serious misinterpretation of the kinetic factors that are influencing the observed isotope effect is to be avoided.

Acknowledgment. We are grateful to Dr. Robert Hanzlik for his helpful comments. This work was supported in part by the National Institute of Health (Grants G.M. 25136 and 36922).

Supplementary Material Available: Experimental and calculational details (1 page). Ordering information is given on any current masthead page.

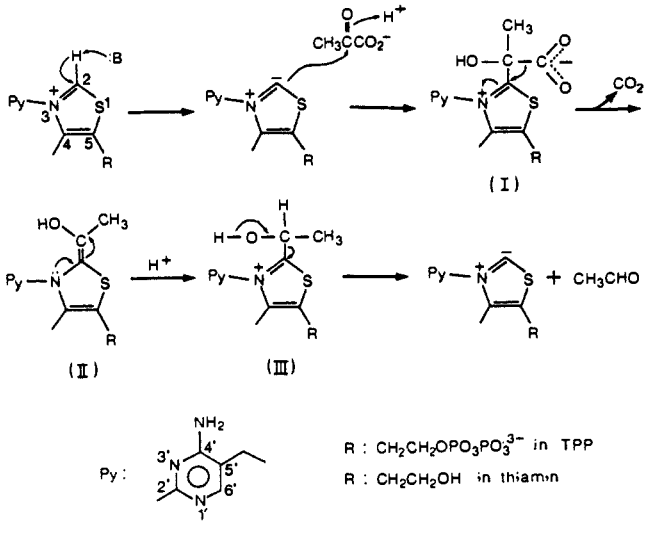
Crystal Structure of Thiamin Thiazolone: A Possible Transition-State Analogue with an Intramolecular N-H···O Hydrogen Bond in the V Form

Whanchul Shin* and Young Chang Kim[1]

Contribution from the Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151, Korea. *Received April 23, 1986*

Abstract: The pyrophosphate ester of thiamin thiazolone (TT) has been proposed to be a transition-state analogue for the thiamin pyrophosphate dependent enzymes due to its high affinity for the apoenzyme and its structural resemblance to the metastable enamine which is assumed to be the immediate product of decarboxylation of the pyruvate adduct of thiamin. TT, $C_{12}H_{16}N_4O_2S$, crystallizes in the monoclinic space group $P2_1/n$ with a = 4.634 (2) Å, b = 12.591 (6) Å, c = 22.291 (10) Å, β = 95.20 (4)°, and Z = 4. The structure was solved by direct methods and refined to an R value of 0.041 for 987 observed reflections measured with Cu Kα radiation on a diffractometer. The molecular conformation of TT is quite different from the S conformation which is characteristic of other C(2)-substituted thiamins. Instead, TT assumes a V conformation (ϕ_T = 104°, ϕ_P = -74°) that has previously been observed only in oxythiamin which is a strong antagonist of thiamin. An intramolecular hydrogen bond between the 4′-amino group and C(2) oxygen stabilizes the V form. This is the first crystal structure showing that the 4′-amino group, whose functional role in thiamin catalysis is not well established, is involved in an intramolecular interaction. The structure of TT suggests that the active conformation of free thiamin in the holoenzyme may be the V form.

Thiamin (vitamin B_1), in the form of the pyrophosphate ester TPP, is a coenzyme in a number of enzyme systems that catalyze decarboxylation of α-keto acids and the transfer of aldehyde or acyl groups.[2] Although as shown in the following scheme Breslow's mechanism[3] depicts the essential features of thiamin catalysis, many details of the enzymatic reactions remain to be



elucidated. One of the intriguing questions is the function of the 4′-amino group which is absolutely required for the enzymatic reactions. Schellenberger[4] proposed that the 4′-amino group actively participates in the catalytic reaction, acting as an acid and a base alternatively, and that thiamin and its C(2) adducts be in the V form[5] in which the 4′-amino group is close to the C(2) active center. Sable et al. also proposed the stable V form of free thiamin from the NMR studies on the model compounds.[6] However, none of the crystal structures of either free thiamin or C(2)-substituted thiamin have revealed the proposed V form. Instead, two other basic conformations have been observed.[7] Free thiamin assumes, with minor exceptions, the F conformation, and C(2)-substituted thiamin has the S conformation. In both conformations, the 4′-amino group is quite distant from the C(2) active center. Even free thiamin compounds that show deviations from the F form do not assume the V conformation but the S conformation. The V form occurs only in oxythiamin, a strong antagonist in which the 4′-amino group is substituted with an oxo group.[8] Undoubtedly the structural characteristics should cor-

