

STACKING INTERACTION OF INDOLE RING WITH THIAZOLIUM RING
AND EFFECT FOR H-D EXCHANGE REACTION OF THIAMINToshimasa Ishida, Miyuki Matsui, Masatoshi Inoue,
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As part of a program to clarify the possible binding mode of tryptophan with thiamin coenzyme, the interaction between the indole and thiazolium rings has been studied by using a model compound, 2-(3,4-dimethylthiazolium-5-ethyl indole-3-propionate, and the prominent stacking formation between both rings has been evidenced by the spectroscopic and X-ray crystallographic methods. The faster H-D exchange reaction of thiamin C2 proton observed in the presence of indole than in the lack would be resulted from the π - π charge-transfer interaction between the indole ring and the thiazolium ring of thiamin.

Thiamin pyrophosphate (TPP) is a coenzyme of the metabolically important enzymes that catalyze the decarboxylation of α -keto acids and the transfer of aldehyde or acyl groups. Although the general outline of the catalytic mechanism has been known for over two decades (1), little is up to now known about the binding mode of the coenzyme to its apoenzymes.

Many reports suggesting that TPP interacts with the tryptophan residue in the active center of the apoenzymes (2-6) led us to investigate the binding mode, at the atomic level, of indole ring to thiamin molecule, in order to clarify which of the two aromatic rings of thiamin does preferentially bind with the indole ring and what kind of force does act in the interaction. X-ray determination of thiamin indole-3-propionate (TIP) perchlorate crystal, an intramolecular model, revealed the prominent stacking formation of indole ring with the pyrimidine moiety of thiamin (7). This suggests that the functional role of the pyrimidine moiety is partly in the binding to the apoenzyme *via* stacking formation with the aromatic amino acid residue.

On the other hand, the spectroscopic studies of TIP in solution, as well as other related tryptophan-thiamin systems (8,9), indicated that the indole ring is also interactable with the thiazolium ring

of thiamin. In order to elucidate the binding mode of these two aromatic rings, we have now determined the crystal structure of a model compound, 2-(3,4-dimethylthiazolium-5-ethyl indole-3-propionate)(DTEIP) iodide. We wish here to report the prominent stacking formation of both the aromatic rings and further to discuss with the effect of indole ring for the H-D exchange reaction of thiamin C2 proton.

MATERIALS AND METHODS

The materials used are thiamin $\text{Cl}\cdot\text{HCl}$, $\text{TIP}\cdot\text{HSO}_4$, DTEIP iodide, 3,4-dimethyl,5-hydroxyethylthiazolium(DHT) iodide, indole and indole-3-propionic acid(IPA). Their chemical formulas are shown in Scheme 1. Among them, TIP, DTEIP and DHT were chemically synthesized.

Spectroscopic measurements

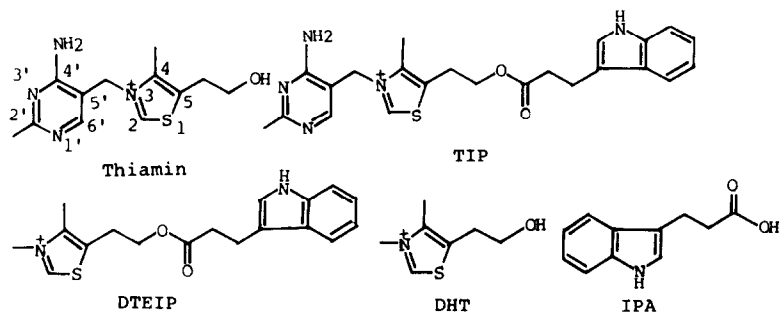
UV measurements were performed in 1-cm 4ml quartz cuvettes on a Hitachi 624 spectrometer. Fluorescence emission spectra(295-nm excitation) were measured on a Hitachi-650-40 spectrometer with a Xenon lamp in 1-cm 4ml glass cuvette. The temperature was held at $25\pm 1^\circ\text{C}$. The sample concentrations were all adjusted to $1.0\times 10^{-4}\text{M}$ and the solvent used was 0.025M phosphate buffer containing 10% ethanol($\text{pH}=6.9$).

