Theoretical Calculations on Thiamine and Related Compounds. II. Conformational Analysis and Electronic Properties of 2-(α-Hydroxyethyl)thiamine[1]

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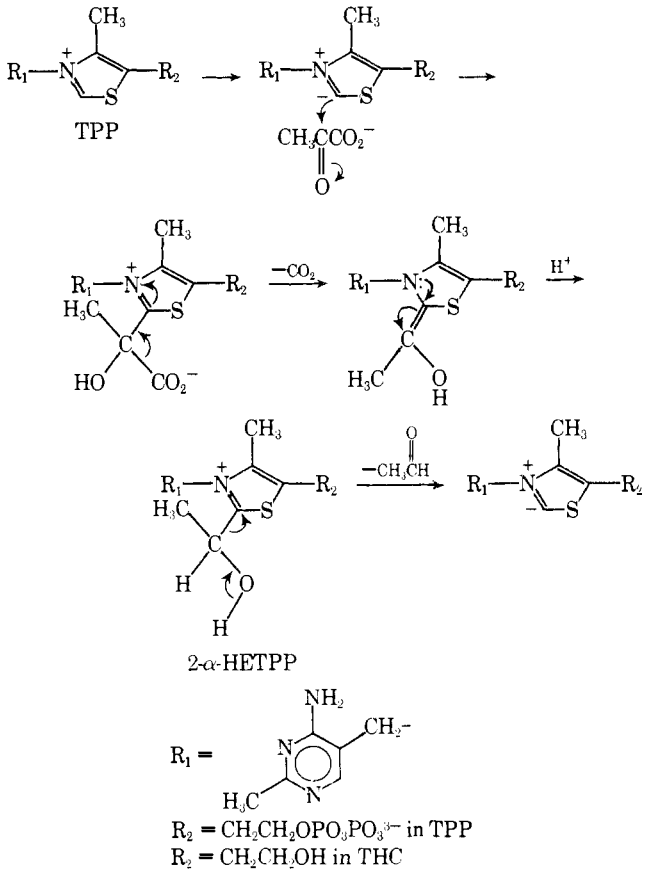
Abstract: Semiempirical potential energy maps were constructed for the definition of the relative disposition of the thiazolium and 4-aminopyrimidine rings in 2-(α-hydroxyethyl)thiamine, a model for the enzymatically decarboxylated intermediate of pyruvate. A comparison of the conformational maps with and without the substituent on the C(2) position of the thiazolium ring indicates that the C(2) substituent greatly decreases the conformational flexibility of the two aromatic rings. Furthermore, the side chain on C(2) has a substantial barrier to rotation around the C(2)–C_α bond, and the conformational region found for this molecule by x-ray crystallography is preferred energetically. Theoretical (extended Hückel and Gaussian STO-3G ab initio) charge densities indicate the thiazolium ring sulfur atom to be positive and the nitrogen neutral or slightly negative (in spite of the formal positive charge on this position).

The isolation of covalently bound stable coenzyme–product or coenzyme–substrate intermediates affords a unique opportunity for observing the changes in coenzyme structure with bound substrate or product.

The thiamine pyrophosphate catalyzed enzymic decarboxylation of pyruvate proceeds according to Scheme I.[2] 2-

Scheme I. Mechanism of Pyruvate Decarboxylation



(α-Hydroxyethyl)thiamine (2-α-HETHC) can be prepared in the laboratory.[3] Its solid-state structure has been determined by x-ray crystallography.[4]

The comparison of the x-ray results on THC,[5] TPP,[6] and 2-α-HETHC allows one to draw some conclusions concerning changes in the coenzyme conformation upon covalently binding the product.

Theoretical studies can add some insight to the problem of enzymatic reactions. Recently I reported on aspects of the electronic structure of THC and TPP[7] and semiempirical conformational maps for the disposition of the two aromatic rings (4-aminopyrimidine and thiazolium) with respect to each other.

In this report results on the conformational maps of 2-α-HETHC will be discussed and compared with maps on THC with respect to the conformational problem discussed above, and the electronic structure of 2-α-HETHC will be presented.

Theoretical Approaches. The results to be discussed were obtained employing a variety of theoretical approaches, this variety being necessitated by the size of the problem. The electronic charges on 2-α-HETHC were first determined employing the extended Hückel (EH) theory[8] with parameters described in the previous calculation.[7]

The conformational maps were generated using a function[7,9]

$$E_{\text{total}} = E_{\text{Lennard-Jones}} + E_{\text{Coulombic}} \qquad (1)$$

where the Lennard-Jones (6–12) potential was employed for nonbonding interaction calculations supplemented by the Coulombic interaction energy. The latter was based on net atomic charges, $E_{\text{Coulombic}} = kq_iq_j/\epsilon r_{ij}$, where k is 332 kcal/mol, q_i and q_j are net atomic charges, and r_{ij} is the distance separating the atoms. For lack of any better numbers a dielectric constant, ϵ, of unity was assumed. This would lead to a maximal electrostatic interaction term. In any real situation with a larger dielectric constant, the Coulombic contribution would be smaller.

