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COMPUTATIONAL STUDIES ON THE DECARBOXYLATION OF 2-(1-CARBOXY-1-HYDROXYETHYL)-3,4-DIMETHYLTHIAZOLIUM DIPOLAR ION, AN ANALOG OF THE COMPLEX OF PYRUVIC ACID AND COENZYME OF PYRUVATE DECARBOXYLASE

Yasushi Nakajima[1]*, Yoshikatsu Sakagishi[1],
Michio Shiibashi[2], Yuuji Suzuki[3] and Hitoshi Kato[4]

[1]Department of Biochemistry, Saitama Medical School,
38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-04, Japan

[2]Department of Physiology, Saitama Medical School,
38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-04, Japan

[3]Saitama College of Health,
519 Kamiokubo, Urawa, Saitama, Japan

[4]Laboratory of Materials Physics, College of Engineering, Kanto Gakuin University,
4834 Mutsuura, Kanazawa-ku, Yokohama 236, Japan

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We have obtained the optimized geometrical structure of 2-(1-carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium dipolar ion and investigated its geometric and electric changes during decarboxylation process by the MNDO-PM3 method, a molecular orbital method. The salient features of the optimized structure are that the dihedral angle of C4-C1-C2-S3 is 5.4 ° and the distance between thiazolium S3 and carboxyl O6 is about 1.8Å (the bond order between S3 and O6 is about 0.4). The lowest energy decarboxylation profile is the following process. First the dihedral angle of C4-C1-C2-S3 becomes about 90°, then the distance between C1-C2 increases while the dihedral angle holds about 90°, and finally the C1-C2 bond disappears. The most remarkable change caused by the 90° rotation is the disappearance of the S3-O6 bond, and this disappearance causes electric changes that prompt the decarboxylation.

The decarboxylation of pyruvate to acetaldehyde by the enzyme pyruvate decarboxylase has an important biomedical role(1). The enzyme is a part of the pyruvate dehydrogenase complex, which catalyzes reaction that the pyruvic acid is oxidatively decarboxylated to acetyl-

* To whom correspondence should be addressed.

Abbreviations used: CHDT, 2-(1-Carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium; CHDTDI, 2-(1-Carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium dipolar ion; CHTP, 2-(1-carboxy-1-hydroxyethyl) thiamin pyrophosphate; τ_1, the dihedral angle of O7-C4-C1-C2; τ_2, the dihedral angle of C4-C1-C2-S3; D_{12}, the interatomic distance of C1 and C2; CONFORMATION-I, a conformation is that τ_2 is fixed on 90°, D_{12} is fixed on 1.7Å, and the other parameters are optimized; CONFORMATION-II, a conformation is that τ_2 is fixed on 0°, D_{12} is fixed on 1.7Å and the other parameters are optimized; CONFORMATION-M, energy minimal conformation.

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CoA within the mitochondrion, and pyruvate dehydrogenase deficiency causes lactic acidosis. In short, this enzymatic decarboxylation has a role for preventing excessive production of lactate resulted by excess of pyruvate.

Moreover, the mechanism of this enzymatic decarboxylation, which requires the coenzyme thiamin pyrophosphate, is interesting. A feature of the enzymatic decarboxylation is that the enzymatic acceleration of the decarboxylation is effected through the transfer of pyruvate coenzyme complex into a region of the enzyme less polar than water. The pyruvate-coenzyme complex, 2-(1-carboxy-1-hydroxyethyl) thiamin pyrophosphate (CHTP), is formed from thiamin pyrophosphate and pyruvic acid by reaction of thiazolium ring, ionized at carbon 2, with the carbonyl group of pyruvic acid. J. Crosby et al reported the kinetics of the non-enzymatic decarboxylation of an analog of CHTP, 2-(1-Carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium (CHDT), in water and organic solvent(2). They compared this non-enzymatic decarboxylation with the enzymatic decarboxylation of CHTP, and then concluded that the enzyme accelerates the decarboxylation of CHDT in water by a factor of at least 10^5 and the enzymatic catalysis is caused by binding of the thiazolium portion of CHTP in a region of the enzyme less polar than water. In other words, for these kinds of decarboxylations of pyruvic acid, both binding of pyruvic acid into thiazolium ring and absence of water around the binding complex are essential.

In spite of such an importance and interest, the stable geometrical structures of both CHTP and CHDT are unknown, not to mention their conformational changes during the decarboxylation process. However, to know the stable geometrical structure and its electric structure is very important to investigate microscopic mechanism of the decarboxylation.