X-ray structural analysis of DTEIP iodide

The slow evaporation of 50% aqueous ethanol solution(0.2M) at $20\pm 1^\circ\text{C}$ afforded yellowish tiny needle crystals. A single crystal with dimension of approx. $0.3\times 0.1\times 0.05\text{ mm}^3$ was used for X-ray analysis. The crystal data are as follows: monoclinic, space group $\text{P2}_1/\text{c}$, $a=7.663(2)$, $b=10.870(4)$, $c=23.100(9)\text{ \AA}$, $\beta=105.20(2)^\circ$, $V=1856.8(11)\text{ \AA}^3$, $D_m=1.613(1)$ (by flotation method in $\text{CCl}_4\text{-C}_6\text{H}_6$ mixture), $D_x=1.632\text{ g}\cdot\text{cm}^{-3}$, and $Z=4$. Intensity data for 3149 independent reflections($2\theta\leq 130^\circ$) were collected on a Rigaku automatic diffractometer with graphite-monochromated $\text{Cu K}\alpha$ radiation by using ω - 2θ scan mode. The structure was solved by the heavy-atom method and refined by the least-squares method up to the present R -factor of 0.065 by using 2935 non-zero reflections.

H-D exchange reaction

The measurements were carried out by Hitachi-Perkin-Elmer spectrometer at $31\pm 1^\circ\text{C}$. The each sample, 0.1M (0.5M for indole molecule), was weighted accurately in NMR tube and 0.8ml of $\text{CD}_3\text{OD}\cdot\text{D}_2\text{O}$ (3:5,v/v) mixture was added. The rate of H-D exchange at the thiazolium C2 proton was followed by integration as a function of time, relative to the pyrimidine C6' proton of thiamin, which was found not to be



Scheme 1

exchangeable under the present conditions. The pH was measured on a Horiba pH meter. The pD was then added to the observed one by 0.4.

RESULTS AND DISCUSSION

Ring stacking interaction in DTEIP molecule

In order to assess the stacking ability between the indole and thiazolium rings of DTEIP molecule in solution, the difference absorption spectrum of this molecule against its two component molecules, IPA and DHT iodide, was measured in the range of 210–360 nm. The result was shown in Figure 1, in which the results of fluorescence measurements are also shown (insert). Compared with the summation of absorbances of the component molecules, that of DTEIP molecule is small in the range of 248–298 nm ($-\Delta\epsilon_{\text{max}} = 1.05 \times 10^3$ at 268 nm). Since the concentration used is enough dilute, this hypochromic effect suggests that the indole ring of DTEIP molecule is, to some extent, intramolecularly stacked with the thiazolium ring in their ground states. This stacking formation was further

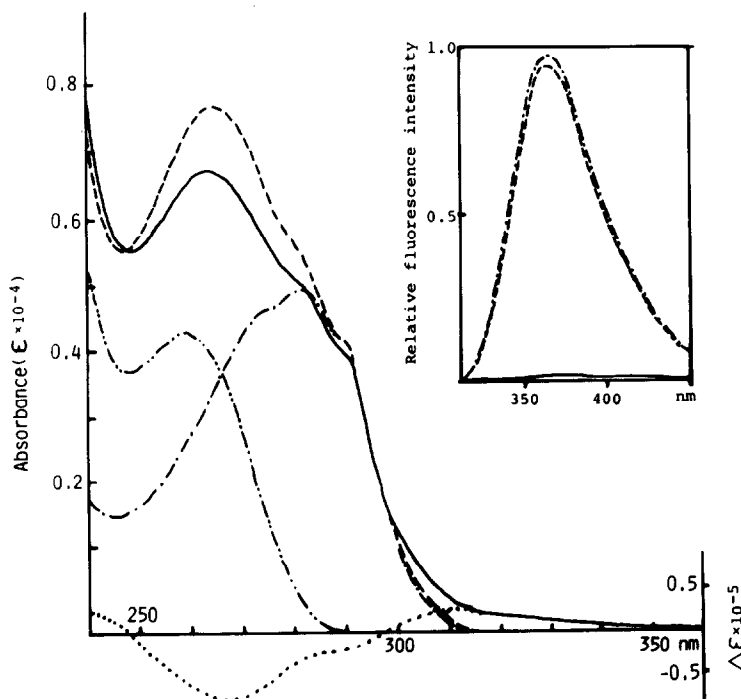


Fig. 1 Electronic absorption spectra of DTEIP iodide(—), DHT iodide(---), IPA(· · ·) and 1:1 mixture of the latter two component molecules(- · - ·). Also indicated is a difference spectrum of DTEIP iodide against DHT iodide plus IPA(· · · ·). Insert: fluorescence emission spectra of IPA alone(---) and coexisting with the equimolar amount of DHT iodide(· · · ·), and DTEIP iodide(—).