The torsional energy contribution was neglected. It was reasoned before[7] that such contribution to the rotation of aromatic rings around the $-CH_2-$ group should be less than 1 kcal/mol. Considering the uncertainty inherent in the use of the potential function in eq 1, the uncertainty of 1 kcal/mol is probably unimportant.

One should preface the discussion of the conformational maps with some very important qualifications. In this study, as the rotations were performed, all interactions between nonbonded atoms (atoms separated by two bonds or more) were summed up and only energy differences from the most stable conformer are quoted. Thus those interactions that

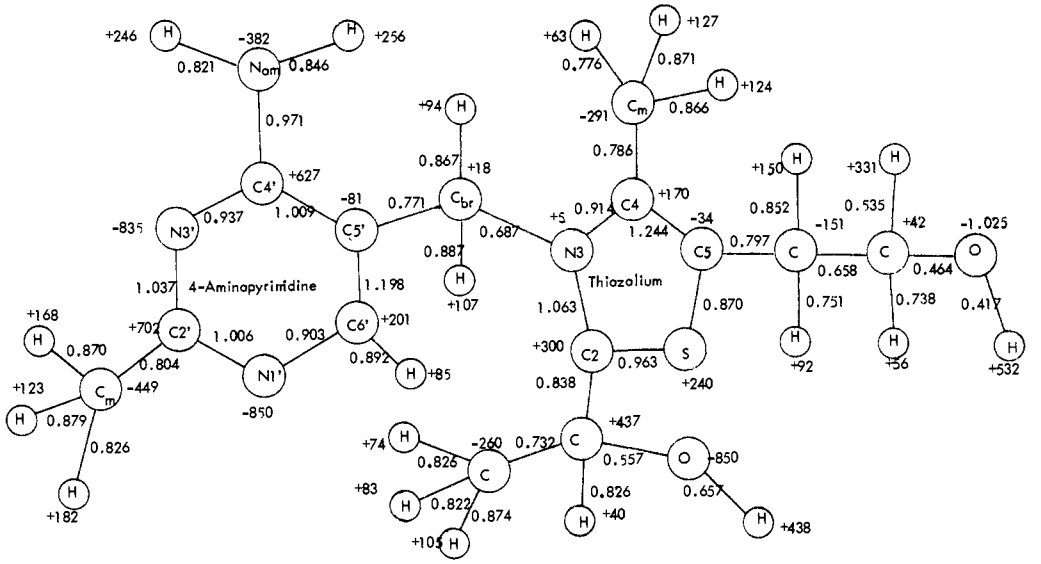
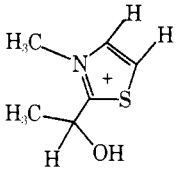


Figure 1. Extended Hückel population analysis on 2-α-HETHC: net charges (×1000) and bond overlap populations.

remain unchanged cancel. The other point which needs clarification is that in the Lennard-Jones (LJ) calculations this study employed contact distances which are usually considered "normal".[10] Depending on the atomic van der Waals radii assumed, the shape of the contours may well change and, in addition, the importance of the Coulombic contribution may change. All this adds up to the conclusion that such conformational maps are helpful in pinpointing very high-energy and relatively low-energy regions. The absolute energy differences, for example, between two low-energy regions should not be taken as proven. This qualification is particularly relevant to the uncertainty regarding contact distances to hydrogen atoms.[10]

In order to check some extended Hückel results on charges and some relative conformational preferences of the 2-(α-hydroxyethyl) side chain, some ab initio results will also be discussed on ion A indicated below



A

(the smallest structural unit incorporating this problem). These calculations were performed in the Gaussian-70 scheme[11] with the STO-3G minimal basis set.[12]

Electronic Structure of 2-(α-Hydroxyethyl)thiamine and of 2-(α-Hydroxyethyl)-3-methylthiazolium Ion. Figure 1 presents the extended Hückel population analysis (net atomic populations and bond overlap populations in the Mulliken scheme[13]) on the 2-α-HETHC ion.