(1) Present address: Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260.
(2) (a) Krampitz, L. O. *Annu. Rev. Biochem.* **1969**, *38*, 213-240. (b) Gallo, A. A.; Mieyal, J. J.; Sable, H. Z. *Bioorg. Chem.* **1978**, *4*, 147-177.
(3) Breslow, R. J. *J. Am. Chem. Soc.* **1958**, *80*, 3719-3726.
(4) (a) Schellenberger, A. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1024-1035. (b) Schellenberger, A. *Ann. N. Y. Acad. Sci.* **1982**, *378*, 51-62.
(5) The conformation of the thiamin molecule is best expressed in terms of the two torsion angles, ϕ_T and ϕ_P, about the bonds from the methylene bridge carbon to the thiazolium and pyrimidine rings, respectively. The torsion angles ϕ_T = C(5′)-C(3,5′)-N(3)-C(2) and ϕ_P = N(3)-C(3,5′)-C(5′)-C(4′). A V conformation is specified by $\phi_T \simeq \pm 90°$ and $\phi_P \simeq \mp 90°$. An F conformation is specified by $\phi_T \simeq 0°$ and $\phi_P \simeq \pm 90°$. An S conformation is specified by $\phi_T \simeq \pm 100°$ and $\phi_P \simeq \pm 150°$. For more details, see: footnote 13 in ref 28 and footnote 4 in ref 8a.
(6) Biaglow, J. E.; Mieyal, J. J.; Suchy, J.; Sable, H. Z. *J. Biol. Chem.* **1969**, *244*, 4054-4062.
(7) Shin, W.; Pletcher, J.; Blank, G.; Sax, M. *J. Am. Chem. Soc.* **1977**, *99*, 3491-3499.
(8) (a) Shin, W.; Pletcher, J.; Sax, M.; Blank, G. *J. Am. Chem. Soc.* **1979**, *101*, 2462-2469. (b) Shin, W.; Pletcher, J.; Sax, M. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1719-1724.

0002-7863/86/1508-7078$01.50/0

Table I. Summary of Crystal Data and Data Collection

formula	$C_{12}H_{16}N_4O_2S$
fw, amu	280.09
a, Å	4.634 (2)
b, Å	12.591 (6)
c, Å	22.291 (10)
β, deg	95.20 (4)
V, Å^3	1295 (1)
space group	$P2_1/n$
Z	4
D(measd), g cm^{-3}	1.433
D(calcd), g cm^{-3}	1.435
absorpt coeff, cm^{-1}	21.47
crystal size, mm^3	0.6 × 0.3 × 0.1
T of date collection, °C	18
wavelength of Cu Kα radiation	1.5418
data collection method	2θ–ω scan
scan speed in 2θ, deg/min	2
scan range in ω, deg	1.3 + 0.6 tan θ
max 2θ, deg	115.0
no. of unique data measd	1764
no. of obsd ($F_o > 6\sigma(F_o)$)	987
no. of variables	236
R_F[a]	0.041
R_{wF}[b]	0.043
goodness of fit[c]	0.79

[a] $R_F = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $R_{wF} = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$. [c] Error in an observation of unit weight, equal to $[\sum w(|F_o| - |F_c|)^2/(NO - NV)]^{1/2}$ where NO = number of observations and NV = number of variables in the least-squares refinement.

relate with the catalytic details, but this has been unsuccessful thus far.

The pyrophosphate ester (TTPP) of thiamin thiazolone (TT) is known to be a transition-state analogue for the TPP-dependent enzymatic reactions due to its structural resemblance to the proposed neutral, metastable enamine (II) and its high affinity for the apoenzyme.[9] It binds much more strongly to the TPP sites of pyruvate dehydrogenases of *E. coli*[9] (at least 20000 times) and bovine kidney[10] and, to a lesser extent, to those of *E. coli* pyruvate oxidase[11] and wheat germ pyruvate decarboxylase[12] than does TPP itself. However, it does not bind so tightly to bakers' yeast transketolase.[13] There are conflicting viewpoints about the nature of the immediate product of the decarboxylation of 2-(α-lactyl)-TPP (I). Kuo and Jordan[14] recently reported a new suicide substrate for brewers' yeast pyruvate decarboxylase that forms an enzyme-bound enamine-like intermediate, supporting the suggestion made earlier by Ullrich and Mannshreck[15] that 2-(α-hydroxyethyl)-TPP (III) is not the true intermediate but rather then enamine is. However, Schellenberger excluded this enamine transition state based on the results of the enzymatic tests on the various 2-oxo acids.[4b] On the other hand, Kruger et al.[12,16] suggested that TT may have a high affinity for the apoenzyme because it resembles an intermediate with lower polarity rather than a transition state.

Whether or not there is an enamine transition state and TT is an analogue of this transition state, X-ray analysis of TT may provide unique information about the structural aspects of thiamin, because no crystal structures of thiamin analogues containing a neutral thiazole moiety instead of a thiazolium ring have yet been determined. The crystal structure of TT may also provide a suggestion concerning the structural requirements for the coenzyme to bind to most of the TPP-dependent enzymes due to its high affinity for the apoenzyme.

