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Molecular Orbital Study of Lactate Dehydrogenase

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The mechanism of the enzymatic reaction of lactate dehydrogenase was studied from the quantum chemical point of view. The charge relay system composed of aspartate, histidine and lactate of the substrate or pyruvate enol of the inhibitor was assumed. The substrate or the inhibitor played a role as a proton donor like serine in the "charge relay system" of α-chymotrypsin. Moreover arginine 171 for the assumed charge relay system was very significant in order to lower the potential barrier of the proton transfer from the substrate or the inhibitor to histidine 195. Last the significance of the HOMO–LUMO interaction between the substrate or the inhibitor and nicotinamide in NAD^+ was shown.

Keywords——lactate dehydrogenase; molecular orbital; structure; charge relay system; enzymatic reaction; LDH; CNDO; ternary complex; pyruvate; lactate

In earlier papers we published the results of molecular orbital studies on the mechanisms of enzymatic reactions of α-chymotrypsin and papain. In α-chymotrypsin the "charge relay system" was necessary for the enzymatic reaction. The "charge relay system" was composed of aspartate (anion), histidine (neutral) and serine (neutral).[2] After a substrate approaches to the active site, the protons belonging to histidine (neutral) and serine (neutral) transfers to aspartate (anion) and histidine (anion), respectively.[2a] However there is not the "charge relay system" in the active site of papain. The active site of papain contains the hydrogen bond system composed of asparagine (neutral), histidine (neutral) and cysteine (neutral).[3] The proton transfer barrier from cysteine (neutral) to histidine (neutral) is very small, though histidine is neutral.[4d] Accordingly, since the proton transfer barrier from cysteine (neutral) to histidine (neutral) is much lower than that from serine (neutral) to histidine (neutral), there is asparagine residue in papain in place of aspartate (anion) in α-chymotrypsin.[4d] In other words the "charge relay system" is necessary to activate serine residue which attacks the substrate. Thiolsubtilisin contains the hydrogen bond system composed of aspartate, histidine and cysteine. The "assumed charge relay system" composed of aspartate (anions), histidine (neutral) and cysteine (neutral) is impossible, since the structure composed of aspartate (neutral), histidine (neutral) and cysteine (anion) is the most stable from the calculations.[4d] Those hydrogen bond systems in the active sites of α-chymotrypsin, papain and thiolsubtilisin are all composed of amino residues in those enzymes. On the other hand the active

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2) *a)* P.B. Sigler, D.M. Blow, B.W. Matthews, and R. Henderson, *J. Mol. Biol.*, **35**, 143 (1968); *b)* T.A. Steitz, R. Henderson, and D.M. Blow, *J. Mol. Biol.*, **46**, 337 (1969); *c)* J.J. Birktoft, B.W. Matthews, and D.M. Blow, *Biochem. Biophys. Res. Commun.*, **36**, 131 (1969); *d)* R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970); *e)* D.M. Blow, J.J. Birktoft, and B.S. Hartley, *Nature* (London), **221**, 337 (1969).

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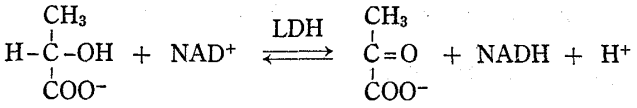
site in dogfish muscle lactate dehydrogenase, which has been studied by Rossmann, *et al.*[5] contains the hydrogen bond system composed of aspartate, histidine and lactate of the substrate or pyruvate enol of the inhibitor in place of serine residue in serine protease. There are two types of lactate dehydrogenases which are present in most animals,[6] and the heart(H) and muscle(M) type lactate dehydrogenase exist as tetramers.[6b,7] Dogfish M_4 lactate dehydrogenase is composed of four identical polypeptide chains with a molecular weight of 36000.[8] LDH catalyzes in the following scheme,



Each subunit is capable of binding one molecule of coenzyme and reacts independently.[9] NAD^+ bound to LDH is activated in the 4-position of the nicotinamide ring and reacts with cyanide some 400-fold faster than free NAD^+ at pH 7.[10] An essential histidine 195 in pig H LDH is 10-fold more reactive than typical enzyme histidine residues or free N-acetylhistidine,[11] and histidine 195 in Dogfish M_4 LDH is homologous to the essential histidine residue in pig H_4 LDH.[12] Moreover the significance of histidine in the LDH reaction was reported in many papers.[13] Arginine 171 as well as histidine 195 determines the specificity of the enzyme for L-lactate.[5,14] Rossmann and his associates tentatively suggest the mechanism described in Fig. 1(a).[14]

