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Ab initio molecular orbital study of the flavin-catalyzed dehydrogenation reaction of glycine – protein transport channel driving hydride-transfer mechanism*

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Abstract. The reaction mechanism of flavin-catalyzed dehydrogenation of glycine has been studied by ab initio molecular orbital calculations using the 6-31G* basis set. 10-Methyl isoalloxazine (10-MIA) has been used as the flavin model compound. The results showed that when we assume a proton transport channel in amino acid oxidase, which is switched on by the substrate anion, the O12-protonated 10-MIA [10-MIAH$^+$(O12)] is generated. The main structure of 10-MIAH$^+$(O12) is one in which the central ring is expressed by an NAD$^+$-like structure, which is favorable for driving the hydride-transfer reaction, i.e., the abstraction of the α-hydrogen of glycine by the hydride-transfer mechanism. We have found that this protonation results in a dramatic lowering of the activation energy of the reaction. The proposed mechanism is summarized as follows: the hydride transfer proceeds via two-electron transfer and synchronous intramolecular proton transfer → intermolecular proton transfer.

Key words: Ab initio molecular orbital calculation – Flavin-catalyzed dehydrogenation – 10–methyl isoalloxazine – Proton transport channel driving hydride transfer – Flavin-glycine complexes

1 Introduction

Flavoproteins(flavoenzymes) catalyze various important biochemical reactions, such as oxidation (oxidase, oxygenase, dehydrogenase), reduction (reductase), electron transfer (both one-electron and two-electron transfers), etc [1]. Among more than 300 kinds of known flavoproteins, D-amino acid oxidase (DAO) is one of the most extensively investigated. DAO catalyzes the dehydrogenation reaction of D-amino acid.

The present work is concerned with the theoretical study of the mechanism of the flavin-catalyzed dehydrogenation (FCDH) of amino acid. The overall reaction of the FCDH of amino acid is expressed as

$$\mathrm{E \cdot FL + NH_2{-}CH'(R){-}COOH} \rightarrow \mathrm{E \cdot FLH_2 + NH{=}C(R){-}COOH}\ , \quad (1)$$

where FL and E represent flavin and apoprotein, respectively. H′ is the α-hydrogen of the substrate C_α—H bond. In general, the FCDH reaction is written as

$$\mathrm{E \cdot FL + SH_2} \rightarrow \mathrm{E \cdot FLH_2 + S}\ , \quad (2)$$

where SH_2 means a substrate. In spite of innumerable studies, the molecular mechanism of the FCDH reaction is far from understood [2]. Many proposals deal with the mechanism of "hydride transfer" from the substrate C_α—H bond [1]. In order to elucidate the molecular mechanism of the FCDH reaction, precise structural information on the active center of flavoprotein is very helpful. The three-dimensional structure of DAO at atomic resolution has recently been solved by Mizutani et al. [3] and independently by Mattevi et al. [4]. Miura et al. [5] proposed an "ionic mechanism" for the FCDH reaction, using their X-ray data of the three-dimensional structure of a substrate-like inhibitor bound active center [3].

In this paper, we report the following conclusive results obtained by ab initio molecular orbital (MO) calculations at the RHF/6-31G* level of theory.

1. In the neutral flavin (Chart 1), O12 is the protonation site, but N5 is a poor position for protonation (Table 1).
2. N5 of flavin is the hydride accepting site (Table 2).
3. The neutral flavin is a poor hydride acceptor. On the contrary, the protonated flavin is a better hydride acceptor than NAD$^+$ (Chart 2 and Table 5).
4. Carbanion is the best electron donor.

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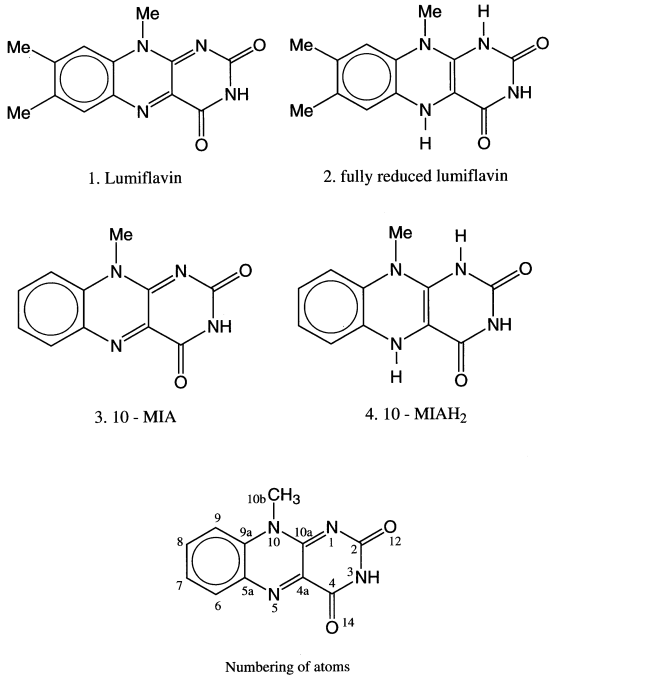

