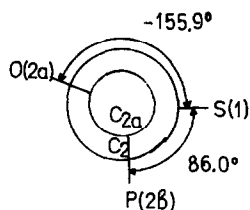
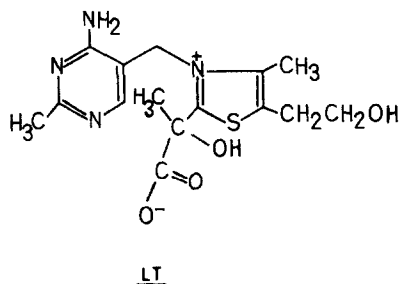Fig. 22. Newman projection along the C(2α)–C(2) bond of PLT·HCl.

Fig. 23. Structure of lactylthiamine (LT).

The structure of (LT) is not known, however. Hence this similarity is not obvious and the conformation of PLT may be the result of the minimization of the steric hindrance of the bulky methylacetylphosphonic group with the pyrimidine ring [15]. The phosphonic methylacetyl ester of TPP is a strong inhibitor of pyruvic dehydrogenase [64], which may be due to the absence of the S(1)···O(2α) interaction.

2.5.3. Factors influencing the stabilization of F, S or V conformations of thiamine

The relative orientations of the pyrimidine and thiazole rings are determined by the torsional angles $\phi_P = N(3)–C(b)–C(5')–C(4')$ and $\phi_T = C(5')–C(b)–N(3)–C(2)$ [13]. Free rotation around the CH_2 bridging group of the two (thiazole and pyrimidine) rings is therefore hindered. This is more pronounced in the case of C(2α)-substituted derivatives of thiamine. As a consequence, only three possible orientations between the two rings have been found in thiamine and its derivatives, constituting the V ($\phi_P = \pm 90°$, $\phi_T = \pm 90°$), the F ($\phi_P = \pm 90°$, $\phi_T = 0°$) and the S ($\phi_P = \pm 150°$, $\phi_T = \pm 100°$) conformations (see also Introduction). Characteristic structures are shown in Fig. 24.

It was suggested [49] that the F and S conformations of thiamine do not differ significantly energetically. Consequently, the forces determining the preferred conformation of thiamine should be very weak [49].

The factors proposed [49,54,56,57,59] to influence the F or S conformations are as follows.

(a) In compounds with polychlorometallic anions, the F form is favored by small metallic ions and smaller non-bonding Cl···Cl distances of the order of 3.4 Å,
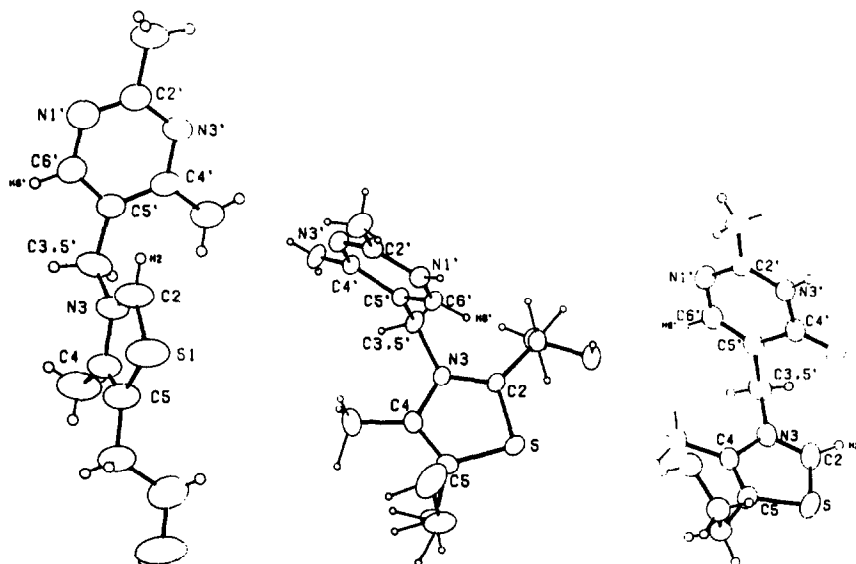
Fig. 24. Representative structures illustrating the three basic conformations F, S and V observed in crystal structure analyses. Reproduced by permission from ref. 15.

