

A Study of the Possible Associations Between Cocarboxylase and Magnesium Ion as Seen in the Crystal Structure of Thiamine · Cl · HCl · $\frac{1}{2}$ [Mg(H₂O)₆]Cl · 2H₂O

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(Received 14 January 1976; accepted 30 April 1976)

An attempt to prepare a magnesium ion complex of thiamine resulted in a structure in which the magnesium ion was coordinated only to water molecules. Its closest association with thiamine is through water molecules that are hydrogen bonded to the β hydroxy oxygen on the C(5) thiazolium side chain. The thiamine molecule assumes the conformation which is characteristic of that seen when C(2) is free of substituents. The bond distances and angles in this structure are similar to those observed in other thiamine structures. Thiamine crystallizes with magnesium as triclinic crystals with space-group symmetry $P\bar{1}$, unit-cell parameters $a = 14.740$ (10), $b = 11.776$ (9), $c = 6.875$ (7) Å, $\alpha = 99.27$ (4), $\beta = 91.59$ (4), $\gamma = 107.06$ (4)° and an elemental composition of C₁₂H₁₈Cl₃Mg_{0.5}N₄OS · 5H₂O per asymmetric unit. The intensities were measured for 3419 reflections with the $\theta/2\theta$ scan technique and Cu radiation. The structure was solved by direct methods using negative quartets and refined by full-matrix least squares to a final R of 0.067 for the 3057 observed reflections.

Introduction

In studies on the effects of thiamine and magnesium deficiencies, Zieve, Doizaki & Stenroos (1968) and Zieve (1969) have shown that the metabolisms of thiamine and magnesium are interdependent and in particular that the activity of transketolase is especially sensitive to levels of thiamine and magnesium both in the deficient state and during the process of recovery.

In various studies of enzymes requiring thiamine and magnesium ion it has been observed that both thiamine pyrophosphate (cocarboxylase) and Mg²⁺ are essential for holoenzyme formation as well as catalytic activity (Morey & Juni, 1968, 1970; Schellenberger & Hübner, 1967; Wittorf & Gubler, 1970). Although it is generally believed that the metal ion binds primarily through the pyrophosphate ester moiety of the coenzyme, the suggestion has been made that there is also direct coordination to the thiamine ring system and that the metal ion activity participates in the catalytic mechanism (Schellenberger, 1967). The magnesium complex of thiamine (without the pyrophosphate ester moiety) was

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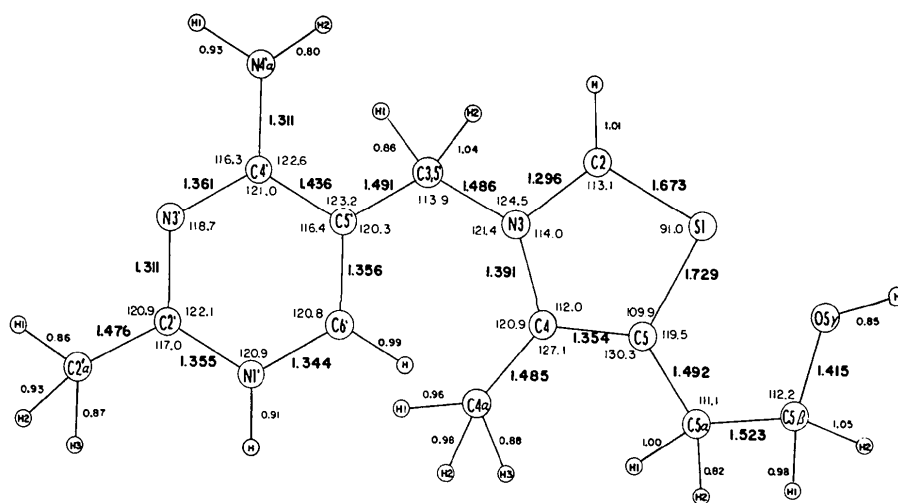


Fig. 1. Schematic representation of thiamine molecule showing the atomic numbering scheme and the interatomic distances (Å) and angles (°). The average estimated standard deviation for bonds between nonhydrogen atoms is 0.005 Å with individual values ranging from 0.004 to 0.006 Å. For angles involving nonhydrogen atoms the average e.s.d. is 0.3° with individual values in the range 0.2 to 0.4°. For bonds involving H atoms the e.s.d.'s range from 0.04 to 0.07 Å and have an average value of 0.05 Å while the e.s.d.'s for angles involving H atoms range from 2.3 to 6.4° with an average value of 3.5°.

prepared to examine the mode of interaction with the thiamine ring system and to study its influence, if any, on the conformation of the thiamine molecule.