LDH is inhibited by the incubation with NAD^+ in the solution containing high pyruvate, and the possible mechanism is shown in Fig. 1(b).[15] The report by Coulson and Rabin

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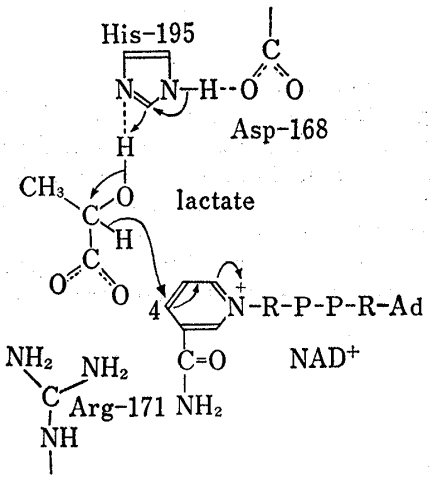

Fig. 1(a). Reaction Mechanism of Dogfish M_4 LDH tentatively Suggested by Rossmann and His Associates

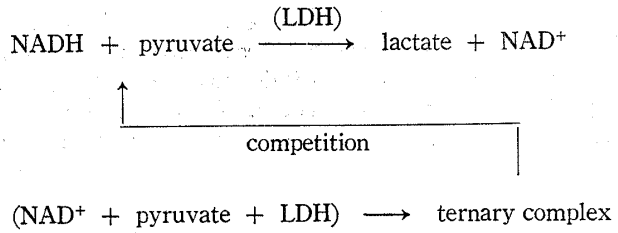

Fig. 1(b). Inhibition of LDH by the Incubation with NAD^+ in the Solution Containing High Pyruvate, and the Possible Mechanism

strongly indicated that the enol form of pyruvate is responsible for excess substrate inhibition,[16] and Griffin and Criddle showed that monomers of lactate dehydrogenase are necessary for abortive ternary complex formation whereas both the active enzyme and the stable inhibited enzyme are tatrameric.[17] The structures of the NAD^+-pyruvate complex was determined by nuclear magnetic resonance, infrared and ultraviolet spectroscopic methods.[18] Moreover the reactions between NAD^+ or the analogues and pyruvate or the analogues have been reported by other authors.[19] Various results of the experiments described above have been studied from the quantum chemical point of view.

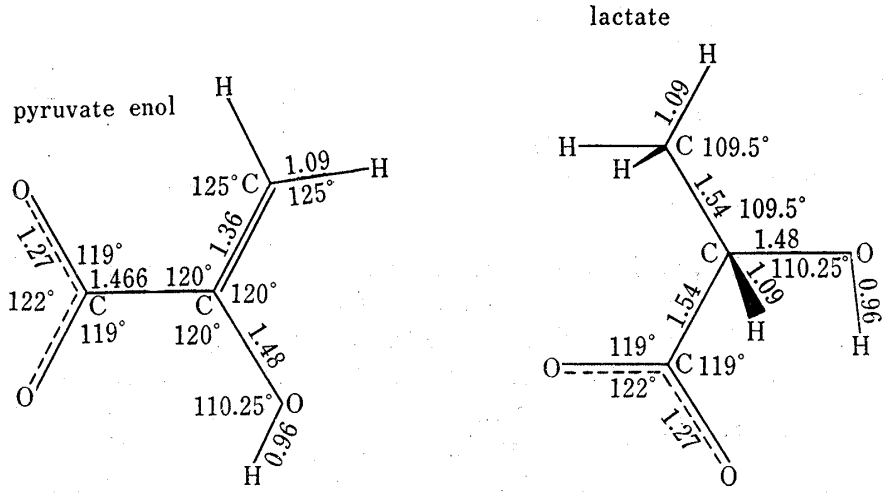

Fig. 2. Structures of Lactate and Pyruvate Enol

In the case of lactate sp^3 carbons are Tetrahedral.