whereas the S form is stabilized when these distances are of the order of 3.9 Å [49] (Table 5). This proposal, however, is not substantiated as there are many exceptions. For example, in the crystal structures of the complexes $Cu(th)Cl_2$ and $Cu(th)Br_2$, the non-bonding $X \cdots X$ distances are 3.867 and 3.995 Å, respectively, while thiamine

TABLE 5

$X \cdots X$ non-bonded distances in the polyhalometal anion–thiamine compounds

Compound	$X \cdots X$ (Å)	Conformation
$(thH)(PtCl_4)$	3.247	F
$(thH)_2(PtCl_4)Cl_2$	3.262	F
$Pt(th)Cl_3$	3.270	F
$(thH)(CuCl_4)$	3.420	F
$Zn(th)Cl_3$	3.700	S
$Cu(th)Cl_2$	3.867	F
$(thH)(CdCl_4)$	4.000	S
$Cd(th)Cl_3$	4.000	S
$Hg(HBT)Cl_3$	3.857	S
$Cu(th)Br_2$	3.995	F
$(thH)(CoCl_4)$	3.720	S
$Zn(th)Br_3$	3.900	S
$Co(th)Cl_3$	3.710	S

is in the F conformation. Also, complexes $Zn(th)Cl_3$, $Co(th)Cl_3$ and (thH) $(CoCl_4)$ have a $Cl \cdots Cl$ distance of 3.7 Å and an S conformation of the ligand.

(b) A common characteristic feature of the structures of thiamine derivatives having the F conformation is the connection of the $N(4'\alpha)H_2$ group with the thiazole ring through hydrogen bonding of the type $N(4'\alpha)H \cdots X \cdots$ thiazole with the aid of a bridging anion (X) [54,56,57], the so-called "one point" anion bridge. The connection through two bridging anions is also possible and is called a "two-point" anion bridge. The hydrogen bonding is of the type $N(4'\alpha)H \cdots X_1-M-X_2 \cdots$ thiazole [54,56,57]. This is rationalized by the pyrimidine–thiazole distance which is too small in compounds with an F conformation to accommodate the "two-point" bridge, whereas in those with an S conformation it is too long for the creation of a "one-point" bridge. It was proposed [54] that carboxylic anions such as aspartate and glutamate used as bridging groups play an important role in the recognition and stability of a certain conformation, F or S, of thiamine in the apoenzyme, depending on the existence of one or two such anions [54] (see Table 6 and Figs. 25, 26).

A similar structural feature was also observed in the structures of thiamine with the F conformation. The interaction was of the "one-point" anion bridge form, but it was not observed in all cases [57].

(c) An additional factor that seems to influence the F but not the S conformation is the binding of thiazole with pyrimidine through hydrogen bonding of an

TABLE 6

$N(4'\alpha)-H \cdots X$ hydrogen bond and $X \cdots$ thiazolium distances in the polyhalometal anion–thiamine compounds

