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Simulation of the Enzymatic Reaction of Dogfish M_4 Lactate Dehydrogenase: A Molecular Orbital Study on the Reactivity of Pyruvate

HIDEAKI UMEYAMA, SETSUKO NAKAGAWA, and TOMOKO NOMOTO

School of Pharmaceutical Sciences, Kitasato University[1)]

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Simulation of the enzymatic reaction of dogfish M_4 lactate dehydrogenase (LDH) was performed. In the enzymatic reaction from L-lactate to pyruvate, the structure in which the proton transfer from L-lactate to His 195 is coupled with the hydride transfer from L-lactate to NAD^+ was simulated. In the reverse reaction, the structure in which the proton transfer from His 195 to pyruvate is coupled with the hydride transfer from NADH to pyruvate was also simulated. Since the proton of His 195 approaches the carbonyl oxygen of pyruvate from the π direction of the pyruvate plane in the simulation, the electronic structure and the reactivity of pyruvate were studied from a quantum chemical point of view, using the *ab initio* LCAO MO method. When the carbonyl carbon of pyruvate is attacked by the hydride ion of NADH, the total π electron density of the carbonyl oxygen of pyruvate increases substantially. Therefore, the proton of His 195 attacks the carbonyl oxygen of pyruvate from the π direction. Moreover, the pyruvate-Arg 171 complex having two nearly parallel hydrogen bonds is more easily attacked by hydride than complexes having other conformations.

Keywords——pyruvate; lactate; lactate dehydrogenase; dehydrogenase; *ab initio* calculation; molecular orbital; structure; enzyme; MO; mechanism

Dogfish M_4 lactate dehydrogenase (LDH) catalyzes the formation of pyruvate from L-lactate with NAD^+ as a coenzyme. L-Lactate has an anionic charge on the carboxyl group, and it forms an ion pair with Arg 171 as shown in Fig. 1.[2)] Charge cancellation between the two ions neutralizes L-lactate. The proton of the hydroxyl group of L-lactate transfers from L-lactate to $N^{\varepsilon 2}$ of His 195, which forms a hydrogen bond with Asp 168.[2)] The nature of the

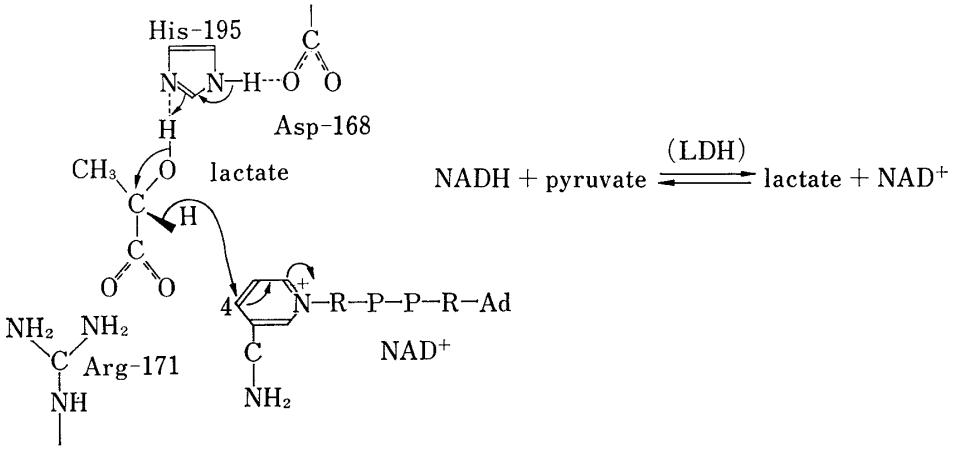

Fig. 1. Reaction Mechanism of Dogfish M_4 LDH tentatively proposed by Rossmann and his Associates[2)]

1) Location: *9-1, Shirokane 5-chome, Minato-ku, Tokyo 108, Japan.*
2) M.J. Adams, M. Buehner, K. Chandrasekhar, G.C. Ford, M.L. Hackelt, A. Liljas, M.G. Rossmann, I.E. Smiley, W.S. Allison, J. Everse, N.O. Kaplan, and S.S. Taylor, *Proc. Nat. Acad. Sci. USA*, **70**, 1968 (1973).

hydroxyl group is similar to that of Ser 195.[3] Umeyama *et al.* reported MO calculations showing that the potential curve of proton transfer from the hydroxyl group to $N^{\varepsilon 2}$ of histidine in LDH was similar in form to that of Ser 195-His 57-Asp 102 found in the active site of serine proteases.[3] In the enzymatic reaction of trypsin, the proton of Ser 195 is affected by $N^{\varepsilon 2}$ of His 57, forming a hydrogen bond with Asp 102,[4] and the interaction between O^{γ} of Ser 195 and the carbonyl carbon of the substrate plays a significant role in accelerating the proton transfer from Ser 195 to His 57.[5] In the enzymatic reaction of LDH, similarly, the proton of the hydroxyl group of L-lactate is affected by $N^{\varepsilon 2}$ of His 195, and the hydride transfer from L-lactate to the 4-position of the nicotinamide ring (NA) of NAD^+ may lower the barrier to proton transfer from L-lactate to His 195. In this paper, thus the coupling structure for proton transfer and hydride transfer in the enzymatic formation of pyruvate from L-lactate is simulated.