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Method

The method used in our calculations is the CNDO/2 (Complete Neglect of Differential Overlap/2) method developed by Pople and Segal.[20] Calculations were carried out using a HITAC 8350 in the National Cancer Center and a HITAC 8700 and 8800 in the Tokyo University Computer Center. The stability of the electronic energy was employed as a check for convergence in the iteration calculation. Since it is not feasible to perform a calculation for the entire lactatedehydrogenase molecule, only the active site was considered. As our previous papers[2] $C(NH_2)_3$ and imidazole were used in place of arginine 171 and histidine 195, respectively. For $C(NH_2)_3$ the distances N–H and N–C are 1.0 Å and 1.33 Å, respectively. The structure of nicotinamide in NAD^+ was shown in the our previous paper.[21] The structures of lactate and pyruvate enol are shown in Fig. 2.[22]

Results and Discussions

Reaction among Lactate, Lactatedehydrogenase and NAD^+

Arginine 171 in the active site of lactatedehydrogenase interacts with lactate. The interacting structure is shown in Fig. 3. Table I shows the total energies at various separations between arginine 171 and lactate for the structure without histidine 157 in Fig. 3. At the separation 3.634 Å between C_1 in Arg-171 and C_4 in lactate the interaction energy was maximum. Histidine 195 interacts with lactate through hydrogen bond between N_1 in His-195 and HO- in lactate. The hydrogen bond system of aspartate 168, histidine 195 and lactate as shown in Fig. 1 is very similar to the "charge relay system" composed of Asp-102, His-57 and Ser-195 in the active site of α-chymotrypsin. Thus we assumed the "charge relay system" composed of Asp-168, His-195 and lactate.[23] The potential energies of the proton transfer from lactate to histidine 157 were calculated without arginine 171 for the structure as shown in Fig. 3. The distance 3.0 Å between N_1 in His-157 and O_3 in lactate was assumed in order

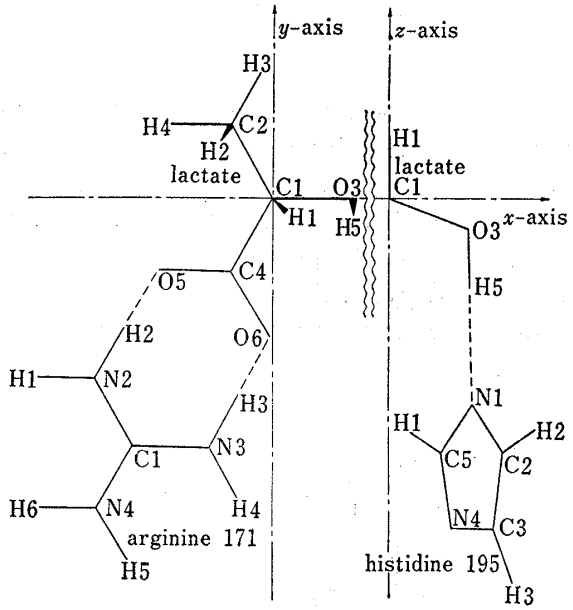

Fig. 3. Structures Composed of Lactate, Arginine 171 and Histidine 195

TABLE I. Total Energy for the Structure without Histidine 157 in Fig. 3 at Various Separations between Cl in Arginine 171 and C4 in Lactate

Separation (Å)	Total energy (eV)
3.334	−3447.18
3.434	−3450.38
3.534	−3451.89
3.634	−3452.40
3.734	−3452.37
3.834	−3452.05
3.934	−3451.62
4.034	−3451.16
4.134	−3450.74
4.234	−3450.35
4.334	−3450.02

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21) H. Umeyama, A. Imamura, and C. Nagata, *J. Theoret. Biol.*, **46**, 1 (1974).
22) L.E. Sutton and B. Phil, "Interatomic Distances," published by the Chemical Society, London, 1958; *Idem*, "Interatomic Distances Supplement," published by the Chemical Society, London, 1965.
23) We must calculate the proton transfer from His-157 to Asp-168. Since the coordinates of His-157 and Asp-168 is not exact completely, the various cases should be considered. These calculations are under investigation.

