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AN X-RAY EVIDENCE FOR THE RING-STACKING INTERACTION BETWEEN INDOLE RING AND THE PYRIMIDINE MOIETY OF THIAMIN: CRYSTAL STRUCTURE OF THIAMIN INDOLE-3-PROPIONATE

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SUMMARY: The crystal structure of thiamin indole-3-propionate was determined by X-ray diffraction as a model for the possible thiamin coenzyme—tryptophan residue interaction at the binding site of thiamin pyrophosphate dependent enzymes. There is an intermolecular stacking interaction of the indole ring with the pyrimidine ring, but not with the positively charged thiazolium ring, of thiamin retaining the characteristic F-conformation. Although this association is due to dipole-dipole interaction between both aromatic rings, charge-transfer interaction cannot be ruled out in solution state because the absorption spectrum shows the characteristic charge-transfer band.

INTRODUCTION

Thiamin pyrophosphate(TPP)* is a coenzyme in a number of enzymatic processes catalyzing the decarboxylation of α-keto acids and the transfer of aldehyde or acyl groups(1). For an understanding of the catalytic mechanisms, the mode of the coenzyme—apoenzyme interaction needs to be detailed.

Information gained from diverse spectral studies of native and chemically modified enzymes(2-8), augmented by model systems (9-11), provides unequivocal evidence for the interaction of TPP with tryptophan residue within the coenzyme-binding hydrophobic

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* Abbreviations used: TPP, thiamin pyrophosphate; TIP, thiamin indole-3-propionate; CNDO/2, complete neglect of differential overlap

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site of numerous apoenzymes, *e.g.* pyruvate oxidase, decarboxylase and transketolase. Although detailed knowledge about the binding mode is lacking, the indole moiety of the tryptophan residue is generally considered to stack with the thiamin ring moiety of TPP coenzyme. Two interaction modes have been proposed: (a) the π-π charge-transfer interaction between the indole ring and the positively charged thiazolium ring and/or pyrimidine ring of thiamin (2-4), having a V-conformation($\phi_T \fallingdotseq \pm90^\circ$, $\phi_P \fallingdotseq \mp90^\circ$)** which facilitates close contact between the 4'-amino group on the pyrimidine ring and the active C(2) atom on the thiazolium ring(9,10,12), (b) a stacking interaction between the indole ring and the pyrimidine ring of thiamin(13), having an F-conformation($\phi_T \fallingdotseq 0^\circ$, $\phi_P \fallingdotseq \pm90^\circ$) which is the characteristic form of thiamin when C(2) is free of substituent(14).

To examine these conflicting viewpoints and to obtain insights into the binding mode of thiamin coenzyme with the apoenzymes, we synthesized thiamin indole-3-propionate(TIP)* as a possible model compound and determined its crystal structure. This is the first example of the indole–thiamin stacking interaction to be investigated directly at the molecular level.

MATERIALS AND METHODS

TIP, as perchlorate hydroperchlorate salt, was synthesized by the esterification and further the oxidative desulfurization of indole-3-propionic anhydride and thiothiamin(15). A solution of crude crystals of $TIP{\cdot}ClO_4{\cdot}HClO_4$ in methanol/ethyl acetate was slowly evaporated at room temperature to afford pale yellow, tiny platelets(mp.139-141°C). A single crystal(dimension: 0.2×0.05×0.1 mm³) was used for X-ray study; it is triclinic and has space group symmetry P$\bar{1}$ with two units of the chemical formula, $C_{23}H_{26}N_5O_2S^+{\cdot}ClO_4^-{\cdot}HClO_4{\cdot}CH_3OH$, in the unit cell. The unit cell dimensions are a=14.960(4), b=14.170(3), c=7.436(2) Å, α=101.79(2), β=102.39(2), γ=94.15(2)° and V=1496.1(6) Å³. The density, as

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** The conformation of thiamin with respect to the methylene bridge carbon joining the thiazolium and pyrimidine rings is defined in terms of the torsion angles of ϕ_T[C(5')-C(35)-N(3)-C(2)] and ϕ_P[N(3)-C(35)-C(5')-C(4')]. Basically three conformations have been proposed for thiamin molecule: V-conformation($\phi_T \fallingdotseq \pm90^\circ$, $\phi_P \fallingdotseq \mp90^\circ$), S-conformation($\phi_T \fallingdotseq \pm100^\circ$, $\phi_P \fallingdotseq \pm150^\circ$) and F-conformation($\phi_T \fallingdotseq 0^\circ$, $\phi_P \fallingdotseq \pm90^\circ$).