LDH catalyzes the reverse reaction from pyruvate to L-lactate with NADH as a coenzyme. A substrate such as pyruvate binds more strongly with LDH in the protonated form of His 195 than in the unprotonated form.[6] In trypsin, moreover, the form His 57(cation)-Asp 102 (anion) was no less stable than His 57(neutral)-Asp 102(neutral) in the presence of hydrogen bonds.[4] Therefore the enzymatic reaction may occur in the form His 195(cation)-Asp 168 (anion). When the proton transfer from the protonated His 195 to pyruvate occurs, the barrier will be large due to the stabilization of the ion-pair structure between His 195 and Asp 168. The hydride transfer from NADH to the carbonyl carbon of pyruvate may play a role in lowering the barrier to proton transfer. In this paper the coupling structure for hydride transfer and proton transfer in the reverse reaction is also simulated. We have already studied the enzymatic reaction in connection with Arg 171 and L-lactate from a quantum chemical point of view,[3] so in this paper the electronic structure and the reactivity of pyruvate are analyzed.

Method

All the calculations were carried out within the closed shell LCAO-SCF approximation using the *ab initio* method. The GAUSSIAN 70 program was used.[7] The basis set was STO-3G.[8] Calculations were carried out using the HITAC M-180 computer at the Institute for Molecular Science and the HITAC 8700 and 8800 computers at the Tokyo University Computer Center.

Energy decomposition analyses in our *ab initio* calculations were carried out using the method of Morokuma *et al.*[9] The intermolecular interaction energy ΔE was divided into five terms: electrostatic (ES), exchange repulsion (EX), polarization (PL), charge transfer (CT), and mixing term (MIX).

Geometries——The structure of L-lactate used in the simulation of the enzymatic reaction was described in another paper,[3] and the structure of pyruvate is shown in Fig. 2. The coordinates of NAD^+, NADH, Arg 171, His 195 and Asp 168 were obtained from X-ray diffraction data.[10] The structure of the active site is shown in Fig. 3. The conformation of the ion-pair between L-lactate and Arg 171 in Fig. 3 is based on that of formic acid and a guanidinium ion; formic acid forms two nearly parallel hydrogen bonds with the guanidinium ion.[11] The ORTEP program of Johnson was used to draw the molecular structures.[12]

3) H. Umeyama, A. Imamura, C. Nagata, and S. Nakagawa, *Chem. Pharm. Bull.*, **25**, 1685 (1977).
4) S. Nakagawa, H. Umeyama, and T. Kudo, *Chem. Pharm. Bull.*, **28**, 1342 (1980).
5) H. Umeyama, A. Imamura, and C. Nagata, *Chem. Pharm. Bull.*, **23**, 3045 (1975).
6) *a*) A.D. Winer and G.W. Schwert, *J. Biol. Chem.*, **234**, 1155 (1959); *b*) J.J. Holbrook and R.A. Stinson, *Biochem. J.*, **131**, 739 (1973).
7) W.J. Hehre, W.A. Lathan, R. Ditchfield, M.D. Newton, and J.A. Pople, GAUSSIAN 70, Quantum Chemistry Program Exchange, Indiana University, 1973.
8) W.J. Hehre, R.F. Stewart, and J.A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).
9) *a*) K. Morokuma, *J. Chem. Phys.*, **55**, 1236 (1971); *b*) K. Kitaura and K. Morokuma, *Int. J. Quantum Chem.*, **10**, 325 (1976); *c*) H. Umeyama and K. Morokuma, *J. Am. Chem. Soc.*, **98**, 7208 (1976); *d*) H. Umeyama and K. Morokuma, *J. Am. Chem. Soc.*, **99**, 1316 (1977).
10) F.C. Bernstein, T.F. Koetzle, G.J.B. Williams, E.F. Meyer, Jr, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, and T. Tasumi, *J. Mol. Biol.*, **112**, 535 (1977).
11) S. Nakagawa and H. Umeyama, *J. Am. Chem. Soc.*, **100**, 7716 (1978).
12) C.K. Johnson, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1971, ORNL-3794.

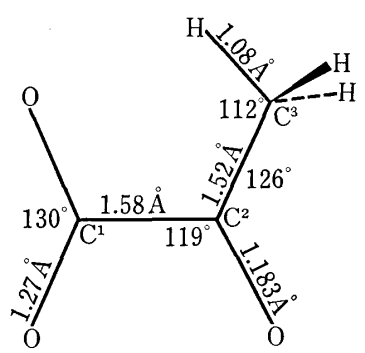

Fig. 2. The Structure of Pyruvate used in the Simulation of the Enzymatic Reaction