to compare with the "charge relay system" in α-chymotrypsin.[24] Table II shows the result. The potential barrier of the proton transfer from lactate to His-157 (anion) was 3.41 eV:

$$-345.90\text{eV} - (-3454.31\text{eV}) = 3.41\text{eV}$$

Since this potential barrier is very high in comparison with the potential barrier 2.46 eV[2d] from Ser-195 to His-57 (anion) in "charge relay system" of α-chymotrypsin, the proton transfer from the complex composed of Arg-171 and lactate to His-157 (anion) was calculated. The structure is shown in Fig. 3. Table III shows the results. The potential barrier from the

Table II. Total Energy for the Proton Transfer from Lactate to Histidine 157 without Arginine 171 at Various Separation from Lactate

Separation (Å)	Total energy (eV)
0.0	−3453.95
0.1	−3454.31
0.2	−3453.86
0.3	−3453.02
0.4	−3452.14
0.5	−3451.41
0.6	−3450.98
0.7	−3450.90
0.8	−3451.07
0.9	−3451.24
1.0	−3451.04

Table III. Total Energy for the Proton Transfer from the Complex Composed of Arginine 171 and Lactate to Histidine 157 (Anion) at Various Separations from Lactate

Separation (Å)	Total energy (eV)
0.0	−4724.67
0.1	−4725.08
0.2	−4724.68
0.3	−4723.95
0.4	−4723.22
0.5	−4722.72
0.6	−4722.59
0.7	−4722.82
0.8	−4723.24
0.9	−4723.60
1.0	−4723.51

complex composed of Ary-171 and lactate to His-157 (anion) was 2.49 eV. This value was almost same as the potential barrier of the "charge relay system" in α-chymotrypsin. Accordingly arginine 171 in lactatedehydrogenase plays an important role in lowering the potential barrier of the proton transfer from lactate to His-157 by 0.92 eV (=3.41 eV−2.49 eV). Since H_1 in lactate transfers from lactate to C_4 in nicotinamide as hydride ion, the highest occupied atomic orbital density and the orbital level were checked. The result is shown in Table IV.

Table IV. Highest Occupied Atomic Orbital Density and Orbital Level of Hl in Lactate at Various Separations of H5 in Lactate from Lactate for the Structure Composed of Lactate, Arginine 171 and Histidine 195 (anion)

Separation (Å)	HO atomic orbital density	HO orbital level (eV)
0.3	0.0125	−6.263
0.4	0.0146	−4.926
0.5	0.0366	−4.964
0.6	0.0496	−4.560
0.7	0.0513	−4.045
0.8	0.0460	−3.600
0.9	0.0410	−3.167
1.0	0.0321	−3.028

24) For the distance between N_1 in His-157 and O_3 in lactate the various values should be considered. However in this report only the value 3.0 Å was used in order to compare with serine-proteases. Those calculations will be given in our next report.

As the separation between H_1 and O_3 in lactate increases the orbital energy becomes more unstable, and hence the transfer of the hydride ion H_1 from lactate to C_4 in NAD^+ will be easy due to the interaction between the highest occupied molecular orbital in lactate and the lowest vacant molecular orbital in NAD^+. The frontier electron density of H_1 in lactate becomes larger as the separation between H_1 and O_3 in lactate increases. However, at the separation 0.7 Å from O_3 in lactate the density is maximum, and hence from the frontier electron density the structure where H_1 in lactate is placed at the distance 0.7 Å from O_3 in lactate is favorable for the hydride ion transfer from lactate to C_4 in NAD^+.[21] In Table V the coordinates of the interacting structure is shown.

Table V. Coordinates of the Structure Composed of Arginine 171, Lactate and Histidine 195

	x	y	z
Lactate			
H1	0.0	0.0	1.09
H2	−0.72532	1.25934	−1.60260
H3	−0.21158	2.14790	−0.14875
H4	−1.75316	1.25871	−0.14979
H5 (initial)	1.40914	0.00142	−1.45253
C1	0.0	0.0	0.0
C2	−0.72532	1.25808	−0.51260
O3	1.39561	0.00086	−0.49263
C4	−0.72583	−1.25718	−0.51406
O5	−1.97798	−1.20443	−0.71959
O6	−0.05408	−2.31520	−0.71959
Arginine 171			
H1	−4.07347	−3.01977	−1.64734
H2	−2.61649	−2.22826	−1.14662
H3	−0.62149	−3.38007	−1.14663
H4	−0.57847	−5.03761	−1.64734
H5	−2.51625	−6.09032	−2.31329
H6	−4.01625	−5.22429	−2.31329
C1	−2.40374	−4.16339	−1.70242
N2	−3.08781	−3.04461	−1.48043
N3	−1.09281	−4.19642	−1.48044
N4	−3.03059	−5.24913	−2.14638
Histidine 157			
H1	0.38120	1.84207	−3.93731
H2	2.52068	−1.85978	−3.93812
H3	2.13422	−1.12798	−6.46575
N1	1.43789	0.00261	−3.49233
C2	2.00685	−0.96212	−4.28178
C3	1.80904	−0.58754	−5.57628
N4	1.12303	0.59881	−5.54943
C5	0.90332	0.94712	−4.27627