The results can be compared to those on the C(2)-unsubstituted THC and TPP systems (Figures 5 and 8, respectively, in ref 7). Quite clearly there is no difference in the charge of the C(2)-H in THC (or TPP) and the total charge on the α-hydroxyethyl side chain of the present study. Yet there is substantial change in the thiazolium ring net charges. Attaching the α-hydroxyethyl group makes S less positive (+0.24 from +0.30), C(2) more positive (+0.30 from +0.15), N less positive (+0.005 from +0.050), C(4) unchanged, and C(5) negative (−0.034 from −0.000). Thus the α-hydroxyethyl group clearly polarizes the thiazolium ring charges. Comparison of chemical shifts of the appropriate carbons in 2-α-HETHC and THC (or TPP) confirms these results. The chemical shift of C(2) is 155 ppm (Me_4Si) in THC and TPP[14] and 179 ppm in 2-α-HETHC.[15]

Of very great interest are the charges at N and S in the thiazolium ring. There is very little evidence from x-ray data indicating chelation of negatively charged species to N,[16] but there is abundant evidence that negative ions electrostatically interact wtih the S atoms.[4,16]

Since any calculated results may be subject to criticism on account of the results being parameter dependent, ab initio calculations were also performed on the smallest model compound incorporating the most structural features of the 2-α-HETHC system (see structure A).

2-(α-Hydroxyethyl)-3-methylthiazolium (2-α-HEMTHZ). The geometry assumed was identical with the one found in 2-α-HETHC with the third hydrogen on the *N*-methyl group, the hydrogens at C(4) and C(5) constructed at 1.09-Å bond length along the C_{br}–C(5′), C(4)–C_m, C(5)–C(6) directions, respectively (see Figure 1 for the numbering system).

The population analysis on this structure is indicated in Figure 2. While the extended Hückel results on the net atomic charges in 2-α-HETHC are quantitatively different from the ab initio values on 2-α-HEMTHZ, the trends in charges are the same according to the two methods. The ring nitrogen is nearly neutral in 2-α-HETHC (EH) and −0.19 charged in 2-α-HEMTHZ (ab initio). In neither case is it positively charged. In this connection one may note that in a number of other ions in which N carries a formal + charge (*N*-methylpyridinium[17] and CH_3-$CH{=}N^+H_2$, $CH_3NH{=}CH_2{}^+$[18]), the nitrogen is also found to be slightly negative according to the ab initio results. It should be emphasized that the quoted values in each case are net atomic populations including overlap populations.[13] The sulfur atom is calculated to have a substantial fractional positive charge in both systems. While the EH method employs d orbitals, the STO-3G ab initio scheme does not; yet the qualitative agreement is present. The positive character of S in numerous x-ray studies has been suggested by various groups.[4,16] The calculations support this conclusion.

Unpublished work of Gallo and Sable indicates that the C(2) has an unusually large chemical shift value reminiscent of a carbonyl carbon in its carbon magnetic resonance spectrum.[15] This would tend to imply the C–N bond polarity as $^{\delta+}C$–$N^{\delta-}$, i.e., the N much less positive (or more nega-

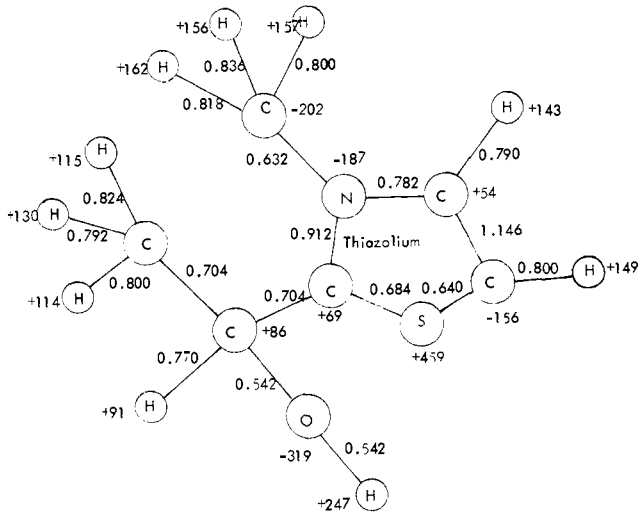


Figure 2. *STO-3G* population analysis on 2-(α-hydroxyethyl)-3-methylthiazolium ion: net charges (×1000) and bond overlap populations.

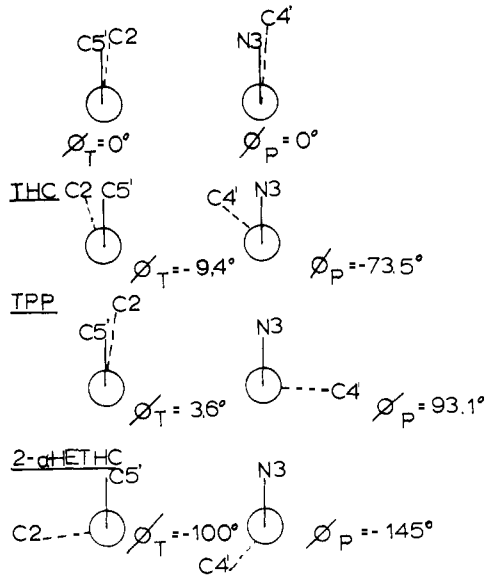


Figure 3. Definition of ϕ_T and ϕ_P dihedral angles.

tive) than C(2). While the EH and ab initio results would go along with this, the previously reported CNDO/2 results (Figure 3, ref 7) are not in accord with this observation.