Compound	Conformation	$N(4'\alpha) \cdots X \cdots$ thiazolium (Å)		
$Cu(th)Cl_2$	F	3.251	3.290	
$Cu(th)Br_2$	F	3.390	3.410	
$Pt(th)Cl_3$	F	3.270	3.300	
$(thH)(PtCl_4)$	F	3.180	3.360	
$(thH)(PtCl_4)Cl_2$	F	3.173	3.320	
$(thH)(CuCl_4)$	F	3.440	3.390	
$Zn(th)(SCN)_3$	F	3.569	3.520	X=S(31)′ ⎫ Fig. 25
$Cd(th)(SCN)_3$	F	3.649	3.480	X=S(21)′ ⎭
$[Mn(th)Cl_2(H_2O)](th)_2Cl_4$	F	3.210	3.440	
Compound	Conformation	$N(4'\alpha) \cdots X_1-M-X_2 \cdots$ thiazolium (Å)		
$(thH)(CdCl_4)$	S	3.306	3.200	
$(thH)(CoCl_4)$	S	3.323	3.190	
$Cd(th)Cl_3$	S	3.395	3.390	
$Zn(th)Cl_3$	S	3.473	3.420	
$Zn(th)Br_3$	S	3.555	3.550	
$Co(th)Cl_3$	S	3.498	3.410	
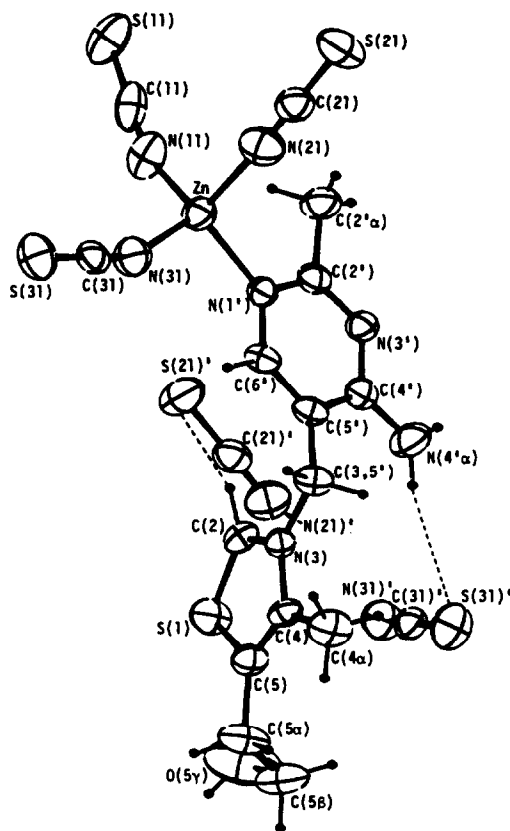
Fig. 25. Crystal structure of Zn(Th) (SCN)$_3$ showing an N(4'α)–H···S(31)'–C(31)'–N(31)'···thiazolium ring interaction ("one-point" anion bridge). Reproduced by permission from ref. 57.

electronegative ion of the type C(2)H···X···pyrimidine and stacking interactions (Fig. 27) [54,57]. Similar interactions may also lead to stabilization of the C(2) derivatives of thiamine (e.g. with pyruvic acid) through an F conformation [57,68].

According to another view [83], the F conformation is stabilized through an electrostatic interaction between the acid proton at C(2) and the aromatic electron density of the pyrimidine ring, the former localized over this ring. A consequence of this is the protection of the C(2) position by the pyrimidine ring in the F conformation, whereas in the S conformation this position is exposed to reaction with the substrate [53]. It is therefore possible that the pyruvic acid is not able to be added to the F conformation, but rather to the S conformation of thiamine. This is in agreement with the fact that the structures of all the intermediates in the enzymatic reactions of thiamine are in the S conformation.