In view of the need for geometric and electronic structural data, we have performed computational studies on CHDT dipolar ion(CHDTDI, see Figure 1), which decarboxylates much more easily than CHDT cation. First we have draw an isoenergetic map of the potential energy surface as function of two dihedral angles, the rotations of which cause the great conformational changing of the whole molecule, to look for some stable conformation. Second full optimization has been performed to obtain the geometric conformation of the lowest energy, and the electric structures of this conformation have calculated. Third we have draw another isoenergetic map to obtain the lowest-energy decarboxylation profile. Moreover, changes of quantum chemical properties along with the lowest-energy reaction profile have been investigated. Finally we have discussed the microscopic mechanism of the decarboxylation of CHDTDI from these data.

METHODS

The geometric and electric structures and isoenergetic maps of CHDTDI were obtained by the MNDO-PM3 method(3)(MOPAC Ver. 6.01(4)), one of the most reliable quantum mechanical semiempirical method. Although the semiempirical methods are approximate, they are advantageous because all the independent geometrical parameters can be varied and optimized at low costs and at short time. Since we must perform enormous calculations to draw isoenergetic maps, the calculation-method for this work should be not only reliable but also efficient. Therefore, we selected the MNDO-PM3 method for this study, rather than the higher application of STO-3G and 4-21G.

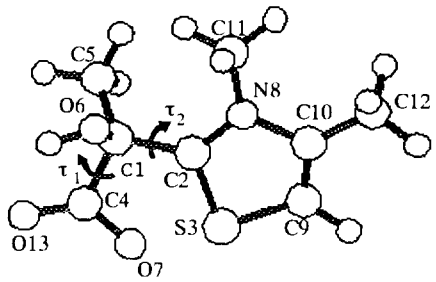

Figure 1. Optimized geometry of CHDTDI. Numbering of the atoms of the CHDTDI is shown. τ_1 is the dihedral angle of O7-C4-C1-C2 and τ_2 is the dihedral angle of C4-C1-C2-S3.

For the search of conformational energy minimum of CHDTDI, the followings were performed. First we drew the isoenergetic map of the potential energy surface as function of dihedral angles of O7-C4-C1-C2(τ_1) and C4-C1-C2-S3(τ_2), by scanning by 15° τ_1 and τ_2 in the range (-90°) - (270°) (except the conformations that decarboxylate by optimization), in order to obtain some low energy conformation of CHDT. Then we used the low energy conformations as initial geometry and fully optimized all geometrical parameters. Geometry of the energy minimum of CHDTDI was thereby obtained.

For the search of the decarboxylation process of CHDTDI, the followings were performed. First we drew the isoenergetic map of the potential energy surface as function of τ_2 and the interatomic distance of C1 and C2 (D_{12}), by scanning by 15° t_2 in the range (-150°) - (150°) and by 0.2 Å D_{12} in the range 1.6 - 2.6 Å. Then we approximately drew the lowest energy reaction profile.

RESULTS AND DISCUSSION

Figure 2 shows the isoenergetic map of potential energy surface as function of τ_1 and τ_2. Its features are the followings. First geometrical conformations decarboxylate on condition of fixing τ_2 on the neighborhood of 90° or -90°. Then this decarboxylation by the τ_2 rotation is somewhat influenced by the τ_1 rotation. Moreover, there is only stable conformation of CHDTDI, τ_1 and τ_2 of which are about -5° and 5° respectively. We have used the stable conformation obtained from Figure 2 and the other conformations near it as initial geometrical structures, and fully optimized all parameters to obtain the conformation of the lowest energy.

The obtained lowest-energy conformation is shown by Figure 1 and its selected geometrical parameters are shown by Table 1. Their features are the followings. First the bond distances of C1-C2, C1-C4, C4-O7 and C4-O13 are 1.51, 1.57, 1.30 and 1.22 Å respectively. These bond lengths indicate that C1-C2 is single bond, C1-C4 is slightly weak single bond, C4-O7 is weak double bond and C4-O13 is double bond. Then τ_1 and τ_2 are -6.2° and 5.4° respectively. These data on τ_1, τ_2 and the other dihedral angles indicate that the plane of carboxyl anion is nearly parallel and close to that of thiazolium ring. Moreover, the interatomic distance of S3...O7 is 1.83 Å. This closeness of S3...O7 suggests existence of some interaction between S3 and O7.

Quantum chemical properties of this lowest-energy conformation are shown by Table 2. Their features are the followings. First The heat of formation of CHDTDI is -76.51 kcal/mol.