Table II. Atomic Coordinates (×10^4) and Equivalent Thermal Parameters of Non-Hydrogen Atoms

atom	x	y	z	U_{eq}[a]
S(1)	2713 (3)	3752 (1)	2192 (1)	0.046
C(2)	1136 (11)	3825 (4)	2882 (2)	0.038
N(3)	-310 (8)	4750 (3)	2907 (2)	0.031
C(4)	-278 (10)	5397 (4)	2386 (2)	0.033
C(5)	1253 (10)	4986 (4)	1965 (2)	0.035
O(2α)	1383 (8)	3130 (3)	3276 (1)	0.049
C(3,5′)	-2140 (11)	4959 (5)	3404 (2)	0.036
C(4α)	-1868 (14)	6419 (5)	2361 (3)	0.048
C(5α)	1827 (13)	5411 (4)	1359 (2)	0.042
C(5β)	862 (12)	4665 (5)	845 (2)	0.044
O(5γ)	-2168 (8)	4552 (3)	801 (2)	0.055
N(1′)	-1067 (10)	7388 (3)	4403 (2)	0.044
C(2′)	1095 (11)	7037 (4)	4783 (2)	0.038
N(3′)	2387 (9)	6096 (3)	4761 (2)	0.036
C(4′)	1446 (10)	5417 (4)	4318 (2)	0.031
C(5′)	-842 (10)	5714 (4)	3884 (2)	0.031
C(6′)	-1969 (13)	6697 (4)	3961 (3)	0.043
C(2′α)	2184 (18)	7768 (5)	5291 (3)	0.052
N(4′α)	2768 (11)	4475 (4)	4317 (2)	0.040

[a] $U_{eq} = {}^1/_3\sum_i\sum_j U_{ij}a^*_i a^*_j a_i a_j$.

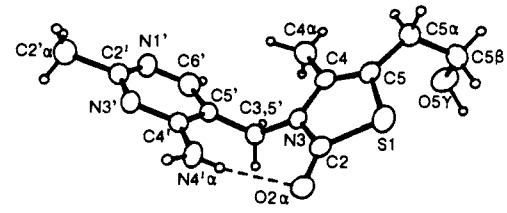


Figure 1. View of the thiamin thiazolone molecule and the atomic numbering scheme.

Experimental Section

TT was prepared following the procedure of Sykes and Todd.[17] Thiamin disulfide (2.5 g) was suspended in isobutyl alcohol (50 mL) and heated at reflux for 2 h. The disulfide dissolved slowly to give a clear, pale-yellow solution which was then cooled and left overnight at 4 °C to yield colorless fine crystals. The crystals were filtered, recrystallized from hot ethanol/H_2O (2:7 v/v) solution at 0.1% concentration, and dried in the air. Colorless needles, suitable for the diffraction experiment, grew from an ethanol solution of the fine crystals by slow evaporation at room temperature over a period of a few weeks.

Oscillation and Weissenberg photographs, which showed systematically absent reflections for $0kl$ when k is odd and $h0l$ when $h + l$ is odd, indicated the crystal to be monoclinic with space group symmetry $P2_1/n$. The unit cell parameters were determined by a least-squares fit of 2θ angles for 20 reflections ($24° < 2\theta < 49°$) measured with Cu Kα radiation on an automated Rigaku AFC diffractometer. The 2θ value used for each reflection was the average of the values for the Friedel pair. Details of the crystal and data collection parameters are summarized in Table I. The intensities were collected with graphite-monochromatized Cu Kα radiation. The background was counted for 12 s at either end of the scan range. Three standard reflections were monitored every 50 reflections throughout the data collection and showed random variations of ±4% with no significant trends. After Lorentz and polarization effects appropriate for graphite-monochromatized ($2\theta_m = 26.5°$) radiation were corrected for, the intensity data were converted to relative structure factor amplitudes. Of the 1764 independent reflections measured with $2\theta < 115°$, 777 (44%) were considered unobserved as defined by $F_o < 6\sigma(F_o)$. No correction for the absorption and extinction effects was made.

The structure was solved by the direct method and refined by full-matrix least squares using the program SHELX 76.[18] Anisotropic refinement converged at $R = 0.041$ for 987 observed reflections and 0.17 for all 1764 reflections. The weighted R was 0.043. The function $\sum w(|F_o| - |F_c|)^2$ was minimized in the refinement. w, the weight of the reflection, was defined by $k/(\sigma^2(F_o) + gF_o^2)$, where $\sigma(F_o)$ was from counting statistics and k and g were optimized in the least-squares procedure ($k = 1.00$ and $g = 0.0043$). The isotropic thermal parameters for the hydrogen atoms were also refined in the last two cycles. Despite the relatively high portion of unobserved reflections due to the weakly dif-