Experimental

Equimolar amounts (1 mmol) of thiamine. Cl. HCl and magnesium chloride hexahydrate were dissolved in an aqueous solution (saturated at 25°C with respect to both components) by heating. The cooled viscous solution yielded crystals, m.p. 188–192°C. Crystallographic analysis determined the composition to be a hydrated adduct of thiamine and magnesium chloride in a 2:1 ratio. The elemental composition for one asymmetric unit requires $C_{12}H_{18}Cl_3Mg_{0.5}N_4OS \cdot 5H_2O$. Crystal data for the triclinic crystals are: space group $P\bar{1}$, $a = 14.740$ (10), $b = 11.776$ (9), $c = 6.875$ (7) Å, $\alpha = 99.27$ (4), $\beta = 91.59$ (4), $\gamma = 107.06$ (4)°, $D_m = 1.34$ g cm⁻³ (by flotation),* $D_c = 1.40$ g cm⁻³ for $Z = 2$, $V = 1122.38$ Å³; Cu $K\alpha$, $\lambda = 1.5418$ Å, $\mu = 49.7$ cm⁻¹.

A crystal with the approximate dimensions 0.6 × 0.2 × 0.1 mm was sealed in a glass capillary and the intensities were collected on a Picker FACS-1 automatic diffractometer by $\theta/2\theta$ scans at 2° min⁻¹ with monochromatic Cu $K\alpha$ radiation (graphite monochromator). Background counts of 20 s with both crystal and counter stationary were taken at the beginning and end of each scan. For the 3419 independent reflections measured within $2\theta \leq 127^\circ$, 326† were designated unobserved on the criterion that $|F|/\sigma(F) \leq 6$, where $|F| = (I/Lp)^{1/2}$, $\sigma(F) = (1/2Lp|F|)\sigma(I)$; Lp is the Lorentz-polarization factor, $I = I_T - kB$, $\sigma(I) = (I_T + k^2B)^{1/2}$, $I_T = (10S + 5)$, $k = t_1/2t_2$, $B = (b_1 + b_2)$, $b_1 = (10C_1 + 5)$, $b_2 = (10C_2 + 5)$, S = number of decacounts accumulated

* The low value for D_m was very likely caused by the loss of water of crystallization during the removal of the viscous mother liquor prior to measurement of the density.

† When the refinement had converged ($R = 0.078$, $R_{obs} = 0.074$), an analysis of the structure factors revealed a few reflections for which there was substantial disagreement between the observed and calculated values. On further inspection of the data four of these were found to have poorly determined background measurements and the remaining two were among the strongest, low-order reflections. An analysis of about 5% of the data which was composed of the reflections having the largest differences ($|F_o| - |F_c|$) indicated systematic errors. The reflections for which $F_o < F_c$ were confined to regions that corresponded to a crystal orientation for which the absorption would be greatest. When $F_o > F_c$ the reflections were restricted to crystal orientations for which absorption would be minimal. Although it was intended to remeasure some of the reflections and to calculate absorption corrections, neither of these could be done because the specimen, which had been stored at ~4°C, recrystallized in the capillary. Although an approximate description of the crystal size, shape and orientation were available, they were not defined accurately enough to warrant calculating an absorption correction. One additional cycle of refinement, in which zero weight was assigned to the 36 reflections having the largest errors, was carried out. In this refinement R dropped from 0.078 to 0.076 for all the reflections and from 0.069 to 0.067 for the 'observed' reflections alone. The shifts in parameters were small; only 13 were greater than 1.0 e.s.d., the largest being 1.42 e.s.d. for the z coordinate of O(W 1).

during the scan requiring time t_1 and C_1 and C_2 are the background decacounts accumulated in time t_2 at each end of the scan range. Three standard reflections measured periodically indicated that the crystal had undergone negligible degradation during the data collection (1% change). Following corrections for background and Lorentz-polarization, the intensities were reduced to structure amplitudes (Picker FACS-1 Disk Operating System, 1972). No corrections were applied for absorption or extinction. An approximate absolute scale was obtained from a Wilson plot (Shiono, 1971) using a composition of $C_{12}H_{18}Cl_3MgN_4OS$ based on density calculations for a 1:1 adduct with thiamine chloride.