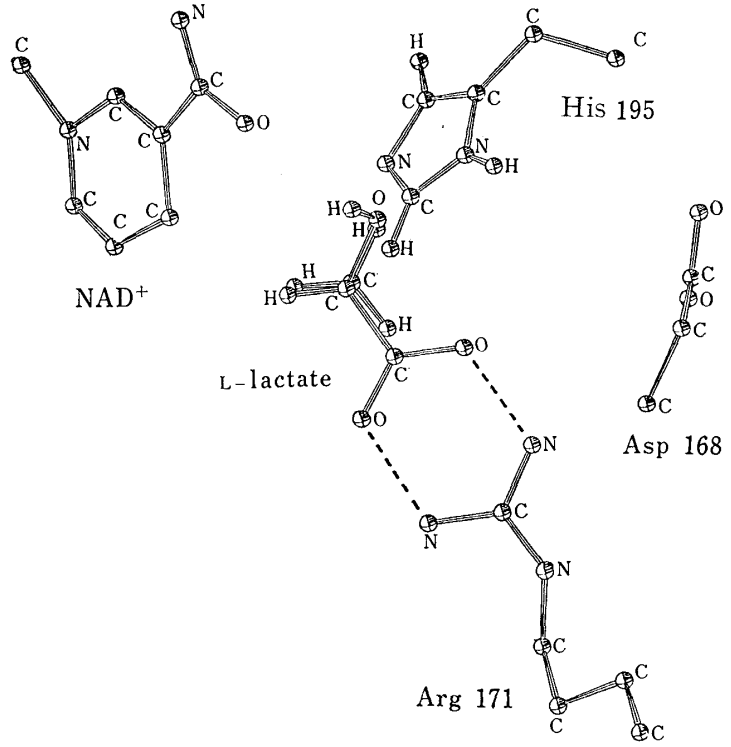

Fig. 3. The Structure of the Active Site of LDH

Results and Discussion

Simulation of the Enzymatic Reaction

The enzymatic formation of pyruvate from L-lactate was simulated. For His 195 in Fig. 3, the side chain was rotated by 20 and −30 degrees around the C^{α}–C^{β} and C^{β}–C^{γ} bonds, respectively, as shown in Table I. Since Umeyama and Nakagawa reported in an MO study on the side chain structure of phenylalanine that the phenyl ring rotates very easily about C^{β}–C^{γ} by ±30 degrees,[13] the rotation around the C^{β}–C^{γ} bond of His 195 should be possible. For Arg 171 and Asp 168, the side chains were rotated as shown in Table I. Figure 4 shows the simulated structure, and the geometry parameters are listed in Table II. In Fig. 4, His 195 forms two hydrogen bonds with Asp 168 and L-lactate, L-lactate forms two nearly parallel hydrogen bonds with Arg 171, and the C^4-position of NAD^+ binds with the CH bond of L-lactate. Thus, the coupling structure for proton transfer from L-lactate to His 195 and hydride transfer from L-lactate to NAD^+ is obtained.

LDH does not catalyze the enzymatic reaction of D-lactate. In Fig. 4, D-lactate in place

Table I. Rotation[a] around the Bond Axes of His 195, Arg 171, Substrates, and Asp 168 from the Structure in Fig. 3

	His 195		Arg 171				Substrate		Asp 168	
	C^{α}–C^{β}	C^{β}–C^{γ}	C^{α}–C^{β}	C^{β}–C^{γ}	C^{γ}–C^{δ}	C^{δ}–C^{ε}	C^1–C^2	C^2–C^3	C^{α}–C^{β}	C^{β}–C^{γ}
L-Lactate	20	−30	50	0	−50	25	−60		85	75
Pyruvate	10	−20	50	10	−50	30	−45	90	40	120

a) Rotation is performed in degrees and in a clockwise direction.

13) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.*, **27**, 2227 (1979).

of L-lactate was superposed so as to fit the hydroxyl group to His 195. The structure is shown in Fig. 5. The transfer of hydride from D-lactate to the 4-position of NAD^+ will not occur, since the hydrogen of the CH group of D-lactate is far from the 4-position. If D-lactate rotates by 120 degrees around the C^1–C^2 bond after proton transfer from D-lactate to His 195, a hydride ion might transfer from D-lactate to NAD^+. Nevertheless, D-lactate is not decomposed by

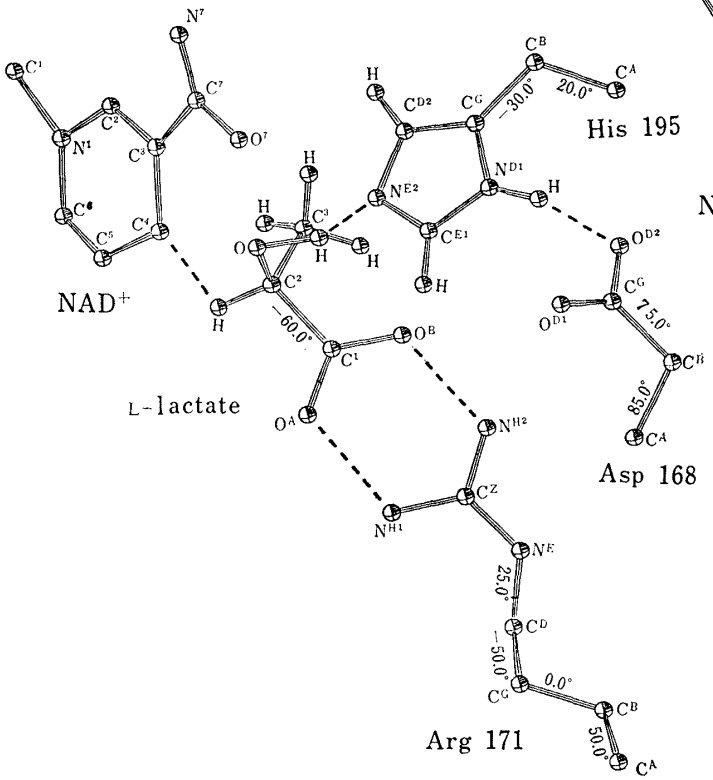

Fig. 4. The Simulated Structure for the Enzymatic Conversion of L-Lactate to Pyruvate