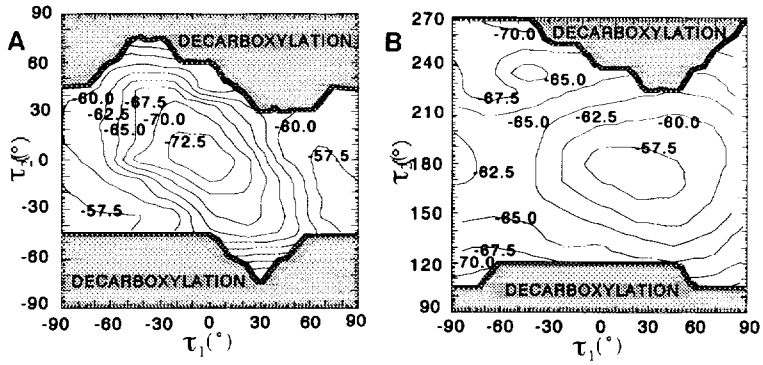

Figure 2. Isoenergetic map of the potential energy surface as function of τ_1 and τ_2. Although both τ_1 and τ_2 are fixed, the other geometrical parameters are optimized. The regions in which the geometrical conformation decarboxylates by optimization are shown. The values of Heat of formation(kcal mol^{-1}) are reported. A: The range of τ_2 is from -90° to 90°. B:The range of τ_2 is from 90° to 270°.

Then the data on the bond orders of C1-C2, C1-C4, C4-O7 and C4-O13 indicate that C1-C2 is single bond, C1-C4 is slightly weak single bond, C4-O7 is weak double bond and C4-O13 is slightly weak double bond, as their bond distances do as above described. This estimation on bond strength of these bonds is also supported by the data of two center energy term. Third the bond order of S3...O7 is 0.44, which fact indicates that weak bond exists between S3 and O7. In addition, atomic net charges of S3 and O7 are .70 and -.47 respectively. These two facts and the closeness of S3..O7 suggested that electrostatic interaction exists between S3 and O7.

Since Figure 2 shows that the decarboxylation is strongly influenced by τ_2 rotation, the decarboxylation process seems to depend on both the τ_2 rotation and the interatomic distance of C1-C2. Figure 3 shows the isoenergetic map of potential energy surface as function of τ_2 and D_{12}. As shown by the arrow of Figure 3, the energy-minimal conformation of CHDTDI decarboxylates along with the energetic advantageous profile as follows: first τ_2 becomes about 90° with increasing of D_{12} to about 1.7Å, then D_{12} increases more and more while τ_2 holds about 90° and finally the bond of C1-C2 disappears. This profile indicates that the 90° rotation of τ_2 is indispensable for the decarboxylation.

Table 1. Selected geometric parameters of the optimised geometry of the CHDTDI

Bond distances (Å)		Bond angles (°)		Dihedral angles (°)	
C1-C2	1.51	O7-C4-C1	113.2	C9-S3-C2-N8	-.2
C1-C4	1.57	C2-C1-C4	103.7	C10-N8-C2-S3	-.5
C1-C5	1.53	S3-C2-C1	117.6	C10-C9-S3-C2	-.8
C1-O6	1.42	C9-S3-C2	85.4	S3-C9-C10-N8	-.1
C4-O7	1.30	N8-C2-C1	126.9	C2-N8-C10-C9	1.0
C4-O13	1.22	O13-C4-C1	124.2	C1-C2-S3-C9	-178.9
C2-S3	1.78	----------		C4-C1-C2-S3	5.4
C2-N8	1.36	----------		O7-C4-C1-C2	-6.2
S3-C9	1.74	----------		O7-C1-C2-S3	2.2
S3...O7	1.83	----------		O13-C4-C1-O7	179.0
N8-C10	1.42	----------		----------	
C9-C10	1.37	----------		----------	

Table 2. Quantum chemical properties of the optimised molecule of *CHDTDI*

HEAT OF FORMATION(kcal/mol)				-76.51
	BOND ORDERS	TWO CENTER ENERGY TERM (eV)		MULLIKEN'S ATOMIC NET CHARGES
C1-C2	0.96	-12.17		
C1-C4	0.84	-14.29	C1	.10
C2-S3	1.13	-16.14	C2	-.42
C2-N8	1.48	-20.60	S3	.70
C2-C9	.09	1.58	C4	.38
C2-C10	.07	1.13	O7	-.47
S3...O7	.44	-7.65	N8	.52
S3-N8	.12	2.77	C9	-.43
S3-C9	.91	-13.15	C10	-.27
C4-O7	1.21	-19.03	O13	-.41
C4-O13	1.74	-24.84	----	------
O7-C9	.13	.53	----	------
O7....O13	.14	1.07	----	------
N8-C10	1.15	-16.87	----	------
C10-C9	1.63	-19.55	----	------

It is important to know the electric changes occurred by the 90° rotation of τ_2, in order to investigate how the 90° rotation of τ_2 promotes the decarboxylation. Firstly we have calculated the interatomic bond orders of both the two following conformations. One conformation is that τ_2 is fixed on 90°, D_{12} is fixed on 1.7Å and the other parameters are optimized (CONFORMATION-I), the other conformation is that τ_2 is fixed on 0°, D_{12} is fixed on 1.7Å and other parameters are optimized (CONFORMATION-II). Then we have compared these interatomic bond orders of CONFORMATION-I, II with those of the energy minimal conformation(CONFORMATION-M, see Figure 1 and Table 1). The obtained Results are as