The structure was solved* by the direct method using a single quartet relationship (Hauptman, 1974) to define the origin and three additional reflections needed for efficient expansion of the basis set to the 361 reflections with $|E|$ values less than 1.61. The successful set of starting phases with their $|E|$ values were as follows: 10,3,4, $\varphi = 0$, $|E| = 3.46$; 9 $\bar{1}$,3, $\varphi = \pi$, $|E| = 3.01$; 051, $\varphi = 0$, $|E| = 2.56$ and 1 $\bar{1}$,2, $\varphi = 0$, $|E| = 2.39$. Three additional reflections were added to the basis set with phases of either 0 or π . Of the eight possible alternative solutions expanded with *MULTAN* (Germain, Main & Woolfson, 1971), the one based on the set 8 $\bar{4}$,2, $\varphi = 0$, $|E| = 1.81$; 9 $\bar{3}$,1, $\varphi = \pi$, $|E| = 2.84$ and 4 $\bar{2}$,1, $\varphi = 0$, $|E| = 2.95$ was clearly indicated as the correct solution according to the *MULTAN* statistical parameters. An E map phased on these reflections revealed the positions of 26 of the 27 heavy atoms. In the refinement the positional parameters of the Mg ion were fixed since the Mg occupied a crystallographic center of symmetry. After convergence of the isotropic refinement at $R = 0.147$, the H atoms were located in a difference map. The structure was refined anisotropically with the B 's of the H atoms fixed at the isotropic value of the atom to which they are attached. The final R values are 0.076 for all 3419 reflections and 0.067 for the 3057 observed values.† The final atomic parameters are listed in

* The multiple solution program (Germain, Main & Woolfson, 1971) failed to produce a solution distinguishable with the usual criteria of high consistency and low R value, and inspection of the E maps failed to reveal any structure. Instead, cosine invariants $\cos(\varphi_m + \varphi_n + \varphi_p + \varphi_q)$ (Hauptman, 1974) with a high probability of being negative were used for selection of a starting set and as an additional acceptance criterion to distinguish a correctly phased set from among the multiple solutions. Cosine invariants were determined for $|E| \geq 1.4$ with the condition that $|E_{m+n}|$, $|E_{m+p}|$ and $|E_{m+q}|$ are ≤ 0.4 . Whenever possible a negative quartet was included in the starting set along with appropriate reflections to ensure propagation of phase relationships. The correctly phased set of E 's was identified among the multiple solutions produced in this fashion as the one showing the highest consistency with the sets of negative cosine invariants.

† A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31861 (21 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. The atomic numbering scheme and the interatomic distances and angles are shown in Fig. 1.

Discussion

The structure, Fig. 2, shows that the Mg ion is octahedrally coordinated to water molecules and is not directly linked to the thiamine molecule. The closest association between the two is through coordinated water molecules *W*(3) and *W*(5) which are hydrogen bonded to the thiazolium side-chain oxygen O(5 γ). The absence of direct coordination with the metal ion is in accord with other recent observations. In the crystal structures of thiamine complexes of CuCl₂ (Caira, Fazakerley, Linder & Nassimbeni, 1974), of tetrachlorodioxouranium (Clemente, Bandoli, Benetollo & Mar-

zotto, 1974) and of CdCl₄ (Richardson, Franklin & Thompson, 1975) the metal ion does not form a direct coordination with thiamine. The NMR spectra of thiamine pyrophosphate with Ni²⁺ under various conditions have been interpreted by Gallo, Hansen, Sable & Swift (1972) as indicating a direct coordination with the pyrophosphate O atoms but only a hydrogen-bond interaction between the pyrimidinium ring and a coordinated water molecule.