Clearly, the results here presented would allow for an electrostatic stabilization of that 2-(α-hydroxyethyl) sidechain conformation which places the OH group such that O and S are as close as possible to each other (since the O is strongly negative and S is positive). However, the overlap population is very near zero according to ab initio and −0.006 according to EH so that the long-range bonding interaction is not indicated according to this criterion. On the other hand, even in the C(2) deprotonated ylide (Figures 4 and 7 in ref 7) the sulfur is positive [and C(2) is negative]. The $^{\delta-}$C(2)-S$^{\delta+}$ bond polarity may be important in aligning the $^{\delta+}$C=O$^{\delta-}$ dipole of the reactant for nucleophilic attack by C(2) on the substrate carbonyl carbon atom in Scheme I.

Conformational Maps for the Disposition of the Two Aromatic Rings in α-HETHC. In the following discussion the definitions proposed by Sax et al.[4] and also adopted by Richardson et al[16] will be employed.

As illustrated in Figure 3 two dihedral angles can be defined by two sets of four atoms, ϕ_T [C(5′)-C_{br} → N(3)-C(2)] and ϕ_P [N(3)-C_{br} → C(5′)-C(4′)], such that rotation around the single bond connecting atoms 2 and 3 is performed. Positive values of the dihedral angles are those which require clockwise rotation of atom 1 (and its environment) to totally eclipse atom 1 with atom 4 (the eclipsed arrangement of atoms 1 and 4 in either group is $\phi_T = \phi_P = 0$).

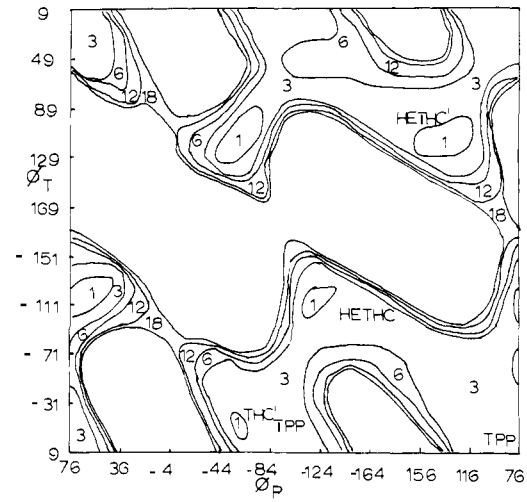


Figure 4. LJ potential map for ϕ_T and ϕ_P rotations in THC.

In this laboratory counterclockwise, 10° incremental rotations were performed around the N → C_{br} bond and then around the C_{br} → C(5′) bond, always rotating the groups attached to the latter atom.

The potential maps are presented for THC in Figure 4 (same as Figure 10 in ref 7) with the convention of ϕ_T and ϕ_P incorporated into the Lennard-Jones (LJ) map for direct comparison with data on 2-α-HETHC (Figures 5 and 6). The conformational angles defining TPP and 2-α-HETHC are also shown. It appears that in the crystal structures of THC, TPP, and α-HETHC both mirror image structures are present. The conformational angles found were THC,[5] ϕ_T = −9.0°, ϕ_P = −76.1° (or +9.0, 76.1); TPP,[6] ϕ_T = 2.7°, ϕ_P = 93.2 (or −2.7, −93.2); and 2-α-HETHC,[4] ϕ_T = −100.3, ϕ_P = −145.6 (or +100.3, +145.6).

LJ map on THC (Figure 4) shows that all six angle combinations are located in relatively low-energy regions (3 kcal or less from the minimum) of the map and, in addition, interconversion of any of these conformations should proceed with very little rotational barrier.

The map for 2-α-HETHC was constructed starting from the crystallographic coordinates.[4] The 2-(α-hydroxyethyl) side chain was assumed to be fixed and the ϕ_T and ϕ_P rotations were performed with only the crystallographic arrangement of the α-hydroxyethyl side chain. The maps were constructed both with and without inclusion of the Coulombic (qq) interaction term. Figures 5 and 6 present the maps without (LJ only) and with inclusion of the Coulombic interaction, respectively. The two enantiomeric sets of ϕ_T and ϕ_P angles found in the crystal structure of 2-α-HETHC are shown to again lie in relatively low-energy regions, but the conformational parameters characteristic of unsubstituted THC and TPP this time fall into high-energy regions (regions at least 18 kcal/mol above the minimum energy conformers).