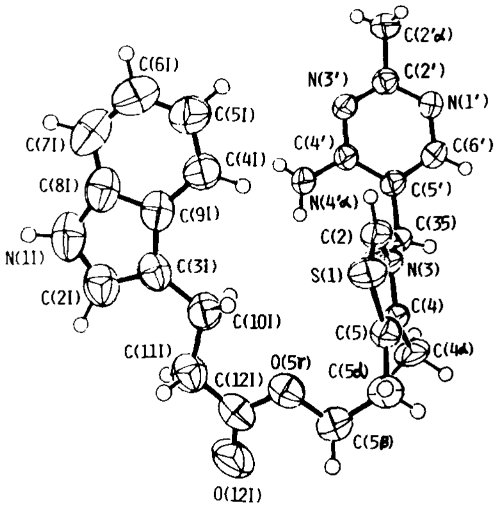

Fig.1 Observed conformation of TIP molecule and atomic numbering

measured by floatation in a mixture of benzene and carbon tetrachloride, is 1.469(3)$g \cdot cm^{-3}$. Intensity data for 5104 independent reflections were collected on a Rigaku automated four-circle diffractometer with graphite-monochromated Cu Kα radiation, using the ω-2θ scan technique; 4477 reflections with $I \geq 2\sigma(I)$ were used for structure determination and refinements. The structure was solved by the heavy-atom method and refined by the least-squares method to a conventional *R* value of 0.095 at this stage.

RESULTS AND DISCUSSION

The conformation of the TIP molecule is shown in Fig.1; the selected torsion angles are listed in Table 1. The TIP molecule exhibits a folded conformation with approximately parallel alignment between the indole and the pyrimidine rings; their dihedral angle is 9.2(2)°. Irrespective of the attachment of the indole-3-propionyl group to the O(5γ) atom, the thiamin moiety takes the

Table 1 Selected torsion angles(°)

C(5')-C(35)-N(3)-C(2) : ϕ_T	5.4(8)
N(3)-C(35)-C(5')-C(4') : ϕ_P	-76.3(7)
S(1)-C(5)-C(5α)-C(5β) : $\phi_{5\alpha}$	99.9(7)
C(5)-C(5α)-C(5β)-O(5γ) : $\phi_{5\beta}$	-57.9(8)
C(5α)-C(5β)-O(5γ)-C(12I)	179.2(6)
C(5β)-O(5γ)-C(12I)-C(11I)	-173.7(6)
C(2I)-C(3I)-C(10I)-C(11I)	-0.6(10)
C(3I)-C(10I)-C(11I)-C(12I)	174.8(6)
C(10I)-C(11I)-C(12I)-O(5γ)	12.0(9)

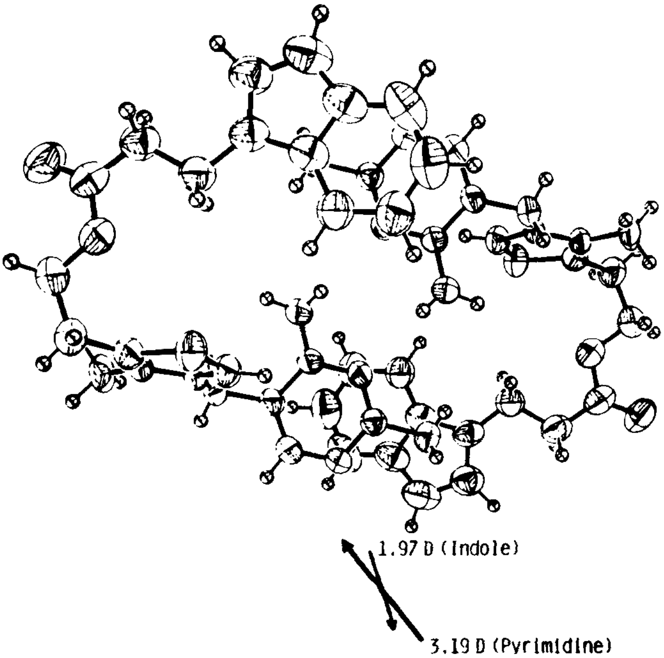

Fig.2 Perspective view of TIP dimer formation due to the stacking interaction between the indole and pyrimidine rings, in which the respective molecules are related by a center of symmetry. The vectors of the permanent dipole moments for the lower aromatic ring pair are represented by arrows; these were calculated by the CNDO/2* method using 3-methylindole and 4-amino-2,5-dimethylpyrimidine molecules.