The three Cl ions are associated with the thiamine molecule or hydrogen bonded to water. Cl(1) accepts a hydrogen bond from N(1'), the usual site of protonation of the pyrimidine ring, and it is a hydrogen-bond acceptor from *W*(1) and *W*(4). Cl(2) accepts four hydrogen bonds, all of which are from thiamine molecules. The hydrogen bond it receives from C(2) is characteristic

Table 1. Positional and thermal parameters

Positional coordinates are designated in fractions of the unit-cell axes; the estimated standard deviations are in parentheses.

(a) Nonhydrogen atoms. Positional coordinates $\times 10^4$; thermal parameters (U_{ij}) $\times 10^3$ are coefficients for the expression:

$$\exp[-2\pi^2(h^2a^{*2}U_{11} + \dots + 2hka^*b^*U_{12} + \dots)].$$

	<i>x</i>	<i>y</i>	<i>z</i>	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S(1)	3055 (1)	2669 (1)	4890 (2)	39 (0.6)	64 (0.7)	50 (0.7)	24 (0.5)	11 (0.4)	24 (0.5)
C(2)	1941 (3)	2696 (4)	5232 (6)	45 (2)	52 (2)	45 (2)	19 (2)	11 (2)	18 (2)
N(3)	1687 (2)	3429 (3)	4275 (4)	32 (2)	35 (2)	33 (2)	8 (1)	4 (1)	4 (1)
C(4)	2391 (3)	4039 (3)	3184 (5)	34 (2)	34 (2)	35 (2)	5 (1)	4 (1)	3 (1)
C(4 α)	2218 (3)	4923 (4)	2015 (7)	41 (2)	44 (2)	65 (3)	11 (2)	7 (2)	19 (2)
C(5)	3194 (3)	3713 (3)	3333 (5)	38 (2)	42 (2)	39 (2)	10 (2)	3 (2)	7 (2)
C(5 α)	4070 (3)	4018 (4)	2246 (7)	37 (2)	49 (2)	62 (3)	10 (2)	13 (2)	15 (2)
C(5 β)	3862 (3)	3436 (4)	72 (7)	56 (3)	53 (2)	60 (3)	17 (2)	25 (2)	14 (2)
O(5 γ)	3414 (2)	2176 (3)	-187 (5)	64 (2)	52 (2)	74 (2)	20 (1)	22 (2)	5 (2)
C(3,5')	719 (3)	3576 (3)	4212 (5)	35 (2)	34 (2)	42 (2)	12 (1)	5 (1)	6 (1)
N(1')	-897 (2)	2494 (3)	8016 (5)	37 (2)	44 (2)	40 (2)	12 (1)	10 (1)	5 (1)
C(2')	-1291 (3)	1306 (3)	7265 (6)	35 (2)	45 (2)	49 (2)	13 (2)	6 (2)	8 (2)
C(2' α)	-2003 (3)	594 (4)	8418 (7)	55 (2)	51 (2)	62 (3)	14 (2)	19 (2)	15 (2)
N(3')	-1062 (2)	802 (3)	5585 (5)	38 (2)	39 (2)	47 (2)	10 (1)	7 (1)	6 (1)
C(4')	-421 (2)	1504 (3)	4548 (5)	31 (2)	35 (2)	43 (2)	12 (1)	4 (1)	5 (2)
N(4' α)	-238 (2)	965 (3)	2849 (5)	52 (2)	38 (2)	49 (2)	6 (1)	12 (2)	-1 (1)
C(5')	32 (2)	2761 (3)	5310 (5)	32 (2)	36 (2)	39 (2)	13 (1)	3 (1)	4 (1)
C(6')	-233 (3)	3205 (3)	7074 (6)	33 (2)	40 (2)	45 (2)	10 (1)	5 (2)	2 (2)
Cl(1)	-1351 (1)	3775 (1)	2045 (1)	40 (0.6)	49 (0.6)	47 (0.6)	12 (0.4)	6 (0.4)	-2 (0.4)
Cl(2)	1196 (1)	1800 (1)	-460 (1)	54 (0.6)	38 (0.6)	35 (0.5)	6 (0.4)	8 (0.4)	3 (0.4)
Cl(3)	-4464 (1)	2751 (1)	5733 (2)	54 (0.7)	61 (0.7)	51 (0.7)	12 (0.6)	4 (0.5)	-2 (0.5)
O(<i>W</i> 1)	-2727 (2)	1801 (3)	4344 (6)	57 (2)	59 (2)	87 (2)	17 (2)	-4 (2)	14 (2)
O(<i>W</i> 2)	-4215 (3)	1695 (3)	-442 (6)	102 (3)	50 (2)	97 (3)	-13 (2)	-40 (2)	27 (2)
O(<i>W</i> 3)	-5579 (3)	827 (5)	2262 (8)	52 (2)	134 (4)	116 (4)	1 (2)	15 (2)	-66 (3)
O(<i>W</i> 4)	-3490 (3)	3922 (3)	2038 (5)	61 (2)	85 (2)	66 (2)	29 (2)	7 (2)	17 (2)
O(<i>W</i> 5)	-3930 (3)	-102 (3)	1857 (7)	72 (2)	46 (2)	118 (3)	20 (2)	-40 (2)	-2 (2)
Mg	-5000	0	0	37 (1)	32 (1)	53 (1)	9 (1)	-1 (1)	2 (1)