(9) Gutowsky, J. A.; Lienhard, G. E. *J. Biol. Chem.* **1976**, *251*, 2863–2866.
(10) Butler, J. R.; Pettit, F. H.; Davis, P. F.; Reed, L. J. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 1667–1674.
(11) O'Brien, T. A.; Gennis, R. B. *J. Biol. Chem.* **1980**, *255*, 3302–3307.
(12) Kluger, R.; Gish, G.; Kauffman, G. *J. Biol. Chem.* **1984**, *259*, 8960–8965.
(13) Shreve, D. S.; Holloway, M. P.; Haggerty, J. C.; Sable, H. Z. *J. Biol. Chem.* **1983**, *258*, 12405–12408.
(14) Kuo, D. J.; Jordan, F. *J. Biol. Chem.* **1983**, *258*, 13415–13417.
(15) Ullrich, J.; Mannshreck, A. *Eur. J. Biochem.* **1967**, *1*, 110–116.
(16) Kluger, R.; Smyth, T. *J. Am. Chem. Soc.* **1981**, *103*, 1214–1216.
(17) Sykes, P.; Todd, A. R. *J. Chem. Soc.* **1951**, 534–535.
(18) Sheldrick, G. M. SHELX. Program for crystal structure determination, University Chemical Laboratory, Cambridge, England, 1976.

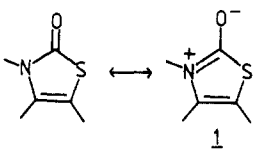
Table III. Bond Distances (Å) and Angles (deg)

S(1)–C(2)	1.764 (5)	S(1)–C(5)	1.752 (5)
C(2)–N(3)	1.348 (6)	C(2)–O(2α)	1.237 (6)
N(3)–C(4)	1.420 (6)	N(3)–C(3,5′)	1.479 (6)
C(4)–C(5)	1.331 (6)	C(4)–C(4α)	1.481 (8)
C(5)–C(5α)	1.498 (7)	C(3,5′)–C(5′)	1.514 (7)
C(5α)–C(5β)	1.519 (7)	C(5β)–O(5γ)	1.406 (7)
N(1′)–C(2′)	1.328 (6)	N(1′)–C(6′)	1.351 (7)
C(2′)–N(3′)	1.330 (6)	C(2′)–C(2′α)	1.510 (8)
N(3′)–C(4′)	1.348 (6)	C(4′)–C(5′)	1.420 (6)
C(4′)–N(4′α)	1.335 (7)	C(5′)–C(6′)	1.360 (7)
N(3)–C(2)–S(1)	109.1 (3)	C(4)–N(3)–C(2)	114.9 (4)
C(4)–C(5)–S(1)	110.9 (4)	C(5)–S(1)–C(2)	91.5 (2)
C(5)–C(4)–N(3)	113.5 (4)	O(2α)–C(2)–S(1)	124.2 (4)
O(2α)–C(2)–N(3)	126.6 (4)	C(3,5′)–N(3)–C(2)	120.3 (4)
C(3,5′)–N(3)–C(4)	124.1 (4)	C(4α)–C(4)–N(3)	119.1 (4)
C(4α)–C(4)–C(5)	127.3 (5)	C(5α)–C(5)–S(1)	118.8 (3)
C(5α)–C(5)–C(4)	130.2 (4)	C(5β)–C(5α)–C(5)	113.3 (4)
O(5γ)–C(5β)–C(5α)	109.8 (4)	N(3′)–C(2′)–N(1′)	126.1 (4)
C(4′)–N(3′)–C(2′)	118.3 (4)	C(4′)–C(5′)–C(3,5′)	122.8 (4)
C(5′)–C(3,5′)–N(3)	115.0 (4)	C(5′)–C(4′)–N(3′)	120.3 (4)
C(5′)–C(6′)–N(1′)	125.6 (5)	C(6′)–N(1′)–C(2′)	114.6 (4)
C(6′)–C(5′)–C(3,5′)	121.9 (4)	C(6′)–C(5′)–C(4′)	115.1 (4)
C(2′α)–C(2′)–N(1′)	117.2 (5)	C(2′α)–C(2′)–N(3′)	116.7 (5)
N(4′α)–C(4′)–N(3′)	116.6 (4)	N(4′α)–C(4′)–C(5′)	123.1 (4)

fracting power of the crystal, the final difference Fourier map was relatively clean, with the highest peak of 0.27 e Å^{-3}. Atomic scattering factors and the terms of the anomalous-dispersion correction were from the International Tables for X-ray Crystallography.[19] The final atomic coordinates are listed in Table II. Structure factors are available as supplementary material.

Description of the Structure

A view of the TT molecule and the atomic numbering scheme is presented in Figure 1. The bond distances and angles are listed in Table III. The molecular dimensions of various thiamin derivatives are well discussed by Cramer et al.[20] The bond distances of the thiazole ring show the expected differences from those of the thiazolium ring. It has been found that the S–C bonds, especially S(1)–C(2), in thiamin have considerable double-bond character, and S(1) bears about 40% of a formal positive charge.[21] Accordingly, the largest variation between TT and thiamin occurs in the S(1)–C(2) bond (1.764 (5) Å) which is lengthened by ~0.09 Å in TT compared to the average value of 1.673 Å for thiamin.[20] The S(1)–C(5) bond (1.752 (5) Å) is also lengthened by ~0.03 Å. The C(2)–N(3) and N(3)–C(4) bonds are also longer by about 0.03 Å than the average values of thiamin, while the C(4)–C(5) bond is shorter by 0.03 Å, reflecting the loss of aromaticity and the increased double-bond character of the C(4)–C(5) bond. However, the exocyclic bonds are not affected by the change in the ionization state of the thiazole ring. The largest variation in the valence angle is in the S(1)–C(2)–N(3) angle. It is decreased by 3.1° in TT, presumably due to the fatter C(2)–O(2α) double bond. The C(2)–O(2α) keto bond distance of 1.238 (6) Å is longer by 0.03 Å than 1.208 Å in the 1,3-thiazolidine-2,4-dione ring.[22] The observed bond distances of TT indicate a significant contribution (estimated to be about 25%) from the zwitterionic resonance structure **1**.