Whenever the C(2) position is exposed in the S conformation or protected by the pyrimidine ring in the F conformation, the angles C(2)–N(3)–C(b) and
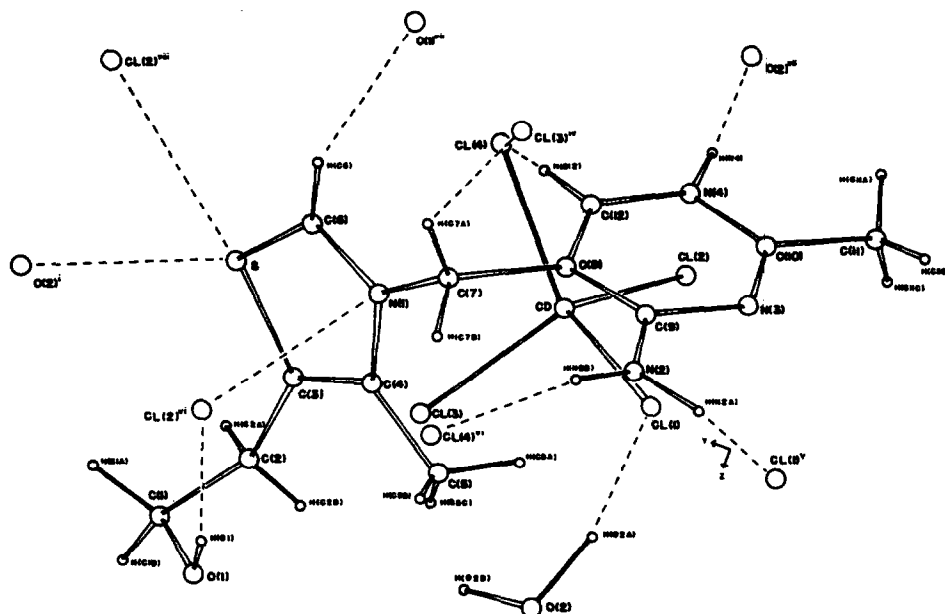
Fig. 26. Crystal structure of (thH) (CdCl$_4$) showing an N(4′α)–H···Cl(4)^{VI}–Cd–Cl(2)^{VI}···thiazolium ring interaction ("two-point" anion bridge). Reproduced by permission from ref. 44.
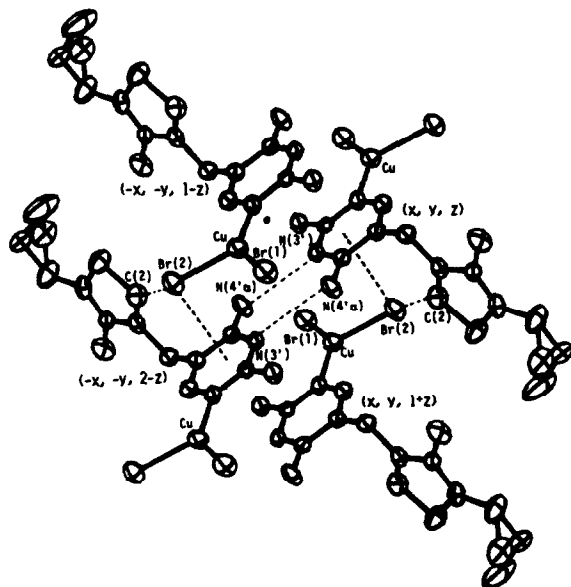

Fig. 27. Plot of four adjacent units of Cu(th)Br$_2$, showing C(2)H···Br(2)···pyrimidine interaction, pyrimidine–pyrimidine stacking and a pair of interbase N(4′α)H···N(3′) hydrogen bonds. Reproduced by permission from ref. 54.

C(4)–N(3)–C(b) are influenced in such a way that in the F conformation the first angle is larger than the second by 1–4° [59] (Table 3).

(d) Hu [59] and Hu and Zang [71] proposed that the conformations of thiamine and its derivatives in their metal complexes are related in the way that the coordinating group approaches the thiazole ring. When a bulky tetrahedral metal complex (X···X > 3.7 Å) approaches the thiazole ring with a trigonal plane, thiamine orients its pyrimidine and thiazole rings to the S conformation to reduce steric hindrance. On the other hand, when a bulky complex anion approaches the thiazole ring with an edge or an apex of the coordinating group, the steric hindrance is smaller. However, when the complex anion is not bulky, the mode of approach does not seem to play any role and the F conformation is preferred (Table 7) [59].

The F and S forms provide the maximum separation between the C(2) and C(4') positions and between the C(6') hydrogen atom and the methyl group at C(4). All the C(2)-substituted derivatives of thiamine are in the S and not the F conformation owing to steric hindrance of the pyrimidine ring and the substituents at this position. Therefore, steric hindrance between the substituents at C(2) and C(4') and also between those at C(4) and C(6') may be factors that determine the preferred conformation of thiamine and its derivatives [18].

Only two thiamine derivatives are in the V conformation. The first is oxythiamine, where the N(4'α)H₂ group is replaced by an oxo group and the second is thiaminethiazolone (TT) (Fig. 28) (see Introduction).