(b) Hydrogen atoms. All values $\times 10^3$. Thermal parameters are coefficients for the expression: $\exp[-(8\pi^2U)\sin^2\theta/\lambda^2]$.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i>		<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i>
H(2)	157 (3)	219 (4)	616 (6)	45	H(2' α 3)	-225 (3)	109 (5)	911 (7)	57
H(4 α 1)	196 (3)	548 (4)	282 (6)	44	H(4' α 1)	19 (3)	134 (4)	201 (7)	46
H(4 α 2)	281 (3)	537 (4)	152 (6)	44	H(4' α 2)	-55 (3)	28 (4)	245 (6)	46
H(4 α 3)	179 (3)	462 (4)	102 (6)	44	H(6')	3 (3)	408 (4)	765 (6)	37
H(5 α 1)	459 (3)	374 (4)	279 (7)	48	H(<i>W</i> 11)	-240 (4)	236 (5)	353 (8)	64
H(5 α 2)	427 (3)	475 (4)	227 (6)	48	H(<i>W</i> 12)	-327 (4)	205 (5)	472 (8)	64
H(5 β 1)	448 (3)	356 (4)	-48 (7)	57	H(<i>W</i> 21)	-443 (4)	174 (6)	-144 (9)	79
H(5 β 2)	346 (3)	380 (5)	-79 (7)	57	H(<i>W</i> 22)	-402 (4)	232 (6)	37 (9)	79
H(5 γ)	282 (3)	207 (5)	-24 (7)	59	H(<i>W</i> 31)	-626 (5)	86 (7)	187 (11)	104
H(3,5'1)	83 (3)	431 (4)	481 (6)	37	H(<i>W</i> 32)	-511 (5)	118 (7)	347 (11)	104
H(3,5'2)	49 (3)	345 (4)	272 (6)	37	H(<i>W</i> 41)	-285 (4)	389 (5)	213 (8)	66
H(1')	-107 (3)	290 (4)	911 (6)	37	H(<i>W</i> 42)	-366 (4)	377 (5)	314 (8)	66
H(2' α 1)	-192 (3)	-10 (4)	846 (7)	57	H(<i>W</i> 51)	-366 (4)	-52 (5)	142 (8)	72
H(2' α 2)	-260 (3)	31 (5)	773 (7)	57	H(<i>W</i> 52)	-355 (4)	46 (5)	232 (8)	72

of thiamine structures and is consistent with the acidic nature of the thiazolium C(2) proton (Krampitz, 1969). This Cl ion also forms an intramolecular bridge by accepting a hydrogen bond from both N(4' α) on the pyrimidinium ring and O(5 γ) on the thiazolium ring. This is a feature that has now been observed in many thiamine compounds which are found in the characteristic ring conformation with the pyrimidine amino and the thiazolium C(5) side chain *syn*-related.* The bridging in the structure containing Cu(Cl₄)²⁺ is nearly identical with that observed here even though the Cl atoms in that structure are coordinated with the Cu atom, whereas here they are free ions, not coordinated to Mg. Cl(3) accepts hydrogen bonds from W(1), W(2), W(3) and W(4). In addition it is in contact with the thiazolium S atom. Although the S contact distance at 3.657 Å is equal to the sum of the van der Waals radii, the association is significant because the Cl ion lies in the plane of the thiazolium ring along the direction of the C(2)–S(1) bond (Pulsinelli, 1970). This tendency for the thiazolium sulfur to associate with negative ions or electronegative atoms is a characteristic of thiamine structures which is of particular significance in the C(2) adduct compounds (Sax, Pulsinelli & Pletcher, 1974).