1

(19) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.
(20) Cramer, R. E.; Maynard, R. B.; Ibers, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 76–81.
(21) Kraut, J.; Reed, H. J. *Acta Crystallogr.* **1962**, *15*, 747–757.
(22) (a) Form, G. R.; Raper, E. S.; Downie, T. C. *Acta Crystallogr., Sect. B* **1975**, *B31*, 2181–2184. (b) Stanković, S.; Andreetti, G. D. *Ibid.* **1979**, *B35*, 3078–3080.

Table IV. Least-Squares Planes[a]

plane	atomic deviations, Å			
1. thiazolone	S(1)*	0.004	O(2α)	−0.015
(σ = 0.010)	C(2)*	−0.008	C(3,5′)	−0.163
	N(3)*	0.009	C(4α)	−0.023
	C(4)*	−0.005	C(5α)	−0.006
	C(5)*	0.0		
2. pyrimidine	N(1′)*	0.0	C(3,5′)	−0.104
(σ = 0.004)	C(2′)*	0.001	C(2′α)	−0.013
	N(3′)*	0.002	N(4′α)	−0.022
	C(4′)*	−0.005	H(6′)	−0.085
	C(5′)*	0.005	H(4′1)	−0.069
	C(6′)*	−0.002	H(4′2)	0.035

eq of the planes
$0.7897x + 0.4530y + 0.4138z = -4.7935$ (1)
$0.7159x + 0.3863y - 0.5817z = 3.0808$ (2)

dihedral angle between the planes 60.0°

[a] Atoms designated by an asterisk (*) were used to calculate the planes. x, y, and z in the equation of the plane are in fractional unit cell coordinates.

Table V. Comparison of Torsion Angles[5] for TT and Oxythiamin

	ϕ_T, deg	ϕ_P, deg
this structure	104.1 (4)	−74.2 (4)
oxythiamin·Cl·HCl·H_2O[8a]		
(A)	105.5 (7)	−62.8 (7)
(B)	−101.5 (7)	64.2 (7)
oxythiamin·Cl·2H_2O[8b]	−103.4 (2)	64.6 (2)

Table VI. Hydrogen Bonding

a b c	a–c, Å	b–c, Å	abc, deg	symmetry for c
N(4′α)–H(1)···O(2α)	2.897 (6)	2.17 (5)	165 (5)	x, y, z
N(4′α)–H(2)···N(3′)	2.992 (6)	2.08 (6)	168 (5)	$1-x, 1-y, 1-z$
O(5γ)–H···N(1′)	2.869 (7)	1.78 (5)	170 (4)	$-^1/_2-x, -^1/_2+y, ^1/_2-z$

Despite differences in the formal electronic charges of the thiazole and thiazolium rings, the molecular dimensions of the unprotonated aminopyrimidine ring in TT are in excellent agreement (within 1σ) with those in thiamin chloride monohydrate,[23] thiamin picrolonate dihydrate,[7] and thiamin nitrate,[24] the only structures containing unprotonated N(1′). The C(2′)–N(1′)–C(6′) angle in thiamin compounds varies widely depending on the protonation state of the pyrimidine ring.[20] The value of 114.6 (4)° in TT is the typically observed one in the unprotonated and metal-bound pyrimidines of thiamin, while this angle in the protonated pyrimidines is about 120°. The two methyl–ring distances are 1.510 (8) and 1.482 (7) Å for C(2′α) and C(4α), respectively. The C(4α) methyl group makes close contacts with O(2α) (3.240 (7) Å) and S(1) (3.603 (6) Å), which are shorter than the predicted van der Waals separations[25] of 3.40 and 3.80 Å, respectively. In contrast, the C(2′α) methyl group is not in a polar environment, the closest electronegative atom being O(5γ) with a separation of 3.565 (8) Å. These differences in the environment suggest that the shorter bond for the C(4α) methyl group might reflect the hyperconjugation effect even though some of the shortening may be the result of thermal motion.