TT presents a strong affinity to the apoenzyme, in contrast to oxythiamine, which is a strong thiamine antagonist. None of these derivatives, however, catalyzes the thiamine enzymatic reactions. The V conformation of TT is stabilized through

TABLE 7

Comparison of the structural parameters in the polyhalometal anion–thiamine compounds

Compound	Conformation	Anion geometry	Approaching manner	X···X (Å)
(thH)(CdCl₄)	S	Tetrahedral	Plane	4.000
Cd(th)Cl₃	S	Tetrahedral	Plane	4.000
Cu(th)Br₂	F	Trigonal Planar	Line	3.995
Zn(th)Br₃	S	Tetrahedral	Plane	3.900
Cu(th)Cl₂	F	Trigonal Planar	Line	3.867
[Mn(th)Cl₂(H₂O)]₂²⁺	F	Square pyramidal	Line	3.714
Co(th)Cl₃	S	Tetrahedral	Plane	3.710
Zn(th)Cl₃	S	Tetrahedral	Plane	3.700
(thH)(CuCl₄)	F	Tetrahedral	Plane	3.420
Pt(th)Cl₃	F	Square planar	Point	3.270
(thH)(PtCl₄)	F	Square planar	Plane	3.243
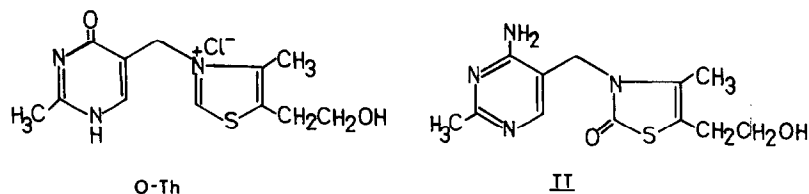
Fig. 28. Structures of oxythiamine (O-th) and thiaminethiazolone (TT).

an intramolecular hydrogen bond between the $N(4'\alpha)H_2$ group and the oxygen atom at C(2) (Fig. 29) [18]. In oxythiamine a similar $C(2)$—$H\cdots O(4'\alpha)$ bond is not observed.

The V conformation is stabilized [18] when the following two conditions are satisfied simultaneously: (a) when the repulsions between the substituents at C(2) and C(4') are minimal and (b) when the size of the substituents is small. Further, HBOT·HCl, which is also a strong thiamine inhibitor, is also in the S conformation (Fig. 30).

Finally, the existence of a substituent at C(2) in the "active aldehyde" intermediate of thiamine, in combination with the existence of a direct M–N(1') bond with bulky or non-metallic ions, should favor the S conformation of thiamine in the holoenzyme [55]. The V conformation of TPP recently found in the crystal structure of transketolase [20] contains Ca^{2+} ions bound through the pyrophosphate group with the apoenzyme.

Fig. 29. View of the thiaminethiazolone molecule and the atomic numbering scheme. Reproduced by permission from ref. 118.

HBOT.HCl

Fig. 30. Structure of HBOT·HCl.

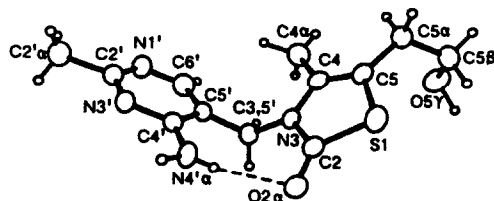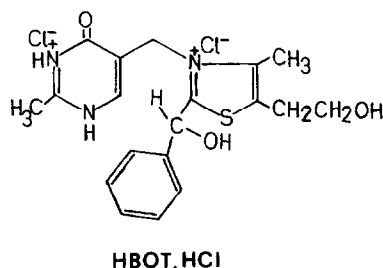### 2.5.4. Ring stacking interactions

Three types of ring stacking interactions have been found in structures of thiamine analogs [77]. (i) The first is the pyrimidine–pyrimidine interaction and a common structural characteristic of TPP·HCl, ThBr·1.5H$_2$O and of the complexes Pt(th)Cl$_3$, Cd(th)Cl$_3$, Zn(th)Cl$_3$, Cu(th)Cl$_2$ and Cu(th)Br$_2$ (Table 8). (ii) The second stacks one of the pyrimidine units with the phenyl ring, observed in the compounds HBT·HCl, Hg(HBT)Cl$_3$ and HBOT·HCl as an intrastrand interaction. (iii) True intermolecular stacking interactions between the pyrimidine and the other aromatic rings were found in the structures of thiamine picrolonate (Fig. 31) [14], thiamine picrate [79] and thiamine indole-3-propionate [85]. These three structures are dimerized through N(4'α)H···N(3') hydrogen bonds (Fig. 32) [77].