The clear implication is that the coenzyme conformation, upon binding pyruvate or acetaldehyde at C(2) of the thiazolium ring, is much more restricted than the unsubstituted one.

(−)-2-(α-Hydroxyethyl)-TPP can be trapped and isolated in the enzymic decarboxylation process.[19] The conver-

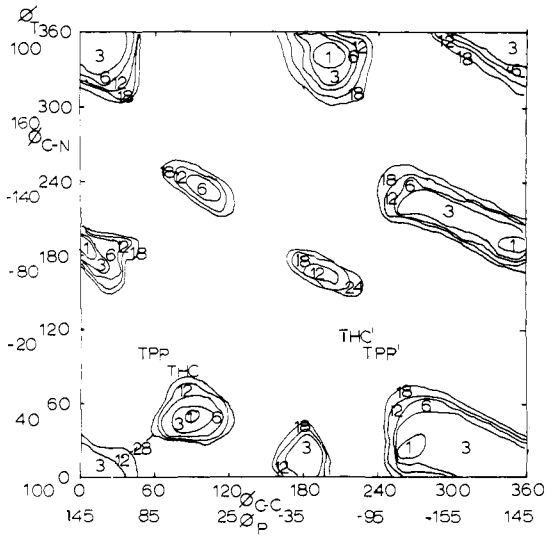


Figure 5. LJ potential map for ϕ_T and ϕ_P rotations in 2-α-HETHC.

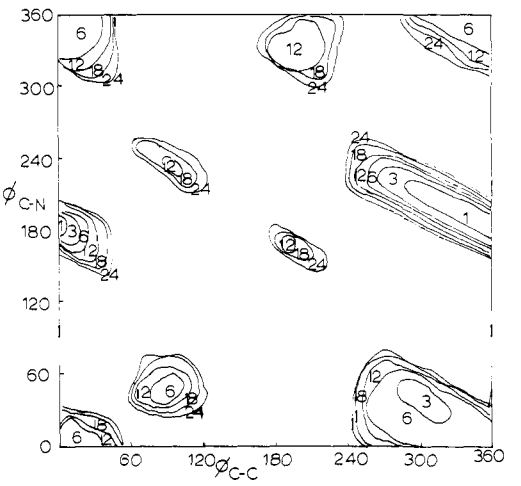


Figure 6. Total energy (LJ + Coulombic) map for 2-α-HETHC; ϕ_T and ϕ_P angles identical with those in Figure 5.

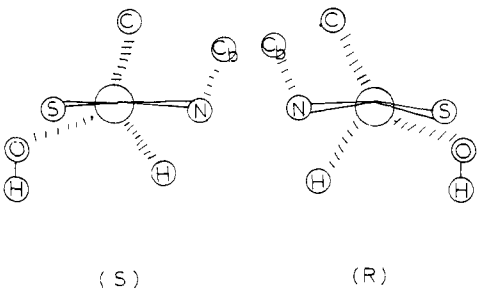


Figure 7. Conformation and configurations of the 2-(α-hydroxyethyl) side chain.

sion of this (−)-α-HETPP to acetaldehyde and TPP by apoenzyme is much slower than the conversion of pyruvate by holoenzyme saturated with TPP; i.e., α-HETPP combines only very poorly with apoenzyme.[19] This may imply that the enzyme accepts the coenzyme TPP in a conformation different from the one found in (−)α-HETPP. The x-ray studies and results here reported suggest that before (or perhaps concurrently with) binding of pyruvate (or acetaldehyde) to TPP there is a conformational change occurring in the coenzyme as well as in the enzyme, in the latter if only to accommodate such changes in the coenzyme.

Inclusion of the Coulombic interactions does not change the maps significantly; their exact role is difficult to assess on account of the uncertainty with respect to the dielectric constant. In any case as a comparison of Figures 5 and 6 suggest, the inclusion of the Coulombic monopole–monopole interactions in the conformational map does not qualitatively change the appearance of the map.

While most thiamine derivatives studied to date fall into the range of conformations similar to that of THC and TPP, the THC–$CdCl_4$ complex[16] has a THC conformation (ϕ = 137, ϕ_T = 110) very similar to that of 2-α-HETHC. As Figure 4 indicates this complex is still in a low-energy region.