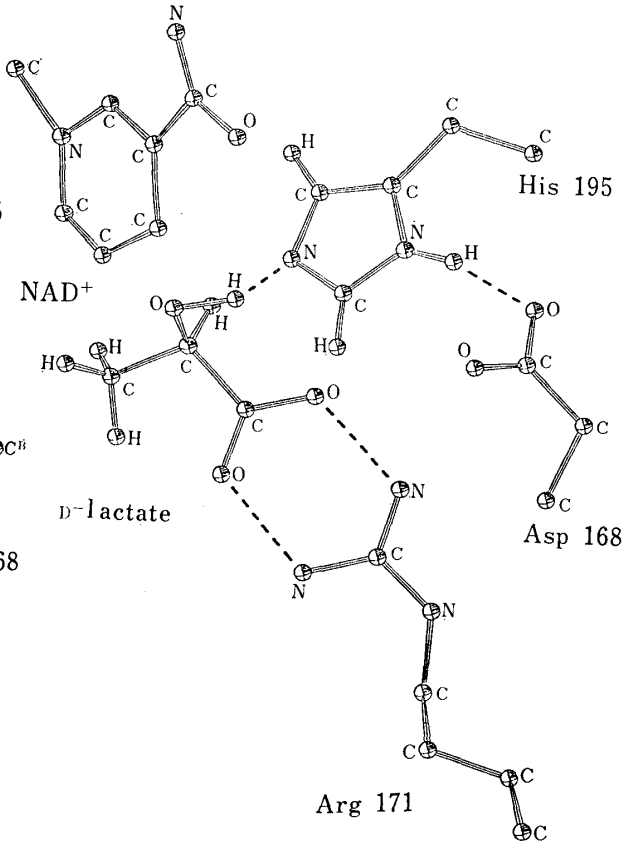

Fig. 5. The Structure in which D-Lactate is superposed in Place of L-Lactate in Fig. 4

TABLE II. Geometries obtained in Simulations of the Enzymatic Conversion of L-Lactate to Pyruvate and the Reverse Reaction

From L-lactate to pyruvate					
L-Lactate-His 195		His 195-Asp 168		L-Lactate-NAD^+	
$\angle OH$–$N^{\varepsilon 2}$	175°	$\angle N^{\delta 1}H^{\delta 1}$–$O^{\delta 2}$	162°	$\angle C^2H$–C^4	89°
$\angle C^2O$–$N^{\varepsilon 2}$	103°	$\angle N^{\delta 1}O^{\delta 2}$–$C^{\gamma}$	118°	$\angle C^2$–C^4C^3	135°
$r(ON^{\varepsilon 2})$	2.33 Å	$r(O^{\delta 2}N^{\delta 1})$	2.78 Å	$\angle C^2$–C^4C^5	74°
				$r(C^2C^4)$	2.08 Å

From pyruvate to L-lactate					
Pyruvate–His 195		His 195-Asp 168		Pyruvate–NADH	
$\angle OH^{\varepsilon 2}$–$N^{\varepsilon 2}$	154°	$\angle N^{\delta 1}H^{\delta 1}$–$O^{\delta 1}$	164°	$\angle C^2$–HC^4	142°
$\angle C^2O$–$N^{\varepsilon 2}$	129°	$\angle N^{\delta 1}$–$O^{\delta 1}C^{\gamma}$	119°	$\angle C^2$–C^4C^3	123°
$r(ON^{\varepsilon 2})$	2.40 Å	$r(N^{\delta 1}O^{\delta 1})$	2.81 Å	$\angle C^2$–C^4C^5	87°
				$r(C^2C^4)$	2.53 Å