This type of hydrogen bonding can be found in many other crystal structures of thiamine derivatives, which do not present any stacking interaction, however, [18,66,74]. In the case of the complex Cu(th)Br$_2$ [54], on the other hand, there exists a stacking interaction not related to the N(4'α)···N(3') one at all (see Fig. 27).

Exceptions to the above type of interaction involving the pyrimidine ring are (a) the structure of thiaminenaphthalene 1,5-disulfonate, which shows a partial stacking interaction between the thiazole and naphthalene rings (Fig. 33) [77], and (b) the structure of thiamine picrate, which displays a partial stacking interaction between the two thiazolium rings [79].

In the complex TPP–Cu^{2+}–phen, on the other hand, no stacking interaction exists between the pyrimidine and the phenanthroline rings, either inter- or intramolecularly [58]. This is mainly due to the existence of a stacking interaction between the phenanthroline rings themselves and also in part to the presence of a nitrate anion located over the pyrimidine [58].

Empirical measurements [84] led to the conclusion that pyrimidine protonation in thiamine indole-3-propionate makes the stacking interaction between the pyrimidine and indole rings less energetically favorable. Crystallographic data show that the deprotonated pyrimidine in thiamine picrolonate [14] and thiamine picrate [79] interact with the aromatic ring while the protonated pyrimidine in thiaminenaphthalene [77] and in the complex TPP–Cu^{2+}–phen [58] do not interact. Exceptions are the structures of HBT·HCl [29] and HBOT·HCl [19], where an intramolecular stacking interaction is present between the protonated pyrimidine

thiamin picrolonate
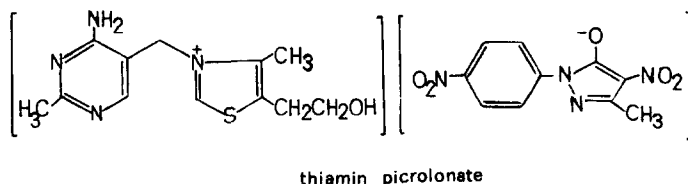
Fig. 31. Structure of thiamine picrolonate.

TABLE 8

Structural parameters of ring stacking interactions

Compound	Conformation	Ring stacking	Ring distance (Å)	Type of interaction
Pt(th)Cl$_3$	F	Pyrimidine–pyrimidine	3.45	Intermolecular
Cd(th)Cl$_3$	S	Pyrimidine–pyrimidine	3.52	Intermolecular
Cu(th)Cl$_2$	F	Pyrimidine–pyrimidine	3.42	Intermolecular
Hg(HBT)Cl$_3$	S	Pyrimidine–phenyl	3.46	Intramolecular
Zn(th)Cl$_3$	S	Pyrimidine–pyrimidine	3.47	Intermolecular
Cu(th)Br$_2$	F	Pyrimidine–pyrimidine	3.46	Intermolecular
TPP–Cu^{2+}–phen	F	Phen–phen	3.3–3.4	Intermolecular
th–naphthalene	F	Partial thiazole–naphthalene		
HBOT·HCl	S	Pyrimidine–phenyl		Intramolecular
HBT·HCl	S	Pyrimidine–phenyl		Intramolecular
Thiamine picrolonate	F	Pyrimidine–nitrophenyl Pyrimidine–pyrazolonate Picrolonate–picrolonate		
TPP·HCl	F	Pyrimidine–pyrimidine		Intermolecular
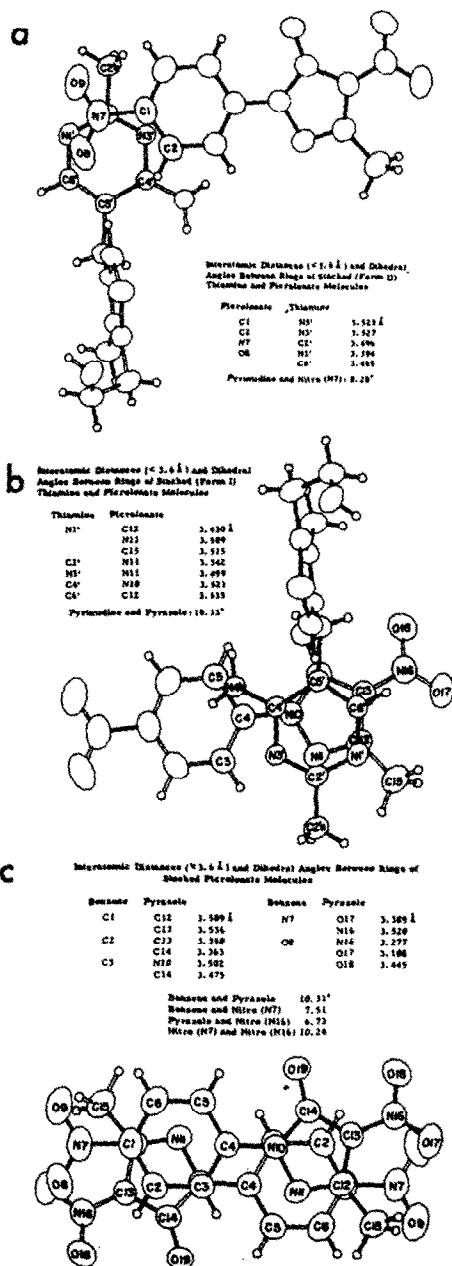
Fig. 32. The three modes of ring stacking which are found in the crystal structure of thiamine picrolonate. The upper molecule is designated by the solid bonds. View (a) shows overlap between the pyrimidine and the nitrophenyl rings. View (b) shows the stacking of the pyrimidine and pyrazolone rings. View (c) shows the overlap of the picrolonate molecules. Reproduced by permission from ref. 14.