Conformation of the 2-(α-Hydroxyethyl) Side Chain. The calculations were performed on 2-(α-hydroxyethyl)-3-methylthiazolium ion (2-α-HEMTHZ). First the *N*-methyl group was rotated with 10° rotational increments in a counterclockwise sense. The results (inspection of closest contacts) indicate that the nearest interactions occur between the *N*-methyl and β-methyl hydrogens but the distances never get smaller than about 2.1 Å for nonbonded hydrogens. Because of the uncertainty associated with the definition of the hydrogen van der Waals radius in various environments, these contact distances imply no serious H–H repulsions. Since one need not vary the *N*-methyl and 2-(α-hydroxyethyl) conformations simultaneously, the *N*-methyl group was assumed to be fixed in the arrangement of the C_{bridge} environment found in 2-α-HETHC.[4]

Next, with this crystallographic arrangement of the *N*-methyl group (derived from 2-α-HETHC), the α-hydroxyethyl group was rotated counterclockwise around the C(2)–C(α) bond in the *R* absolute configuration of the molecule (see Figure 7). In order to see if any rotational barriers are due to unfavorable repulsions with hydrogens, both 1.0 and 1.2 Å were used for van der Waals radii of hydrogen in the LJ type calculation (Figures 8 and 9). The ab initio charges obtained on the crystallographic conformation of the 2-(α-hydroxyethyl) group were employed in calculating Coulombic interactions with a dielectric constant of one.

There are two major barriers for rotating the 2-(α-hydroxyethyl) group and two nearly equal energy minima. However, the minimum near the crystallographic conformation is considerably broader than the other one. Statistically, the distribution of conformations in the broader minimum range should be very much greater than in the steep valley at rotation angle 150° counterclockwise from the crystallographic one. In addition, however, the barrier to conformational interconversion appears to be very high even with the lower (1 Å) hydrogen van der Waals radius.

The calculations thus predict that such side chains should in general be found to have a conformation near that found in 2-α-HETHC.[4]

Ab initio calculations were performed on the unrotated and 150° rotated 2-(α-hydroxyethyl) ions to see if the LJ potential predictions hold up. The unrotated conformer was calculated to be 0.4 kcal/mol more stable than the 150° rotated one. It would be attractive to associate this excess stability with the favorable $^{\delta-}O\text{–}S^{\delta+}$ interaction and the Coulombic (qq) contribution appears to be favoring the zero-rotated conformer also (Figure 8). Yet this author is hesitant to assign overwhelming significance to such electrostatic factors on account of the lack of positive finite overlap population between the two atoms.

Summary and Critical Evaluation of Results

Although molecular orbital and semiempirical calculations have been performed on a number of conformational problems relevant to biochemical structure and function, it is well worth considering the objections one may raise to such studies.

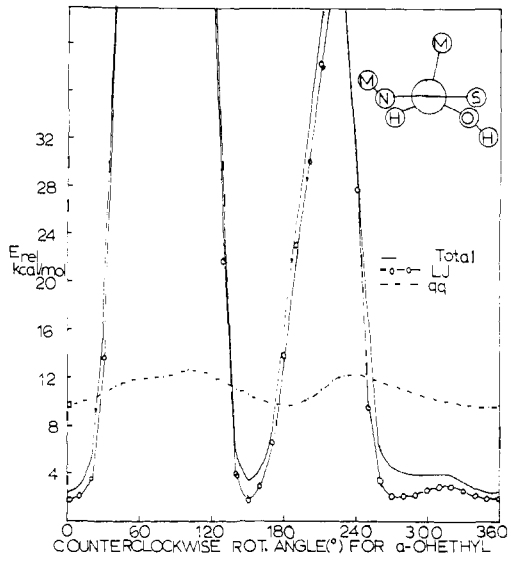


Figure 8. Conformational map for rotation of the 2-(α-hydroxyethyl) side chain in 2-α-HEMTHZ; hydrogen van der Waals radius equals 1.2 Å; LJ and *qq* contribution and total energy map as shown.

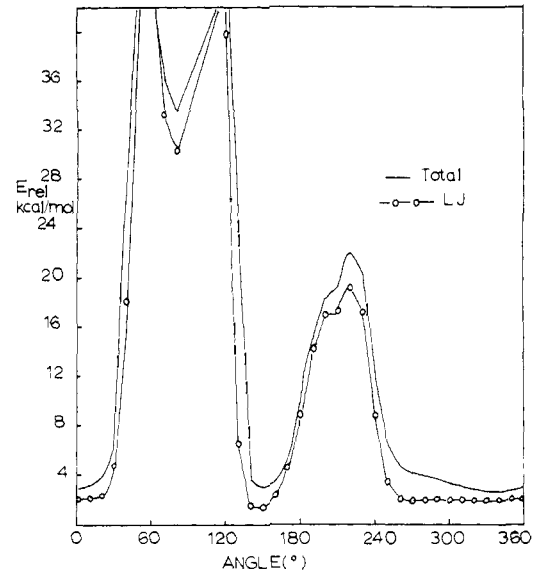


Figure 9. Conformational map for rotation of the 2-(α-hydroxyethyl) side chain in 2-α-HEMTHZ; hydrogen van der Waals radius equals 1.0 Å; LJ and total energy maps shown; Coulombic contribution same as in Figure 8.