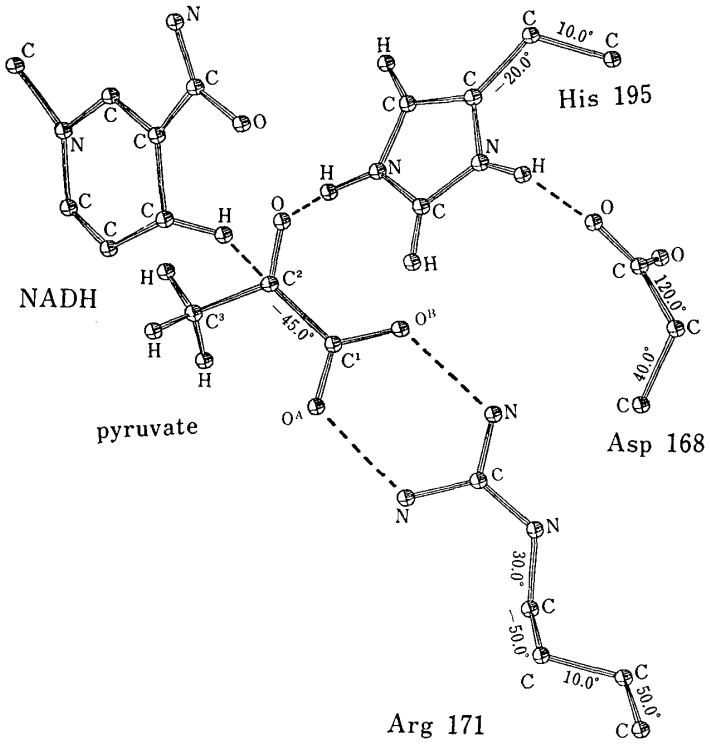

Fig. 6. The Simulated Structure for the Enzymatic Conversion of Pyruvate to L-Lactate

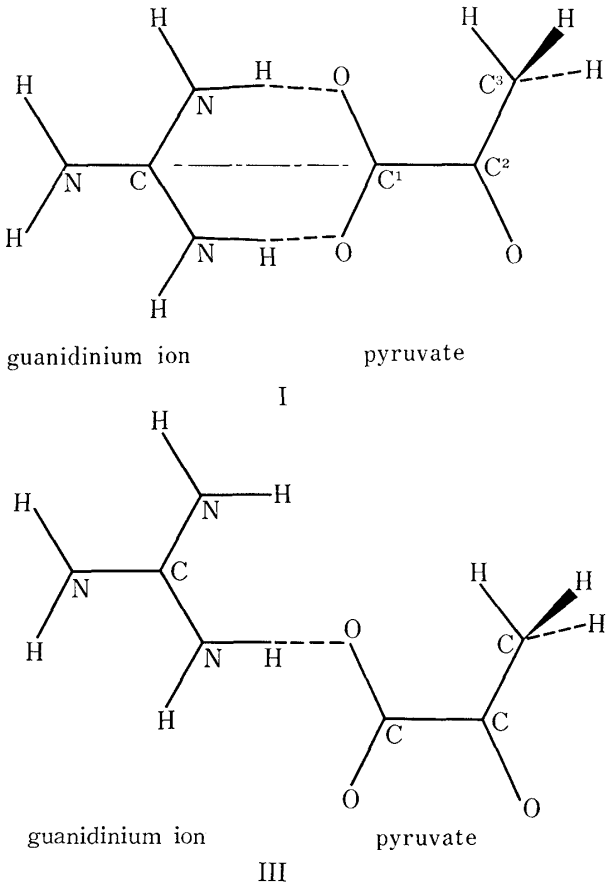

Fig. 7. Interacting Structures between Pyruvate and a Guanidinium Ion

LDH. In the enzymatic reaction, therefore, the proton transfer and the hydride transfer may be concerned.

The reverse reaction from pyruvate to L-lactate must also be simulated in order to study the enzymatic reaction of LDH. For His 195, Arg 171, Asp 168, and pyruvate, bond rotations were performed as shown in Table I. Figure 6 shows the simulated structure. The details of the conformation are shown in Table II. His 195 forms two hydrogen bonds with Asp 168 and pyruvate, pyruvate forms two nearly parallel hydrogen bonds with Arg 171, and the CH group at the 4-position of NADH binds with the carbonyl carbon of pyruvate. In Fig. 6, the proton transfer from His 195 to pyruvate is coupled with the hydride transfer from NADH to pyruvate. The structure in which the proton of His 195 approaches the oxygen of pyruvate from a direction perpendicular to the carbonyl (C^3C^2O) plane of pyruvate is interesting, and is analyzed by *ab initio* quantum chemical calculations in the next section.

Electronic Structure and Reactivity of Pyruvate

The electronic structure and the reactivity of pyruvate in the enzymatic reaction of LDH were studied. X-Ray diffraction data showed that the sodium salt of pyruvate has a dihedral angle of 30 degrees for the OC^1C^2O chain.[14] In the *ab initio* calculations, the structure obtained from the X-ray diffraction data is less stable by 0.9 kcal/mol than the planar structure in which the dihedral angle for the OC^1C^2O chain is zero. Due to the small size of the difference, the

14) S.S. Tavale, L.M. Pant, A.B. Biswas, *Acta Cryst.*, **14**, 1281 (1961).

use of the planar structure of pyruvate, as shown in Fig. 2, is appropriate in the simulation.[15]

Since pyruvate forms an ion-pair structure with Arg 171, some interacting structures between a guanidinium ion and the planar structure of pyruvate were calculated. The structure I, in which the guanidinium ion forms two nearly parallel hydrogen bonds with pyruvate (Fig. 7), the structure II in which the guanidinium ion is rotated around the C–C^1 bond of pyruvate by 90 degrees, and the structure III in which the guanidinium ion forms a single hydrogen bond with pyruvate, as shown in Fig. 7, were calculated.[16] The results shown in Table III. The interaction energy is largest in the structure I.[17] It originates from *ES*, and is larger by 56 and 33 kcal/mol than those in structures II and III, respectively, due to *CT* and *ES*. Therefore Arg 171 will form two nearly parallel hydrogen bonds with pyruvate in the enzymatic reaction of LDH.