evidenced by the appearance of charge-transfer band at above 298nm ($\lambda_{\text{max}}=313\text{nm}$, $\Delta\epsilon_{\text{max}}=350$). The existence of the band was already expected from the yellow coloration of DTEIP molecule compared with the colorless component molecules, and would be resulted from the π -electron transfer from the indole ring to the thiazolium ring. On the other hand, the fluorescence emission spectrum of indole ring observed in IPA molecule was scarcely measured in DTEIP molecule (99.4% quenching at 365nm). Taking into account that there is no fluorescence at the fully stacked state of indole ring with the acceptor ring such as nucleic acid base(10), this prominent quenching clearly indicates the majority of DTEIP molecules existing in the stacked state of both aromatic ring in their excited states.

The crystal structure of DTEIP molecule, on the other hand, showed the extensive intermolecular overlappings of these two aromatic rings as shown in Figure 2. There are two kinds of stacking modes of the indole ring with respect to the central thiazolium ring. Both the aromatic rings are parallelly stacked to each other (the dihedral angles are $3.3(2)^\circ$ for both pairs), and are stabilized by the normal van der Waals contacts between the overlapping atoms of respective rings (the average interplanar spacing is 3.50 \AA for the upper pair and 3.74 \AA for the lower pair). The stacking mode of the upper pair is similar to the one proposed by the theoretical consideration(9), although the orientation of indole ring is almost opposite with respect to its long axis. This stacking mode, *i.e.* the overlapping of thiazolium ring to the center of indole ring, would be energetically the most stable one.

Effect of indole ring on the H-D exchange of thiamin C2 proton

The present spectral and X-ray evidences of the indole-thiazolium ring stacking formation led us to investigate the effect of indole ring on the rate of H-D exchange reaction of thiamin thiazolium C2 proton.

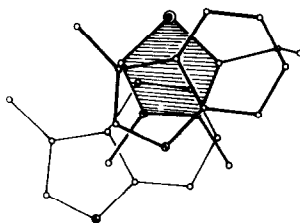
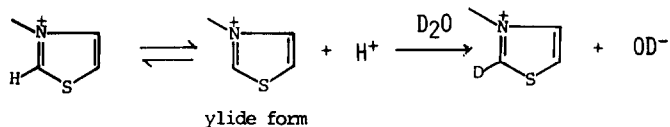


Fig.2

Stacking mode of nearest-neighboring indole-thiazolium ring pairs observed in DTEIP iodide crystal, projected perpendicular to the central thiazolium ring(shaded). For the sake of clarity, the molecular fragments far from the stacking pairs are omitted.



Scheme 2

The reaction is generally representable by Scheme 2 and is considered to be subject to lyate ion catalysis(11-14). The rate constants for thiamin-indole systems were measured by assuming the first-order reaction. The results are shown in Figure 3. Contrary to the previous report(8), the rate constant of TIP or thiamin coexisting with indole is ca. 1.9 or 1.7 times faster than that of thiamin alone. This result implies that the indole ring