The bond lengths and angles shown in Fig. 1 are in generally good agreement with those observed for other thiamine structures, although there is one that requires comment. The bond to the pyrimidine methyl group, C(2')–C(2' α), at 1.476 Å, is significantly shorter (5 σ) than expected for an *sp*²–*sp*³ single bond (Lide, 1962; Dewar & Schmeising, 1960) even though some of the shortening is undoubtedly the result of thermal motion. This short bond is similar to that found in the structure of TPP.HCl (Pletcher & Sax, 1972) where the methyl group was situated in an environment of electronegative atoms, some at distances less than the sum of van der Waals radii. In this structure also (Fig. 2),

the C(2' α) methyl group is surrounded by the O atoms of water and O(5 γ) and by the chloride ion Cl(2) which is closer than the van der Waals distance (Table 2). In a structure such as thiamine.Cl.H₂O (Pletcher, Sax, Sengupta, Chu & Yoo, 1972) where the C(2' α) methyl group is not surrounded by electronegative atoms, this C–C bond has a value (not corrected for thermal motion) of 1.496 (4) Å.

The relative conformation of the thiazolium and pyridinium rings, which is described by the torsion angles (ϕ_T and ϕ_P) about the bonds to methylene C linking the two ring systems, is similar to that usually found in thiamine structures unsubstituted at C(2). [These angles and their reference values are defined in Sax, Pulsinelli & Pletcher (1974) and in Pletcher & Sax (1972).] In spite of the substantial apparent freedom of rotation about the bonds to the methylene bridge C, stable conformations as observed in crystal structure analysis are confined to a narrow range of these torsion angles. The principle conformation, which has been observed for thiamine when C(2) is unsubstituted, is defined by the parameters $\phi_T \simeq 0^\circ$, $\phi_P \simeq (\pm) 90^\circ$ and is hereinafter designated by F. For this structure $\phi_T = -2.0$ and $\phi_P = -77.4^\circ$. The other conformation observed is that defined by $\phi_T \simeq (\pm) 100^\circ$, $\phi_P = (\pm) 150^\circ$ and is hereinafter designated by S. S was first observed in a structure of thiamine which contained a substituent on the C(2) position.* Adding the substituent increases the energy of F relative to S. Jordan (1974) has drawn attention to the fact that a relatively low energy path exists between the F conformations which differed by 180° in ϕ_P (*i.e.* $\phi_T \simeq 0$, $\phi_P \simeq 90$ and $\phi_T \simeq 0$, $\phi_P \simeq -90$). Of perhaps even greater significance is the fact that there is a low-energy path between the two major conformations, F and S. In fact, the path between F+ and F– always passes through at least two of the S conformations. The F conformation has now been observed in 11 of the 12 crystal structures† containing

* When this conformational aspect was initially observed in TPP.HCl (Pletcher & Sax, 1966, 1972), the C(5) side chain and the pyrimidine amino group were described as *cis*-related when directed toward the same side of the thiazolium ring and *trans*-related when directed towards opposite sides. Since the groups in these two respective arrangements are more appropriately described as being *syn*- and *anti*-related, this latter terminology has been adopted.

* The *N*-benzylthiazolium.Br structure (Power, Pletcher & Sax, 1970) had previously been observed to have the S conformation also, but the absence of substituents on the phenyl ring (especially the 4'-NH₂ group) results in less restrictive rotational parameters.

† Thiamine, as the tetrachlorocadmate salt, has recently been observed (Richardson, Franklin & Thompson, 1975) in the S conformation.