Hydride is transferred from NADH to pyruvate. Since the carbonyl carbon of pyruvate acts as an electrophile, the LUMO of pyruvate, the frontier electron density (C_r^2) of $2p_\pi$ of the

Table III. Interaction Energies in kcal/mol and Energy Decomposition Analyses of Guanidinium Ion-Pyruvate Complexes using an STO-3G Basis Set

	I[a]	II[b]	III[c]
ΔE	−161.0	−104.7	−128.3
ES	−152.1	−98.8	−112.2
EX	96.6	12.2	48.3
PL	−7.9	−4.5	−6.8
CT	−69.5	−12.2	−36.2
MIX	−28.1	−1.5	−21.5

a) The structure in which the guanidinium ion forms two nearly parallel hydrogen bonds with pyruvate.
b) The structure in which the guanidinium ion is rotated around the C-C^1 bond of pyruvate by 90 degrees from the structure I.
c) The structure in which the guanidinium ion forms a single hydrogen bond with pyruvate in structure I.

Table IV. Electrophilic Reactivity of Pyruvate and Pyruvate–Guanidinium Ion Complexes

Structure	LUMO[a]	C_r^{2b}	Density[c]
Isolated structure	0.490	0.516	5.847(0.845)
I	0.299	0.354	5.814(0.888)
II	0.355	0.431	5.838(0.869)
III	0.342	0.422	5.827(0.877)

a) LUMO level (Hartrees) in π MO's of pyruvate.
b) MO electron densities of the carbonyl carbon of pyruvate at LUMO in π MO's.
c) Total electron densities of the carbonyl carbon of pyruvate. Numbers in parentheses are total π electron densities.

15) When pyruvate forming two hydrogen bonds with an ammonium ion is internally rotated around the C^1–C^2 axis by 30 degrees, the planar structure is more stable than the rotated one by 0.7 kcal/mol. Therefore, even though pyruvate forms an ion pair, it internally rotates very easily around the C^1–C^2 axis by 30°. Moreover, rotation of only 30° does not affect the LUMO, the frontier electron density of $2p_\pi$ of the carbonyl carbon in the LUMO, or the total electron density of the carbonyl carbon; the changes in these values are only 0.007 Hartrees, 0.021, and −0.003, respectively.
16) In structure I, the intermolecular distance was obtained from the results of geometry optimization between a guanidinium ion and formic acid given in reference 11. In structure III, the guanidinium ion is rotated about the hydrogen bond between the hydrogen of the guanidinium ion and the oxygen of pyruvate by 180 degrees.
17) The structure interacting within the van der Waals distance and similar to structure I except for the distance between the carbon of the guanidinium ion and the carboxyl carbon of pyruvate was less stable than structure I by 83 kcal/mol, and the interaction energy of the former was due to ES (−78 kcal/mol).

carbonyl carbon in the LUMO, and the total electron density of the carbonyl carbon are given in Table IV. When pyruvate forms the three ion-pair structures with the guanidinium ion, the LUMO levels decrease markedly, accelerating the nucleophilic reaction of hydride ion from NADH. However, the frontier electron densities in the ion-pair structures decrease, and unfavorably affect the attack of the hydride. Although the LUMO and the frontier electron density are reactivity indexes in connection with the charge transfer energy, the total electron density is related to the electrostatic interaction. The total σ electron density of the carbonyl carbon decreased markedly on ion-pair formation, and hence, these decreases will contribute to the nucleophilic reaction of the hydride ion. The results in Tables III and IV indicate that structure I in Fig. 7 is more favorable than structures II and III. The interaction energy between hydride and the ion-pair structure I was calculated and energy decomposition analyses were performed. Hydride approaches the carbonyl carbon from a direction perpendicular to the pyruvate plane.[18] The results are shown in Table V. The interaction energy is −18 kcal/mol, and *ES*, *CT*, and *PL* contribute to the complex formation. *MIX* is also large, due to a coupling interaction between *CT* and *PL*.[19] The electrostatic interaction energy between the hydride and guanidinium ion is −64 kcal/mol, and, hence, *ES* is negative, despite the electrostatic repulsion between hydride and pyruvate. The *ES* terms of the complexes between hydride and the ion-pair structures II and III in Fig. 7 were calculated in comparison

Table V. Interaction Energy in kcal/mol and Energy Decomposition Analysis between H^- and the Complex in which the Guanidinium Ion Forms Two nearly Parallel Hydrogen Bonds with Pyruvate, using an STO-3G Basis Set

	Energy[a]
ΔE	−17.7
ES	−37.0(−64.1)[b]
EX	154.4
PL	−17.0
CT	−29.2
MIX	−88.8

a) H^- approaches the carbonyl carbon of pyruvate at the distance of 1.5 Å. The value of 1.5 Å was obtained from calculations of the interaction energies between H^- and the complex composed of pyruvate and ammonium ion.
b) This shows the electrostatic energy between H^- and the guanidinium ion. Pyruvate was excluded.