The least-squares planes for the thiazolone and pyrimidine rings are listed in Table IV. The magnitudes of the atomic deviations from the thiazolone ring are larger than those for the pyrimidine ring. This differs from most thiamin structures in which deviations from the pyrimidine ring are usually larger than those for the thiazolium ring and may reflect the nonaromatic nature of the

(23) Pletcher, J.; Sax, M.; Sengupta, S.; Chu, J.; Yoo, C. S. *Acta Crystallogr., Sect. B* **1972**, *B28*, 2928–2935.
(24) Ishida, T.; Tanaka, A.; Inoue, M. *Acta Crystallogr., Sect. C* **1984**, *C40*, 437–439.
(25) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441–451.

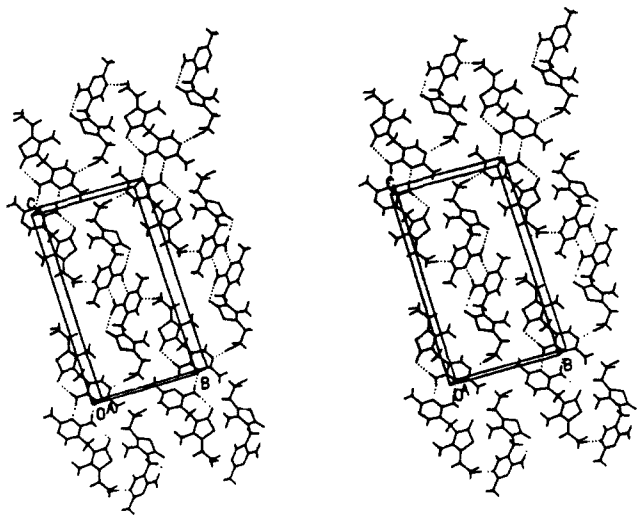


Figure 2. Stereoscopic view showing the packing of the thiamin thiazolone molecules in the unit cell. Dotted lines indicate the hydrogen bonds.

thiazolone ring. N(3) deviates by 0.068 Å from the plane formed by C(2), C(4), and C(3,5'), and the sum of valence angles around N(3) is 359.3°. These are indicative of a near-sp^2-hybridization of N(3). The methylene bridge C(3,5') deviates significantly from the two planes (0.163 and 0.104 Å from the thiazolone and pyrimidine planes, respectively), maybe to relieve the torsional contacts with O(2α) and N(4'α). The torsion angles of C(3,5')–N(3)–C(2)–O(2α) and C(3,5')–C(5')–C(4')–N(4'α) are –8.3° and 4.5°, respectively. The dihedral angle between the two planes is 60.0°. TT assumes a V conformation which is very similar to that of oxythiamin. The torsion angles of TT and oxythiamin are compared in Table V. An intramolecular N(4'α)–H···O(2α) hydrogen bond (Table VI) stabilizes the V conformation of TT, whereas there is no comparable C(2)-H···O(4'α) hydrogen bond in oxythiamin. Concomitantly, with the N–H···O hydrogen bond, one of the hydrogen atoms of C(3,5') is close to O(2α) [C···O = 2.852 (6), H(3,5'2)···O = 2.35 (6) Å, C–H···O = 113 (4)°]. This interaction may be, albeit very weak, an attractive C–H···O interaction as Taylor and Kennard[26] have pointed out. C(2)–O(2α)···H(4'α1) and C(2)–O(2α)···H(3,5'2) are 101 (6)° and 75 (6)°, respectively, while H···O(2α)···H is 60 (6)°. The conformation of the hydroxyethyl side chain is similar to that found in thiamin compounds when there is no close S···O electrostatic interaction. $\phi_{5\alpha}$ = S(1)–C(5)–C(5α)–C(5β) = 56.7° and $\phi_{5\beta}$ = C(5)–C(5α)–C(5β)–O(5γ) = 65.0°. S(1) no longer bears a partial positive charge, and consequently there are no electronegative atoms around S(1).

Figure 2 shows the packing drawing of the structure. Although crystal structures of thiamin analogues usually contain counteranions, there is no counteranion in this structure because TT is uncharged. The crystal packing forces consist of two unique intermolecular hydrogen bonds (see Table VI). The N(4'α)–H···N(3') hydrogen bonds dimerize two molecules related by a center of symmetry at ($^1/_2$, $^1/_2$, $^1/_2$). The formation of dimer across a center of symmetry between N(3') and N(4'α) is a common hydrogen-bonding scheme in thiamin structures. The dimers are cross-linked by the O(5γ)–H···N(1') hydrogen bonds to form hydrogen-bonded hexamers. These hydrogen bonds make a two-dimensional hydrogen-bonded molecular layer. Besides the hydrogen-bonding interactions, there are no unusually close contacts in the structure.

Discussion

The present structure is the first X-ray example of a thiamin analogue with an intact aminopyrimidine ring showing the V form in which the 4'-amino group is involved in an intramolecular hydrogen bond and the only crystal structure that remotely resembles the active V model[4] proposed by Schellenberger. It appears very unlikely that the conformation of TT is influenced by the crystal packing forces, since the V conformation is stabilized by an intramolecular hydrogen bond and the hydrogen-bonding scheme in the crystal lattice is very similar to that of thiamin even with the different conformation and electronic structure in the thiazole moiety.