Until quite recently, the principal problem facing those interested in both experimental and theoretical approaches to conformational problems has been the gap between experimental (solution of greatest interest) data and theoretical "free state" results. A few attempts have appeared in the literature which explicitly incorporated solvent effects into the calculations; yet, it will be at least several more years before such efforts can provide "chemical quality" data, that is, data of accuracy equal to the experimental one.

It is necessary then to identify those theoretical results which should be subject to relatively little solvent effect and those which could easily change upon solvation or upon changes in the dielectric constant of the medium being considered.

This study presents theoretical results of two types: conformational and electron density (the latter derived from wave functions). In a conformational problem there are two basic quantities of interest: the relative energies of the conformers and the barrier height separating them. While both of these quantities are subject to solvent effects without a doubt, the relative conformer stabilities in general will be very much more difficult to predict. This has been evident in the literature from calculations on rather small ions even with extended basis sets. The existence of major barriers to rotation can, on the other hand, be safely predicted and while the barrier heights are solvent dependent and difficult to define, their existence is certainly not a function of medium polarity. After all, the regions of high energy predicted simply by adding up van der Waals radii of nonbonded atoms, using a more complete calculation here employed or relying on molecular orbital studies, should all be qualitatively the same. The intervention of solvent is unlikely to reduce nonbonded repulsions.

Based on this premise and the figures presented one can conclude that α-HETHC is much less flexible (has many fewer conformational regions accessible to it) than the unsubstituted THC or TPP with respect to the relative disposition of the thiazolium and 2-methyl-4-aminopyrimidine rings. The suggestion (speculation) is that perhaps the enzyme can accommodate a large range of TPP conformations but before the substrate is attached, TPP has to assume one of the low-energy conformational regions presented in the Figure 5. Similarly, the α-hydroxyethyl side chain has only two regions of relative stability for rotating it with respect to the thiazolium ring. Again, the barrier to rotation appears to be high enough to make such rotations prohibitive at room temperature.

The reader should note that no claim is made to define which of the stable regions should be predominant since such preferences indeed could vary with changes in solvent. It is, however, a fortunate coincidence (and perhaps more) that the conformations found by x-ray crystallography always fall into low-energy regions as calculated by the simple Lennard-Jones (6-12) potential.

Next a few thoughts concerning the calculation of charge densities and their relation to experiment are in order. While thermodynamic properties (equilibrium constants for, e.g., ionization, tautomerization, and molecular association) may be very strongly solvent dependent (as vividly demonstrated by recent comparison of gas phase and solution basicities),[20] it appears unlikely that the intrinsic electronic structures of ions and molecules are. One knows that many functional groups undergo subtle electronic changes upon solvation such as the spectroscopic changes accompanying hydrogen bonding. Yet there is no evidence from theory that attaching a hydrogen bonded water molecule seriously changes the electronic structure of a large molecule. Furthermore, theoretical "free state" calculations are capable of reproducing solution spectral characteristics. For example, they accurately reflect changes as gross as covalent attachment of a proton to a nucleic base in water[21]—certainly more significant a change than adding a neutral noncovalently bound solvent molecule. It is then reasonable to assume that while the solvent may cause subtle changes in the electronic structure, it is highly unlikely and to this author's knowledge unprecedented that the polarity of a bond would be reversed on account of a change in solvent alone. One should also note in a related context the many successful correlations of experimental magnetic resonance parameters with simple charge or spin densities calculated for the "free state" of a system.[22]

The electron density calculations in this study indicate the S atom to be positive as also deduced by x-ray crystallography,[4,16] and the thiazolium N to be nearly neutral or even slightly negative. Attaching the α-hydroxyethyl side chain increases the positive charge at C(2) of thiazolium

and sets up a $^{\delta+}C(2)-N(3)^{\delta-}$ bond polarity. Carbon magnetic resonance results show the C(2) to have a positive charge nearly that of carbon in a C=O bond with a polarity for the C(2)–N(3) bond reminiscent of C=O bonds.[15]

Finally, the inflexible nature of the 2-α-HETHC ion suggests that knowledge of the absolute configuration at the C(2α) atom in the side chain produced in the enzymic reaction would give desirable detail concerning the stereochemical pathway of the intermediate steps in the reaction.