Reaction among Pyruvate Enol, Lactatedehydrogenase and NAD^+

Arginine 171 interacts with pyruvate enol in place of lactate. The interacting structure is shown in Fig. 4. Table VI shows the total energies at the various separations between C_1 in Arg-171 and C_4 in pyruvate enol for the structure without histidine 195 in Fig. 4. The most stable structure obtained was at the separation 3.56 Å between C_1 in Arg-171 and C_4 in substrate. Histidine 195 interacts with pyruvate enol. In order to compare this result with that of the "charge relay system" of α-chymotrypsin the distance 3.0 Å between N_1 in

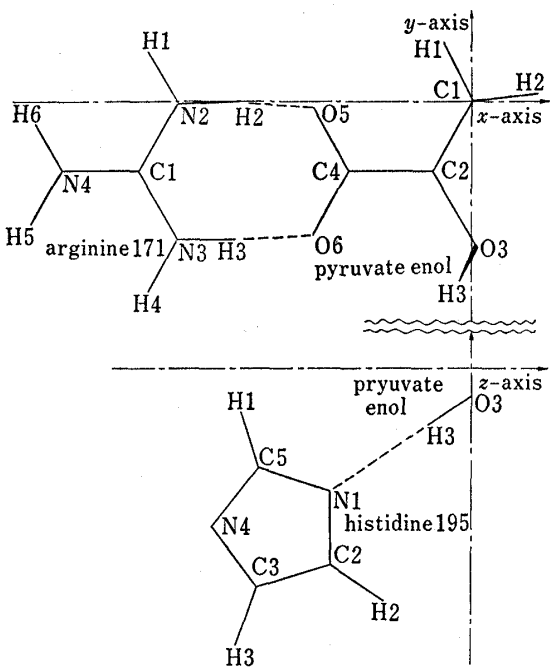

Fig. 4. Structures Composed of Pyruvate Enol, Arginine 171 and Histidine 195

TABLE VI. Total Energy for Structure Composed of Arginine 171 and Pyruvate Enol without Histidine 195 in Fig. 4 at the Various Separation between Cl in Arg-171 and C4 in Pyruvate Enol

Separation (Å)	Total energy (eV)
3.16	−3399.14
3.26	−3402.95
3.36	−3404.81
3.46	−3405.53
3.56	−3405.61
3.66	−3405.34
3.76	−3404.94
3.86	−3404.48
3.96	−3404.05
4.06	−3403.65
4.16	−3403.31

TABLE VII. Total Energy for the Proton Transfer from Pyruvate Enol to Histidine 195 (Anion) without Arginine 171

Separation (Å)	Total energy (eV)
0.0	−3407.28
0.1	−3407.66
0.2	−3407.21
0.3	−3406.41
0.4	−3405.55
0.5	−3404.87
0.6	−3404.51
0.7	−3404.48
0.8	−3404.70
0.9	−3404.90
1.0	−3404.72

TABLE VIII. Total Energy for the Proton Transfer from the Complex Composed of Arginine 171 and Pyruvate Enol to Histidine 195 (Anion) at Various Separations from Pyruvate Enol

Separation (Å)	Total energy (eV)
0.0	−4677.79
0.1	−4678.20
0.2	−4677.83
0.3	−4677.11
0.4	−4676.41
0.5	−4675.94
0.6	−4675.84
0.7	−4676.08
0.8	−4676.53
0.9	−4676.90
1.0	−4676.82