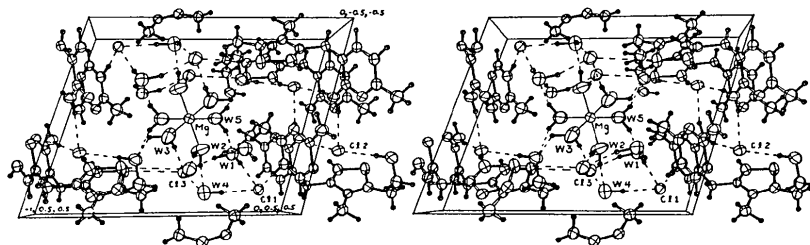


Fig. 2. Stereoscopic (Johnson, 1965) packing diagram of unit cell viewed along the *c* axis. The thiamine molecule with the solid bonds and the labeled ions and water molecules correspond to those atoms whose coordinates are listed in Table 1. The hydrogen-bonding scheme is depicted by the dashed lines.

Table 2. Selected distances (Å) and angles (°)

(a) Hydrogen bonds

<i>a</i>	<i>b</i>	<i>c</i>	<i>a-b</i>	<i>b-c</i>	<i>a-c</i>	$\angle a-b-c$
C(2)—H(2)·····Cl(2) ⁱⁱⁱ			1.01	2.49	3.406	151.7
O(5 γ)—H(5 γ)·····Cl(2) ⁱ			0.85	2.31	3.164	178.8
N(4' α)—H(4' α 1)·····Cl(2) ⁱ			0.93	2.31	3.222	166.4
N(4' α)—H(4' α 2)·····Cl(2) ⁱⁱ			0.80	2.50	3.278	164.3
N(1')—H(1')·····Cl(1) ⁱⁱⁱ			0.91	2.22	3.123	171.2
O(W1)—H(W11)·····Cl(1) ⁱ			0.96	2.31	3.257	168.6
O(W1)—H(W12)·····Cl(3) ⁱ			0.95	2.24	3.193	175.9
O(W2)—H(W21)·····Cl(3) ^{iv}			0.76	2.44	3.140	152.9
O(W2)—H(W22)·····O(W4) ⁱ			0.82	1.97	2.782	172.7
O(W3)—H(W31)·····O(5 γ) ^{vi}			1.04	2.40	3.108	124.4
O(W3)—H(W32)·····Cl(3) ⁱ			1.01	2.17	3.063	145.8
O(W4)—H(W41)·····Cl(1) ⁱ			0.95	2.26	3.207	174.7
O(W4)—H(W42)·····Cl(3) ⁱ			0.83	2.45	3.253	162.2
O(W5)—H(W51)·····O(5 γ) ⁱⁱ			0.75	2.14	2.845	157.9
O(W5)—H(W52)·····O(W1) ⁱ			0.75	2.00	2.712	161.0

(b) Close contacts and C(2' α)-methyl nearest neighbors

C(2)—S(1)·····Cl(3) ^v	1.673	3.658	—	163.1
C(5)—S(1)·····Cl(3) ^v	1.728	3.658	—	99.8
C(2' α)—H(2' α 1)·····Cl(2) ^{vii}	0.86	3.02	3.805	152.6
C(2' α)—H(2' α 2)·····O(W1) ⁱ	0.93	3.17	3.607	110.9
C(2' α)—H(2' α 2)·····O(W3) ^{viii}	0.93	2.62	3.447	148.0
C(2' α)—H(2' α 3)·····O(W3) ^{viii}	0.87	3.34	3.447	89.9
C(2' α)—H(2' α 3)·····O(W5) ⁱⁱⁱ	0.87	3.28	3.737	115.7
C(2' α)—H(2' α 1)·····O(5 γ) ^{vii}	0.86	3.19	3.725	122.6
C(2' α)—H(2' α 2)·····O(W2) ⁱⁱⁱ	0.93	3.42	3.902	114.8
C(2' α)—H(2' α 3)·····O(W2) ⁱⁱⁱ	0.87	3.19	3.902	141.2
C(2' α)—H(2' α 3)·····Cl(1) ⁱⁱⁱ	0.87	3.34	3.991	134.1

(c) Magnesium coordination

O(W2)—Mg—O(W3) ⁱ	2.068	2.042	2.856	88.0
O(W2)—Mg—O(W5) ⁱ	2.068	2.045	2.952	91.7
O(W3)—Mg—O(W5) ⁱ	2.042	2.045	2.947	92.3