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References and Notes

(1) Presented, in part, at the 6th Northeast Regional Meeting of the American Chemical Society, Burlington, Vt., August 1974. Experimental work on thiamine is supported by NIH Grant AM-17495 and the Rutgers University Research Council.
(2) L. O. Krampitz, *Annu. Rev. Biochem.*, **38**, 213 (1969).
(3) For a recent report see G. E. Risinger, W. E. Gore, and K. E. Pulver, *Synthesis*, **9**, 659 (1974).
(4) M. Sax, P. Pulsinelli, and J. Pletcher, *J. Am. Chem. Soc.*, **96**, 155 (1974).
(5) J. Kraut and H. J. Reed, *Acta Crystallogr.*, **15**, 747 (1962).
(6) J. Pletcher and M. Sax, *J. Am. Chem. Soc.*, **94**, 3998 (1972).
(7) F. Jordan, *J. Am. Chem. Soc.*, **96**, 3623 (1974).
(8) R. Hoffmann, *J. Chem. Phys.*, **39**, 1397 (1963).
(9) F. Jordan, *J. Theor. Biol.*, **41**,375 (1973).
(10) A. J. Hopfinger, "Conformational Properties of Macromolecules", Academic Press, New York, N.Y., 1973, p 41.
(11) Quantum Chemistry Program Exchange, Indiana University, Bloomington, Ind., program no. 236 on an IBM 370-158.
(12) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).
(13) R. S. Mulliken, *J. Chem. Phys.*, **23**, 1833 (1955).
(14) A. A. Gallo and H. Z. Sable, *J. Biol. Chem.*, **249**, 1382 (1974).
(15) H. Z. Sable and A. A. Gallo, private communication and Abstracts of the FASEB Meeting, 1975.
(16) M. F. Richardson and K. Franklin, and D. M. Thompson, *J. Am. Chem. Soc.*, **97**, 3204 (1975).
(17) F. Jordan, *J. Am. Chem. Soc.*, **97**, 3330 (1975).
(18) F. Jordan, *J. Phys. Chem.*, **80**, 76 (1976).
(19) H. Holzer, *Angew. Chem.*, **73**, 721 (1961).
(20) E. M. Ernett, *Acc. Chem. Res.*, **6**, 404 (1973).
(21) W. Hug and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **95**, 2803 (1973); **96**, 665 (1974).
(22) See, for example, J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, Chapter 4.

Kinetic Studies of the Helix–Coil Transition in Aqueous Solutions of Poly(α-L-glutamic acid) Using the Electric Field Pulse Method

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Abstract: Relaxation phenomena were studied in aqueous solutions of poly(α-L-glutamic acid) under various conditions using the electric field pulse method with detection by conductivity change. The relaxation time has a maximum value at the midpoint of the helix–coil transition but does not depend on the polymer concentration, degree of polymerization, kind of counterion, or electric field density. Some possible mechanisms are discussed and the observed relaxation phenomenon is attributed to the helix–coil transition. Using Schwarz's theory the rate constants of the helix growth step are estimated. The activation parameters are also calculated from the temperature dependence of the rate constants. It is found that the helix growth process is not diffusion controlled but is limited by an accompanying large decrease of activation entropy.

The conformational transitions of biopolymers are known to play an important role in their functions in biological systems. The helix–coil (H–C) transition of synthetic polypeptides has been studied extensively as a useful model for conformational transitions of biopolymers. The equilibrium properties of the H–C transition have been studied in detail both experimentally and theoretically. Many kinetic studies have been made by the various relaxation methods such as the temperature-jump,[2,3] ultrasonic absorption,[4–7] and dielectric relaxation techniques.[8,9] However, the dynamic features of the H–C transition still are not clear.

Burke et al.[10] have estimated limits for the relaxation time of the H–C transition of poly(α-L-glutamic acid) (PGA) as $5 \times 10^{-8} < \tau < 10^{-5}$ by combining their result of ultrasonic absorption with that of the temperature-jump method by Lumry et al.[2] Subsequently, relaxation phenomena due to H–C transitions have been observed experimentally by using the ultrasonic absorption method for poly(α-D-glutamic acid) by Inoue[6] and for PGA by Barksdale et al.[7] The maximum relaxation times near the midpoint of the H–C transition have been estimated as 1.1×10^{-6} sec at 30°C and 1×10^{-6} sec in 0.03 *M* NaCl at 37°C, respectively. Recently, the present authors[11] have studied the H–C transition of PGA by a modified temperature-jump method employing optical rotation to follow the transient, and the maximum relaxation time of 3.6×10^{-6} sec has been estimated. Previously we[12] have observed a relaxation in aqueous solutions of PGA by means of the electric field pulse (EFP) apparatus with detection by electric conductivity change and have attributed it to the H–C transition from the dependence of the relaxation time on pH and polymer concentration. The assignment of the relaxation mechanism, however, has not been conclusive enough to be confirmed.

The purpose of the present investigation is to confirm the assignment of the observed relaxation to an H–C transition and to obtain detailed information of the dynamic picture of the H–C transition.