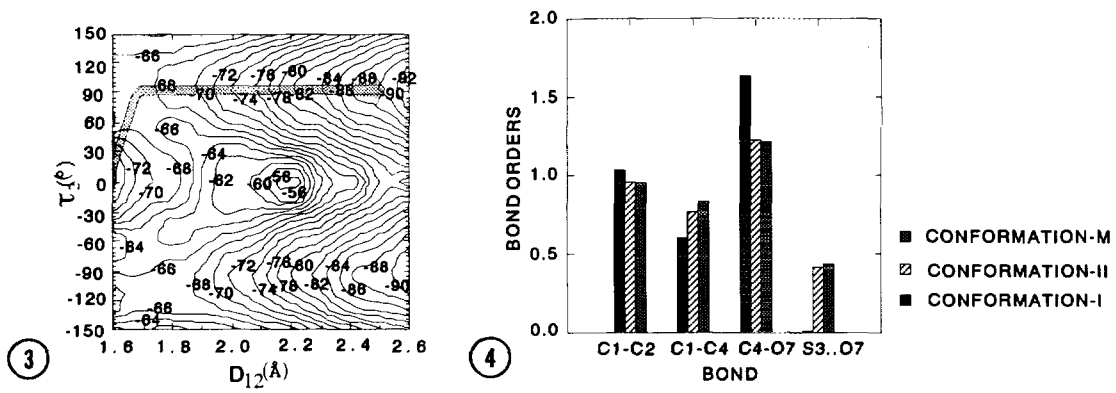

Figure 3. Isoenergetic map of the potential energy surface as function of D_{12} (interatomic distance C1 and C2) and τ_2. Although both D_{12} and τ_2 are fixed, the other geometrical parameters are optimized. The arrow is the lowest energy reaction profile of the decarboxylation process. The values of Heat of formation(kcal mol^{-1}) are reported.

Figure 4. Bond orders of C1-C2, C1-C4, C4-O7 and S3..O7 in CONFORMATION-M,-I and -II. CONFORMATION-M is energy minimal conformation(see Figure 1. and Table 1.) CONFORMATION-I is that τ_2 is fixed on 90°, D_{12} is fixed on 1.7Å and the other parameters are optimized. CONFORMATION-II is that τ_2 is fixed on 0°, D12 is fixed on 1.7Å and the other parameters are optimized.

follows: (1) there is little difference in the bond orders between CONFORMATION-I and CONFORMATION-M, except the bond order of C1-C4, (2) the bond order of C4-O7 of CONFORMATION-I is 1.63 while that of CONFORMATION-M is 1.21, (3) the bond order of C1-C2 of CONFORMATION-I is 1.04 while that of CONFORMATION-M is 0.96, (4) the bond order of S3...O7 of CONFORMATION-I disappears while that of CONFORMATION-M is 0.44, and this disappearance occurs by the dissociation of S3...O7 caused by the 90° rotation of τ_2, (5) The bond order of C1-C4 of CONFORMATION-I is .61 while that of CONFORMATION-II is .77, nevertheless the bond distance of C1-C4 of CONFORMATION-I is the same as that of CONFORMATION-II. The summary of these results is shown in Figure 4.

From above described results, we have discussed the microscopic decarboxylation process of CHDTDI. In CONFORMATION-M, a part of orbital electron of O7 is attracted by the positive charge of S3 and there is a weak electrostatic bond between O7 and S3, as shown by Table 1 and 2. Although this conformation is the most stable of CHDTDI, the 90° rotation of τ_2 easily occurs as shown by Figure 3. This 90° rotation of τ_2 gets the distance of S3...O7 to increase to 3.5Å, and hence the electrostatic bond of S3-O7 disappears as shown by Figure 4. In other words, the increase of the distance of S3...O7 weakens attraction between positive charge of S3 and a part of orbital electron of O7, and that of O7 becomes free. Then a part of orbital electron of C4, which has interacted with that of C1 before, makes π-bond with that of O7. Through the process as above described, the double bond of C4-O7 of carboxyl anion becomes stronger and the bond of C1-C4 becomes weaker in CONFORMATION-I(see Figure 4). Finally the increase of the distance of C1-C4 in CONFORMATION-I causes the decarboxylation, as shown Figure 3.

We have finally concluded that (1) the decarboxylation of CHDTDI occurs through the 90° rotation of τ_2, and (2) this 90° rotation of τ_2 gets the electrostatic bond of S3...O7 to disappear and this disappearance causes electric changes that prompt the decarboxylation.

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