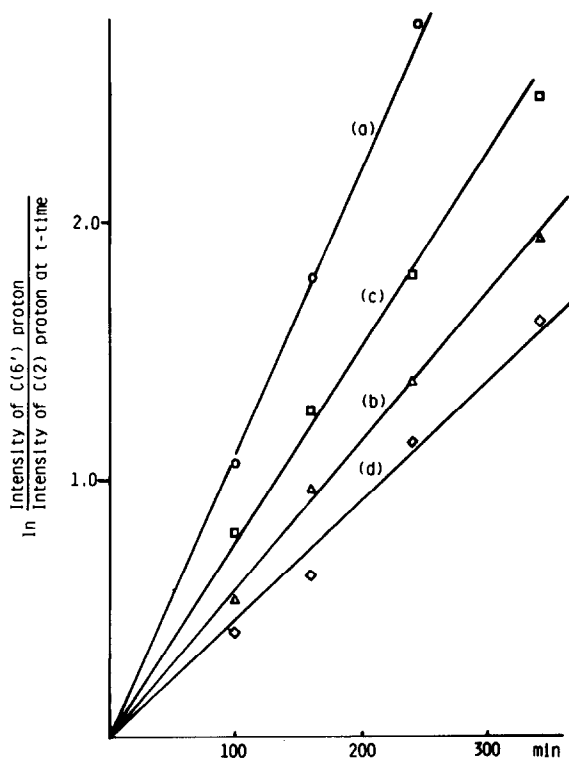


Fig.3

First-order plots for the H-D exchange reactions. The decrease of thiazolium C2 proton of TIP·HSO₄(a) was measured in the coexistence of thiamin·Cl·HCl(b) (pD=4.4, 0.1M). The proton decrease of thiamin·Cl·HCl(0.1M) was measured in the absence(d) or presence(c) of indole molecule(0.5M). In the case of (c), a slight amount of indole was precipitated upon mixing with thiamin. The pD value of (c) and (d) was checked to be same to each other(=4.0). The apparent rate constants obtained by least-squares fit are $11.1 \times 10^{-3} \text{ min}^{-1}$ for (a), $5.8 \times 10^{-3} \text{ min}^{-1}$ for (b), $7.6 \times 10^{-3} \text{ min}^{-1}$ for (c) and $4.6 \times 10^{-3} \text{ min}^{-1}$ for (d).

may accelerate the ylide formation of thiamin. Since the indole does not act as a base, the observed effect would be due to the stacking formation between the indole and thiazolium rings: π -electron transfer from indole ring to the thiazolium ring would discourage the recombination of ylide form. The detailed kinetic study is now progress.

Previously we reported the prominent stacking formation of indole ring with the pyrimidine moiety of thiamin(7), suggesting that the role of tryptophan residue is partly in the binding of thiamin coenzyme. Present study suggests further the possibility of the tryptophan residue playing a role in accelerating the catalytic reaction of TPP molecule, because the reaction is done through the ylide formation(1). The tryptophan residue may be liable to transfer from the pyrimidine moiety to the thiazolium one of thiamin by the conformational change in the holoenzyme(15).

REFERENCES

1. Breslow, R. (1958) J. Amer. Chem. Soc. 80, 3719-3726.
2. Wittorf, J.H. and Gubler, C.J. (1970) Eur. J. Biochem. 14, 53-60.
3. Heinrich, C.P., Noack, K. and Wiss, O. (1971) Biochem. Biophys. Res. Commun. 44, 275-279.
4. Ullrich, J. and Wollmer, A. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1635-1644.
5. Kochetov, G.A., Meshalkina, L.E. and Usmanov, R.A. (1976) Biochem. Biophys. Res. Commun. 69, 839-843.
6. O'Brien, T.A. and Gennis, R.B. (1980) J. Biol. Chem. 255, 3302-3307.
7. Inoue, M., Hirano, H., Sugiyama, K., Ishida, T. and Nakagaki, M. (1982) Biochem. Biophys. Res. Commun. 108, 604-609.
8. Biaglow, J.E., Mieyal, J.J., Suchy, J. and Sable, H.Z. (1969) J. Biol. Chem. 244, 4054-4062.
9. Farzami, B., Mariam, Y.H. and Jordan, F. (1977) Biochemistry, 16, 1105-1110.
10. Mutai, K., Gruber, B.A. and Leonard, N.J. (1975) J. Amer. Chem. Soc. 97, 4095-4104.
11. Breslow, R. and McNelis, E. (1959) J. Amer. Chem. Soc. 81, 3080-3082.
12. Haake, P., Bausher, L.P. and Miller, W.B. (1969) J. Amer. Chem. Soc. 91, 1113-1120.
13. Kemp, P.S. and O'Brien, J.T. (1970) J. Amer. Chem. Soc. 92, 2554-2555.
14. Crosby, J. and Lienhard, G.E. (1970) J. Amer. Chem. Soc. 92, 5707-5716.
15. Jordan, F. (1974) J. Amer. Chem. Soc. 96, 3623-3630.