Symmetry code

(i)	<i>x</i>	<i>y</i>	<i>z</i>	(v)	<i>x</i> +1	<i>y</i>	<i>z</i>
(ii)	− <i>x</i>	− <i>y</i>	− <i>z</i>	(vi)	<i>x</i> −1	<i>y</i>	<i>z</i>
(iii)	<i>x</i>	<i>y</i>	<i>z</i> +1	(vii)	− <i>x</i>	− <i>y</i>	− <i>z</i> +1
(iv)	<i>x</i>	<i>y</i>	<i>z</i> −1	(viii)	− <i>x</i> −1	− <i>y</i>	− <i>z</i> +1

thiamine which is unsubstituted at C(2). Both F⁺ and F[−] appear in each of the crystal structures since they all crystallize with centrosymmetric space-group symmetry; they must also be in equilibrium in solution. Although it has been suggested that the F conformation is stabilized by packing forces, this does not seem a very likely possibility in view of the variety of packing modes and intermolecular interactions observed in the 11 crystal structures. A more reasonable premise is that F is the stable conformation.

The Mg ion exhibits distorted octahedral coordination. The geometrical details of the hydrated Mg ion are given in Table 2(c). The values found in this structure compare reasonably well with those reported by Whitaker & Jeffery (1970) although the Mg—O bonds tend to be somewhat shorter. The average Mg—O distance in this structure (2.052 Å) is at the low end of the range of average values (2.062–2.083 Å) for hydrated Mg ions tabulated by Whitaker & Jeffery (1970).

We wish to acknowledge the help of the Computer Center of the University of Pittsburgh which provided

the computer time for this study. This study was supported in part by NIH grant AM 18898.

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A Method to Determine Heavy-Atom Positions for Virus Structures

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(Received 6 January 1976; accepted 10 May 1976)

Small spherical virus particles consist of multiples of 60 chemically identical protein subunits related by icosahedral symmetry. If the orientation of the virus has been previously established, then Patterson self-vectors can be calculated between heavy atoms attached specifically to equivalent points in each subunit. A systematic search of all reasonable positions within the subunit and a comparison with the known difference Patterson map would thus establish heavy-atom positions relative to the particle center. This method was used successfully on two heavy-atom derivatives of satellite tobacco necrosis virus [Lentz, Strandberg, Unge, Vaara, Borell, Fridborg & Petef, *Acta Cryst.* (1976), **B32**, 2979–2983].

Introduction

Low-resolution X-ray diffraction data are now available for a number of small spherical viruses, such as turnip yellow mosaic virus (Klug, Longley & Leberman, 1966), tomato bushy stunt virus (TBSV; Harrison, 1971), satellite tobacco necrosis virus (STNV; Åkervall *et al.*, 1971), and southern bean mosaic virus (SBMV; Johnson, Rossmann, Smiley & Wagner, 1974). Data extension to atomic resolution is presently in progress. The solution of the phase problem will depend upon the molecular replacement technique (Rossmann, 1972; Bricogne, 1974; Argos, Ford & Rossmann, 1975), the isomorphous replacement technique, or both. Heavy-atom derivatives have been prepared and analyzed for TBSV (Harrison & Jack, 1975), STNV (Lentz *et al.*, 1976), and SBMV (Wagner, 1974).

The presence of several heavy-atom sites within the crystallographic asymmetric unit has, in general, been

a limitation to the isomorphous replacement technique. The interpretation of the TBSV heavy-atom difference Patterson synthesis was a complex task (Harrison, 1971; Harrison & Jack, 1975). Yet when heavy-atom sites are related by non-crystallographic symmetry as in virus structures, the problems of interpreting difference Patterson syntheses can be lessened as only those sites in the non-crystallographic asymmetric unit need be determined. Argos & Rossmann (1974) have shown the feasibility of solving systematically a difference Patterson synthesis for a tetrameric protein with 222 molecular symmetry. Their methods have now been extended for use in virus structures with 532 symmetry in the asymmetric unit, resulting in 60 non-crystallographic symmetry-related subunits in the crystal unit cell. Such viruses have $T=1$ symmetry (Caspar & Klug, 1962), the simplest of all situations. One virus with such simplicity is STNV and the subsequent paper (Lentz *et al.*, 1976) describes the success of the method for the STNV data.