His-195 and H_3 in pyruvate enol was used. The potential energies of the proton transfer without arginine 171 in Fig. 4 were shown in Table VII. The potential barrier of the proton transfer from pyruvate enol to histidine (anion) is 3.18 eV at the separation 0.7 Å from O_3 in pyruvate enol. This barrier is very high in comparison with the potential barrier 2.46 eV[2d] from Ser-195 to His-57 (anion) in the active site of α-chymotrypsin. Since the proton transfer from pyruvate enol to His-195 (anion) is seemed to be possible, the proton transfer from the complex composed of arginine 171 and pyruvate enol to histidine 195 was calculated. Table VIII shows the result. Figure 4 shows the structure. The potential barrier is 2.36 eV and this value is almost same as the proton transfer barrier of the "charge relay system" in α-chymotrypsin. Accordingly arginine 171 is very significant amino acid residue to lower the potential barrier of the proton transfer from pyruvate enol to histidine 195; the lowered energy of the potential barrier was 0.82 eV (−3.18 eV—2.36 eV). Table IX shows the coordinates of the structure composed of arginine 171, pyruvate enol and histidine 195. Table X shows

Table IX. Coordinates of the Structure Composed of Arginine 171, Pyruvate Enol and Histidine 195

	x	y	z
Pyruvate enol			
H1	−0.46065	0.98788	0.0
H2	1.08585	0.09500	0.0
H3 (initial)	−0.16387	−2.97243	−0.78000
C1	0.0	0.0	0.0
C2	−0.68000	−1.17779	0.0
O3	0.06000	−2.45951	0.0
C4	−2.14600	−1.17779	0.0
O5	−2.76171	−0.06703	0.0
O6	−2.76170	−2.28856	0.0
Arginine 171			
H1	−5.61500	0.84004	0.0
H2	−4.11500	−0.02598	0.0
H3	−4.11500	−2.32961	0.0
H4	−5.61499	−3.19564	0.0
H5	−7.60999	−2.04382	0.0
H6	−7.60999	−0.31177	0.0
C1	−5.78000	−1.17780	0.0
N2	−5.11500	−0.02598	0.0
N3	−5.11500	−2.32961	0.0
N4	−7.10999	−1.17780	0.0
Histidine 157			
H1	−0.01801	−2.72563	−4.02594
H2	−1.47839	−5.89587	−1.55507
H3	−1.77941	−6.62030	−4.09640
N1	−0.63957	−4.06239	−2.43749
C2	−1.20459	−5.31116	−2.43377
C3	−1.35867	−5.68196	−3.73532
N4	−0.88483	−4.65260	−4.50571
C5	−0.45000	−3.67288	−3.70354

Table X. Highest Occupied Molecular Orbital and Atomic Orbital Density of C1 and C2 in Pyruvate Enol for the Structure Composed of Arginine 171, Histidine 195 and Pyruvate Enol at Various Separations of H3 in Pyruvate Enol from Pyruvate Enol

Separation (Å)	HO energy level of pyruvate enol (eV)	HO atomic orbital density of	
		C1	C2
0.0	−4.173	0.0011	0.0000
0.1	−4.257	0.0024	0.0000
0.2	−4.340	0.0046	0.0001
0.3	−4.623	0.0110	0.0005
0.4	−4.910	0.0311	0.0023
0.5	−4.962	0.0807	0.0082
0.6	−4.588	0.1279	0.0137
0.7	−4.034	0.1631	0.0167
0.8	−3.677	0.2012	0.0188
0.9	−3.299	0.2333	0.0203

the highest occupied molecular orbital and the atomic orbital densities of C_1 and C_2 in pyruvate enol. As the separation between the proton H_1 and O_3 in pyruvate enol increases the orbital level becomes higher, and hence due to the interaction between HOMO in the inhibitor and LUMO in NAD^+ the complex between pyruvate enol and NAD^+ will be formed. Atomic orbital densities of C_1 and C_2 in pyruvate enol become larger as the separation between H_3 and O_3 in pyruvate enol increases, and hence pyruvate enol becomes easier to react with C_4 in NAD^+[21] after the proton transfer from the inhibitor to histidine 195. In comparison with the frontier orbital densities of C_1 and C_2 in pyruvate enol, C_1 in the inhibitor is more active than C_2, and hence C_1 in the inhibitor and C_4 in NAD^+ is thought to react each other due to HOMO–LUMO interaction.

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