Table VI. Electrostatic Energies in kcal/mol between H^- and the Complex composed of the Guanidinium Ion and Pyruvate by using an STO-3G Basis Set

Structure	*ES*
I	−37.0
II	−18.3
III	−21.2

18) Using an ammonium ion in place of the guanidinium ion, the interaction energies between hydride and a structure similar to the ion-pair structure I were calculated at various distances between hydride and the carbonyl carbon of pyruvate. Hydride approaches the carbonyl carbon from a direction normal to the pyruvate plane. The interaction energy at a distance of 1.5 Å was minimum, and its value is −173 kcal/mol. *ES*, *EX*, *CT*, and *PL* of the energy decomposition terms are −172, 93, −69, and −6 kcal/mol, respectively. Thus, the value of 1.5 Å was used in the calculations of ΔE between hydride and the guanidinium ion-pyruvate complex.
19) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.*, **27**, 1524 (1979).

with the complex between hydride and the ion-pair structure I.[20] The results are shown in Table VI. *ES* in structure I is greater than those in structure II and III. Therefore hydride attacks the carbonyl carbon of pyruvate more readily in I than in II or III.[21] When the hydride transfers from NADH to pyruvate, the proton transfers simultaneously from $N^{\varepsilon 2}$ of His 195 to the carbonyl oxygen of pyruvate from a direction perpendicular to the pyruvate plane or from a direction coincident with a lone-pair orbital of the oxygen, as shown in Fig. 8.[23]

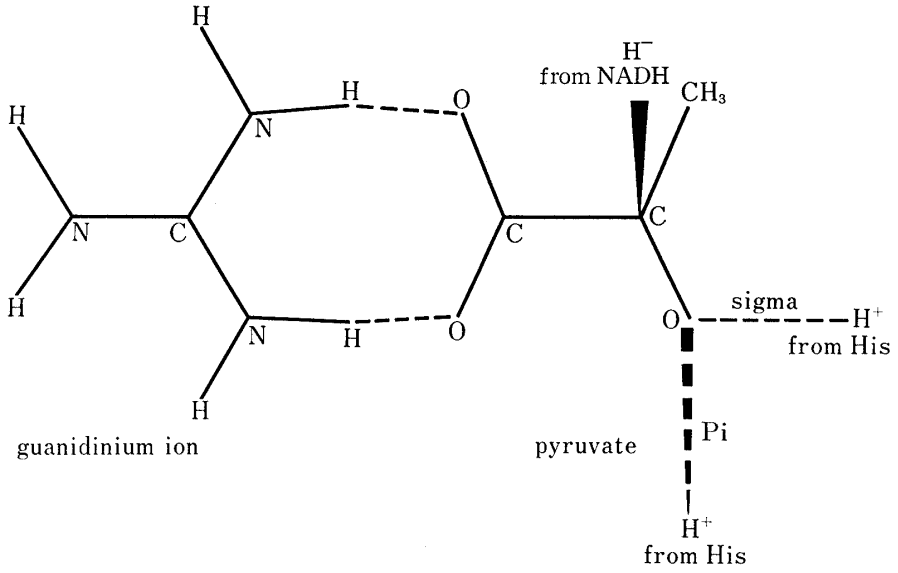

Fig. 8. The Structure in which the Proton of His 195 Attacks the Carbonyl Oxygen of Pyruvate

Table VII. Interaction Energies in kcal/mol and Energy Decomposition Analyses between Hydride and the System composed of a Guanidinium Ion, Pyruvate and a Proton

	(1)	(2)		(2)−(1)
ΔE	−216.3	−283.5	$\Delta\Delta E$	−67.2
ES	−157.8	−166.8	ΔES	−9.0
EX	146.4	138.2	ΔEX	−8.2
CT	−53.7	−104.9	ΔCT	−51.2
$PL+MIX$	−151.2	−149.9	$\Delta(PL+MIX)$	1.3

(1) The proton approaches the carbonyl oxygen of pyruvate on the same plane as pyruvate; the proton interacts with σ electrons of the carbonyl oxygen.
(2) The proton approaches the carbonyl oxygen of pyruvate from the direction normal to the plane of pyruvate; the proton interacts with π electrons of the carbonyl oxygen.

20) Similar calculations of some interacting structures between hydride and the ion-pair structures composed of pyruvate and ammonium ion indicated that only *ES* contributed to the difference among these interactions. Accordingly, comparisons among the *ES* terms should lead to valid conclusions.
21) Umeyama reported *ab initio* calculations showing that the order of electrophilic reactivity of the nicotinamide ring of NAD^+ as a coenzyme for hydride was 4-position>2-position>6-position. The differences between the 4-position and 2-position and between the 4-position and 6-position were 5 and 7 kcal/mol, respectively.[22] Thus, the selectivity among the positions is determined by differences of within a few kcal/mol.
22) H. Umeyama, *Chem. Pharm. Bull.*, **28**, 1317 (1980).
23) The distances between the proton and the carbonyl oxygen of pyruvate were obtained from the results of geometry optimization between formaldehyde and an ammonium ion by *ab initio* MO calculations.[24] The distances between the proton from the σ direction and the carbonyl oxygen and between the proton from the π direction and the carbonyl oxygen were 1.66 and 1.73 Å, respectively.
24) H. Umeyama, S. Nakagawa, T. Nomoto, and I. Moriguchi, *Chem. Pharm. Bull.*, **28**, 745 (1980).