The fact that the bond distances of the pyrimidine moiety of TT and those of various thiamin compounds are very similar suggests that major changes in the inductive effects of the thiazolium moiety in thiamin may not exert significant effects on the electronic structure of the pyrimidine ring. It has also been found in oxythiamin that major changes in the pyrimidine ring did not exert any significant effects on the electronic structure of the thiazolium ring.[8] These indicate that long-range structural effects between the two ring systems in thiamin may not be present.

TT and oxythiamin assume an almost identical V conformation even though the corresponding two-ring systems are quite different from each other. Although free thiamin is believed to be conformationally flexible, it always crystallizes either in the F form or in the S form but not in the proposed V form. It is interesting to note that the F and S forms are the two extreme conformations that provide maximum separations between the substituents at C(2) and C(4') and between C(6')–H and the C(4α) methyl group. Obviously, any thiamin analogue with a bulkier substituent than hydrogen at C(2) cannot assume the F form due to the steric hindrances between the pyrimidine ring and the substituent. Thus far, crystal structures of three C(2)–substituted thiamin analogues have been determined. These include 2-(α-hydroxyethyl)thiamin,[27] 2-(α-hydroxybenzyl)thiamin,[28] and phosphalactylthiamin,[29] all of which assume the S conformation instead of the proposed V form. 2-(α-Hydroxybenzyl)oxythiamin[30] also assumes an identical S conformation. The fact that the V form has not been observed in thiamin and its C(2)-substituted analogues with the only exceptions of oxythiamin and TT suggests that the steric interactions between the substituents at C(2) and C(4'), and subsequently those at C(4) and C(6'), may be the determining factors for the specific conformations. All of the crystallographic results are consistent with an assumption that the V form can be observed only when two conditions are satisfied simultaneously, that is, only when repulsions, especially between the substituents at C(2) and C(4'), are relieved and, at the same time, the size of the substituents is small. It has been suggested by Sax and Pletcher that an electrostatic interaction between the acidic C(2) proton, which is located on top of the pyrimidine ring, and the pyrimidine π electrons may be the stabilizing force for the F form in free thiamin.[31] However, this does not account for the exceptional cases of three thiamin structures[32] occurring in the S form because the C(6') proton, which is above the thiazolium ring in the S form, is not acidic. Furthermore, all four of the C(2) adducts which seemingly can utilize an intramolecular N–H···O or O–H···O hydrogen-bonding interaction in the V form appear in the S form. These suggest that the observed S conformations may be the result of minimizing the repulsive interactions between the substituents at C(2) and C(4') and subsequently those at C(4) and C(6'). This interpretation also holds for the F conformation in which the attractive interactions between C(2)–H and the π electrons can be utilized additionally. It is increasingly clear that the various conformers of thiamin may have small but significant energy differences, enough to be resolved in the crystal structures, and the observed crystal conformations may be the representations

(26) Taylor, R.; Kennard, O. *J. Am. Chem. Soc.* **1982**, *104*, 5063–5070.

(27) Sax, M.; Pulsinelli, P.; Pletcher, J. *J. Am. Chem. Soc.* **1974**, *96*, 155–165.

(28) Pletcher, J.; Sax, M.; Blank, G.; Wood, M. *J. Am. Chem. Soc.* **1977**, *99*, 1396–1403.

(29) Turano, A.; Furey, W.; Pletcher, J.; Sax, M.; Pike, D.; Kluger, R. *J. Am. Chem. Soc.* **1982**, *104*, 3089–3095.

(30) Shin, W.; Pletcher, J.; Sax, M. *J. Am. Chem. Soc.* **1979**, *101*, 4365–4371.

(31) Turano, A.; Pletcher, J.; Furey, W.; Sax, M. *Ann. N. Y. Acad. Sci.* **1982**, *378*, 91–106.

(32) (a) Richardson, M. F.; Franklin, K.; Thompson, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3204–3209. (b) Maclaurin, C. L.; Richardson, M. F. *Acta Crystallogr., Sect. C* **1983**, *C39*, 854–856. (c) Reference 20.

of an intricate balance of the various intramolecular interactions, rather than the results of crystal packing forces.

It is important to know the "active" conformation of thiamin, apart from the "stable" conformation, because it can be directly correlated with the functional property of the 4'-amino group. The present structure along with the high affinity of TT for the apoenzyme suggests that the conformation of the enzyme-bound free thiamin may be the V form. If TT assumes a different conformation upon binding, the intramolecular hydrogen bond has to be broken and it would be energetically unfavorable. Although the most stable conformation of free thiamin seems to be the F form, a conformational change to V may take place upon binding to the apoenzyme. The energy barrier between the different conformers is known to be small as shown by semiempirical energy calculations[33] (less than 6 kcal/mol) and by NMR studies.[6] Therefore, conversion of F or S to V via concerted rotation of the two rings about the methylene bridge can be achieved easily. It is worthwhile to notice that oxythiamin in the V form can also bind to the apoenzyme as strongly as thiamin does although it cannot release the product.[4a]