(a)

(b)

Fig. 33. Perspective view of thiaminenapthalene-1, 5-disulfonate showing the partial ring stacking interaction. Reproduced by permission from ref. 77.

and the phenyl ring and the structure of TPP·HCl [10] with an intermolecular interaction of the protonated pyrimidine ring.

Based on the above, a proposal that the stacking interactions between the rings may be a dominant factor in the coenzyme–apoenzyme and coenzyme–substrate relationships has also been made [29]. Finally, the fact that the various rings can be stacked together in many complexes of thiamine with metal ions indicates that complex formation may facilitate such interactions and thereby the coenzyme–apoenzyme or coenzyme–substrate interactions. This is substantiated in the crystal

structure of transketolase [20], where pyrimidine is stacked between the phenyl-alanine and tyrosine aromatic rings. Thiazole, on the other hand, interacts hydro-phobically with the apoenzyme [20].

3. CONCLUSIONS

The main conclusions and observations made in this review are as follows. (a) The ready formation of complexes of metal ions with "active aldehyde" derivatives of thiamine having a direct metal–N(1') bond to pyrimidine possibly suggests that the intervention of the metallic ions follows the formation of the "active aldehyde" intermediates in the enzymatic action of thiamine. (b) The S conformation is preferred in thiamine whenever the C(2) of thiazole is substituted due to steric hindrance (as in all the intermediates of the enzymatic cycle) or whenever a bulky coordination group approaches the N(1') site again owing to steric hindrance either in solution or in the solid state. As a result, the S conformation seems to be preferred when thiamine interacts in its active aldehyde form with the substrate in the presence of bivalent metal ions. (c) The role of the sulfur atom of thiazole and of the $S \cdots O(2\alpha)$ electrostatic interaction in the S conformation of thiamine seems to be very important in the enzymatic action of thiamine. It not only influences several stages of the decarboxylation of pyruvate [28], but also facilitates metal ion complexation with N(1') by delocalizing the positive charge of the N(3) to it. (d) The V conformation of TPP is also very important and this assigns a proton acceptor role to the $NH_2(4'\alpha)$ group of pyrimidine approaching the C(2)H position of thiazolium, with the metal (Ca^{2+}) coordinated through the pyrophosphate oxygens to the apoenzyme. (e) The S conformation may succeed the original V conformation of TPP once the "active aldehyde" derivative intermediate is formed. (f) Metal binding to TPP may take place through both the N(1') of pyrimidine and the pyrophosphate oxygens, thereby connecting it with the apoenzyme.

Further research is obviously required with the aim of confirming the above conclusions.

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