characteristic F-conformation(ϕ_T= 5.4°, ϕ_P= -76.3°). This has frequently been observed in thiamin derivatives free of substituent on the C(2) atom(14), suggesting the inherent stability of the F-conformation for the thiamin molecule. The dihedral angle between the pyrimidine and the thiazolium ring planes is 72.1(2)°. The conformation of the C(5)-C(5α)-C(5β)-O(5γ) linkage is also in the range found in related compounds(14), although relatively large variations were seen in the values of $\phi_{5\alpha}$ and $\phi_{5\beta}$. The conformation of the TIP molecule is further characterized by a curved planar formation of the C(5α)-C(5β)-O(5γ)-C(12I)-C(11I)-C(10I)-C(3I) bond sequence; the *trans zig-zag* chains of C(5α)-C(5β)-O(5γ)-C(12I) and C(3I)-C(10I)-C(11I)-C(12I) bond sequences are linked to each other with *cis* orientation; the dihedral angles of the carboxyl

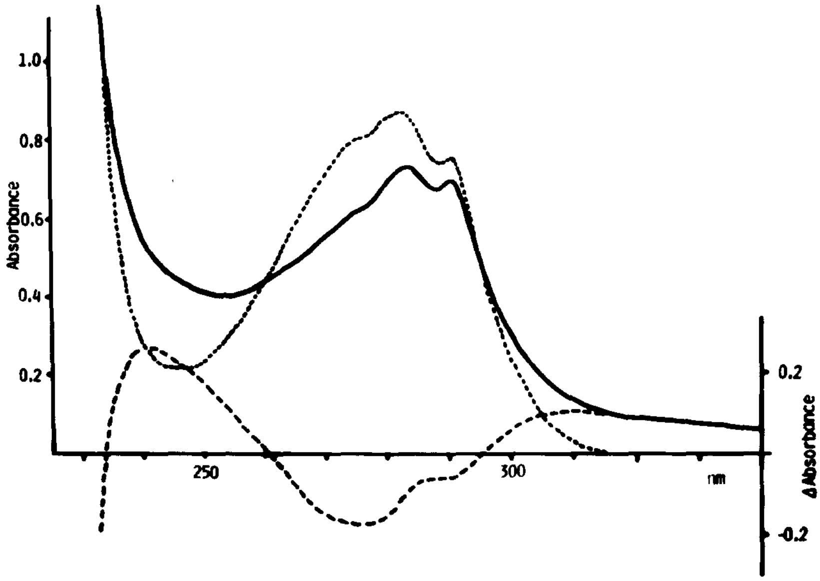

Fig.3 Absorption spectra of TIP·ClO_4·$HClO_4$ minus thiamin·ClO_4·$HClO_4$(——) and indole-3-propionic acid(···), and their difference spectrum(---) in 0.025M phosphate buffer containing 20% ethanol solution(pH=7.8). The spectra were measured three times, and were averaged, using 0.15×10^{-3}M solution of samples.

group with the indole ring and with the thiazolium ring are 10.8(2) and 79.1(2)°, respectively.

Although the π-π charge-transfer interaction between the indole and thiazolium rings has been considered an essential force for thiamin coenzyme—apoenzyme binding(2-4,9,10), our model compound clearly demonstrates the importance of the stacking interaction between the indole ring and the pyrimidine ring. The dimer formation produced by the stacking interaction is shown in Fig.2. The dihedral angle between the two aromatic rings is 9.2(2)° and the average interplanar spacing in the area of overlap is 3.557 Å.

We propose that this association is ascribable to the dipole-dipole interaction between the indole and pyrimidine rings, because the dipole moments of respective aromatic rings are fully coupled in their ground states, as is obviously from Fig.2. Therefore, it is reasonable to posit that the stacking interaction of tryptophan residue and the pyrimidine ring of thiamin coenzyme with a stable F-conformation is important in the binding to enzymes(11,14).

However, based on the UV difference spectrum of TIP and its reference molecules, the π-π charge-transfer interaction between the indole ring and the pyrimidine and/or thiazolium ring cannot be ruled out. As shown in Fig.3, the positive broad band above 295 nm tailing into 375 nm(λ_{max}=310 nm) could be a charge-transfer band resulting from electron transfer from the indole ring to the acceptor aromatic ring of thiamin(16).

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