From the kinetic studies of the binding processes of TTPP to pyruvate decarboxylase, Kruger et al. concluded that the high affinity of TTPP for the apoenzyme is mainly due to its lower polarity and that TTPP resembles an intermediate rather than a transition state.[12] The present study shows that the conformational characteristics of TT, as well as the neutral thiazole moiety, certainly contribute to its low polarity since one of the amino hydrogens is engaged in an intramolecular hydrogen bond. Apart from the question of whether TTPP is a transition-state analogue or not, the present study suggests that the conformational differences do exist between thiamin and TT and thus may affect the binding efficiency. If the active form of free thiamin is the V form, one of the functions of the apoenzyme may be to hold the coenzyme in the V conformation. If this is the case, the 4'-amino group can directly participate in the catalytic process, or at least serve to direct the incoming substrate properly. However, further studies have to be done to know whether the 4'-amino group is directly involved in acid-base catalysis.

Acknowledgment. We thank the referees for the valuable comments. This work was supported by a grant from the Korea Science and Engineering Foundation.

Registry No. Thiamine thiazole, 490-82-4.

Supplementary Material Available: Tables S1-S3 listing anisotropic thermal parameters, coordinates of the hydrogen atoms, and bond distances and angles involving the hydrogen atoms (3 pages); tables of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

(33) Jordan, F. *J. Am. Chem. Soc.* **1974**, *96*, 3623-3630.

NMR Studies of the Binding of Sodium and Calcium Ions to the Bile Salts Glycocholate and Taurocholate in Dilute Solution, as Probed by the Paramagnetic Lanthanide Dysprosium

Elizabeth Mukidjam,[†] Stephen Barnes,[†‡⊥] and Gabriel A. Elgavish*[‡§]

Contribution from the Departments of Pharmacology and Biochemistry, Cardiac NMR Laboratory, Division of Cardiovascular Disease, and The Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.
Received February 21, 1986

Abstract: The role of calcium in the biliary and intestinal milieu may be quite central, forming physiologically important complexes of bile salts. The binding of Ca^{2+} to bile salts in aqueous solution, in particular to glycocholate and taurocholate, has been investigated by using Ca^{2+}-specific electrodes. The reported dissociation constants are surprising since they suggest that the sulfonate-bearing taurocholate has a greater affinity for Ca^{2+} than the carboxylate-bearing glycocholate. Hydroxyl involvement in Ca^{2+} binding to glycocholate has also been suggested (Moore, E. *Hepatology* **1984**, *4*, 228S-243S). This may bring geometrical contraints on the molecule, which may result in a thermodynamic preference for binding in the case of taurocholate vs. that in the glycocholate system. In the present study we have used a paramagnetic NMR approach in which the lanthanide ion Dy^{3+}, an isomorphous replacement for Ca^{2+}, causes concentration-dependent stereospecific changes in the bile salt ^1H chemical shifts. The dysprosium-induced effects have been modulated by the addition of $CaCl_2$ or NaCl. Analysis of the data by nonlinear computer programs has enabled calculation of the metal-bile salt dissociation constants for Dy^{3+}, Ca^{2+}, and Na^+ complexes. For each metal ion the dissociation constant of the metal-bile salt complex was 2-6 times larger for taurocholate than for glycocholate. These data are consistent with the idea that the Ca^{2+} ion has a greater affinity for the carboxylate group than for the sulfonate group. The intrinsic shifts of ^1H resonances, which are sensitive to changes in the position of the Dy^{3+} ion, decrease gradually for protons the further they are from the carboxylate end, indicating metal binding to the carboxylate group. These intrinsic shifts are unaltered in the presence of $CaCl_2$ or NaCl, suggesting that Ca^{2+} competes for binding to the anionic group and not to any other site on the bile salt molecule.

Bile salts are the most important natural detergents. They have amphiphilic properties and in aqueous solution, above their critical micellar concentration, undergo self-association, forming micellar complexes.[1,37] In bile they form mixed micellar aggregates with phospholipids and cholesterol,[2] while in the small intestines micellar aggregates of bile salts with fatty acids and glycerides occur.[3] These micellar species have a multifaceted role in the gastrointestinal system. They disperse triglycerides, enhance the activity of lipases, and solubilize fatty acids and monoglycerides, the

[†] Department of Pharmacology.
[‡] Department of Biochemistry.
[§] Division of Cardiovascular Disease.
[⊥] The Comprehensive Cancer Center.

(1) Small, D. M. In *The Bile Acids, Chemistry, Physiology and Metabolism*; Nair, P. P., Kritchevsky, D., Eds.; Plenum: New York, 1971; Vol. I, pp 249-356.
(2) Carey, M. C.; Small, D. M. *J. Clin. Invest.* **1978**, *61*, 998-1026.
(3) Hofmann, A. F. *Biochem. J.* **1963**, *89*, 57-68.

0002-7863/86/1508-7082$01.50/0