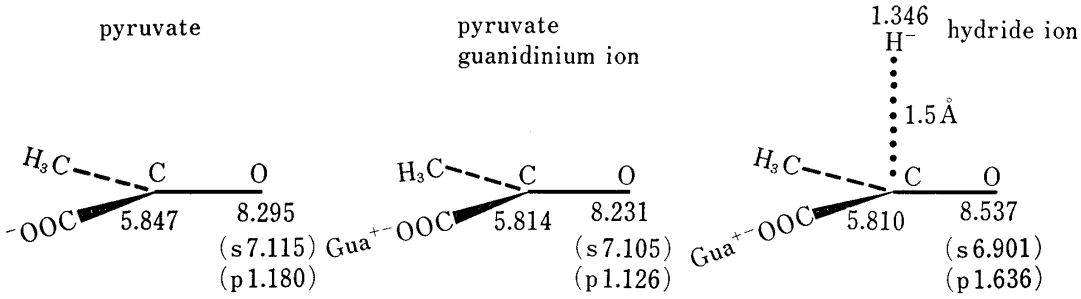

Fig. 9. Total Electron Densities of Pyruvate and the Pyruvate–Guanidinium Ion Complex

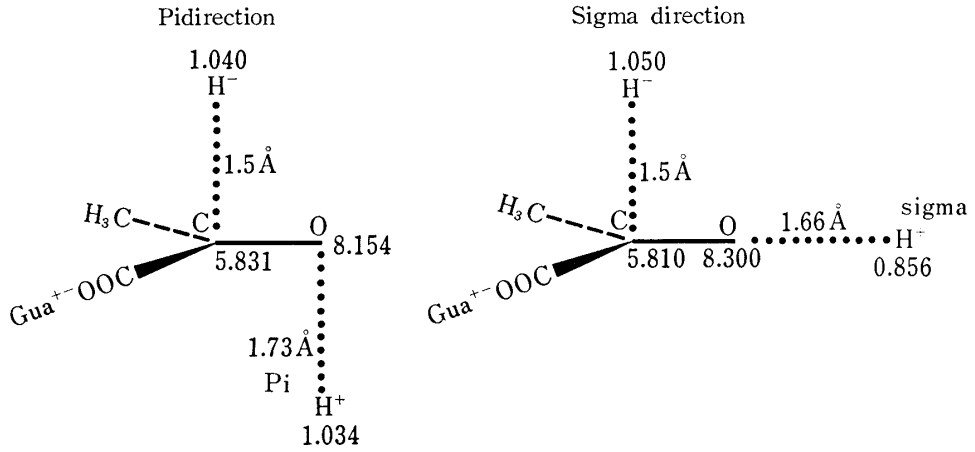

Fig. 10. Total Electron Densities of the Pyruvate–Guanidinium Ion Complex in which the Carbonyl Oxygen is attacked by the Proton from the π or σ Direction of the Carbonyl Plane

The interaction energies between the proton and the structure composed of structure I and hydride were calculated. The results are shown in Table VII, together with the energy decomposition analyses. Proton approach from the π direction is much more favorable than from the σ direction. The difference between the interaction energies is 67 kcal/mol, and it is largely due to ΔCT. In order to analyze the preferred approach from the π direction, σ (s) and π (p) total electron densities of the carbonyl group of the pyruvate moiety in structure I are shown in Fig. 9. The approach of hydride to the carbonyl carbon of pyruvate increases the total electron density by 0.306. The increase of the total π electron density contributes substantially to this. Electrons of hydride transfer to π orbitals of the carbonyl oxygen, and electrons in σ orbitals of the carbonyl oxygen decreases, probably due to the repulsive interaction between π and σ electrons. Figure 10 shows the total electron densities. Protons coming to the carbonyl oxygen from the π and σ directions receive 1.034 and 0.856 electrons, respectively. The larger increase of the total electron density of the proton from the π direction explains the greater interaction energy in the approach from the π direction, as shown in Table VII. In the enzymatic reaction, thus, the proton of His 195 will attack the carbonyl oxygen of pyruvate from the π direction.

Conclusion

1. The LDH-catalyzed enzymatic conversion of L-lactate to pyruvate with NAD^+ as a coenzyme and the reverse reaction with NADH as a coenzyme were simulated as shown in Figs. 4 and 6.

2. The carbonyl group of pyruvate will form two nearly parallel hydrogen bonds with

Arg 171. The electrophilicity of the carbonyl carbon of pyruvate increases substantially upon formation of the ion-pair complex.

3. Quantum chemical calculations indicated that the proton of His 195 approaches the carbonyl oxygen of pyruvate from the π direction of the carbonyl group, as shown in Fig. 6.

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