Chart 1

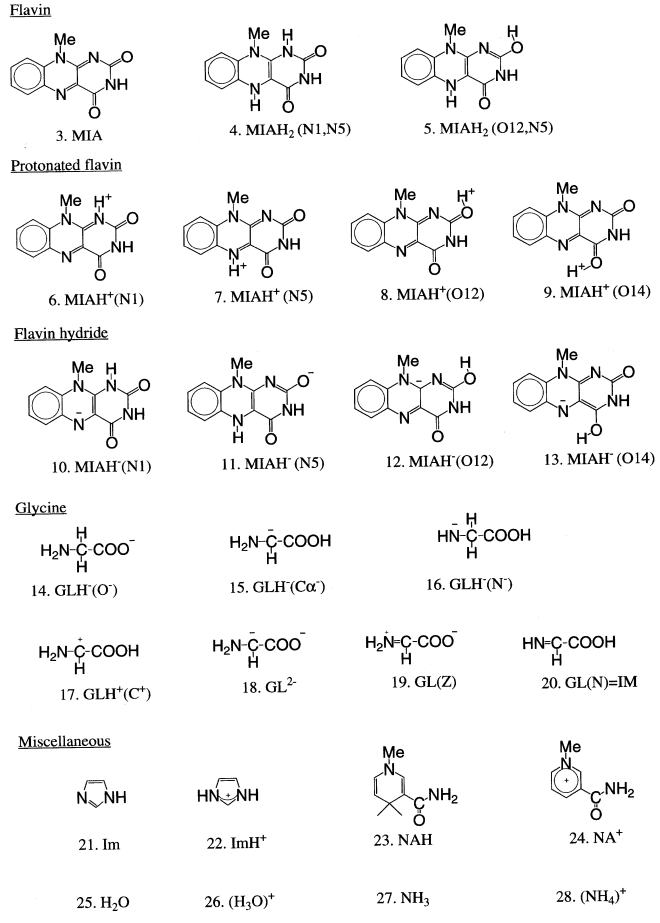

Chart 2

Based on these theoretical results, we studied the reaction mechanism of the FCDH of glycine, using 10-methyl isoalloxazine (MIA) as a flavin model compound.

In previous reports [6, 7], we obtained a "direct hydride-transfer mechanism" by ab initio calculations, starting from a $N5\cdots H'—C_\alpha$ hydrogen-bonded complex of 12-protonated flavin [$MIAH^+$(O12) in Chart 2] with glycine. We moved H′ toward N5, keeping the $N5\cdots C_\alpha$ distance constant (3 Å). Then at the middle point of the $N5\cdots C_\alpha$ distance (1.5 Å), two-electron transfer suddenly took place followed by proton transfer. The energy barrier for this reaction is 36 kcal/mol when we assume the starting state to be a "T-state" in enzyme chemistry [8].

In the present study, the complex at the $N5\cdots H'$ distance of 1.5 Å was partially optimized, freezing $MIAH^+$(O12) at the RHF/3-21G geometry. Then we obtained complex III shown in Fig. 1. In complex III, 1.7 negative charges [two-electron transfer ($2e^-$) + back-charge transfer of $0.3e^-$] are transferred from glycine to flavin. Furthermore intramolecular proton transfer, $NH_2—CH_2—COO^- \rightarrow NH_2—CH^-—COOH$, took place. This result suggests that hydride transfer should proceed by a new mechanism: "intramolecular proton transfer and synchronous two-electron transfer → intermolecular proton transfer".

We also examined other representative mechanisms: the "carbanion mechanism" proposed by Massey and Ghisla [9, 10] and the "ionic mechanism" of Miura et al. [5].

Table 1. Calculated proton affinities (kcal/mol) of 10-methyl isoalloxazine (*10-MIA*)

Process	Proton affinity[a]	
	RHF/6-31G*	RHF/6-31G*// 3-21G
6[b]. $MIAH^+$(N1) – 3. MIA	233.3	233.3
7. $MIAH^+$(N5) – 3. MIA	214.5	214.6
8. $MIAH^+$(O12) – 3. MIA	236.5	236.2
9. $MIAH^+$(O14) – 3. MIA	221.6	221.1

[a] Proton affinity of X is given by the difference of the total electronic energy of X and that of XH^+; 1 a.u. = 627.51 kcal/mol
[b] Number and total electronic energy should be referred to Table 3

Table 2. Calculated hydride affinities (kcal/mol) of some species at the RHF/6-31G* level of theory

Process	Hydride affinity[a]
10[b]. $MIAH^-$(N1) – 3. MIA	358.9
11. $MIAH^-$(N5) – 3. MIA	383.9
12. $MIAH^-$(O12) – 3. MIA	335.9
13. $MIAH^-$(O14) – 3. MIA	356.3
14. $MIAH_2$(N1, N5) – 6. $MIAH^+$(N1)	496.5
5. $M1AH_2$(O12, N5) – 8. $MIAH^+$(O12)	482.0
23. NAH – 24. NA^+	475.8

[a] Hydride affinity of X is given by the difference of the total electronic energy of X and that of XH^-; 1 a.u. = 627.51 kcal/ml
[b] Number and total electronic energy should be referred to Table 3

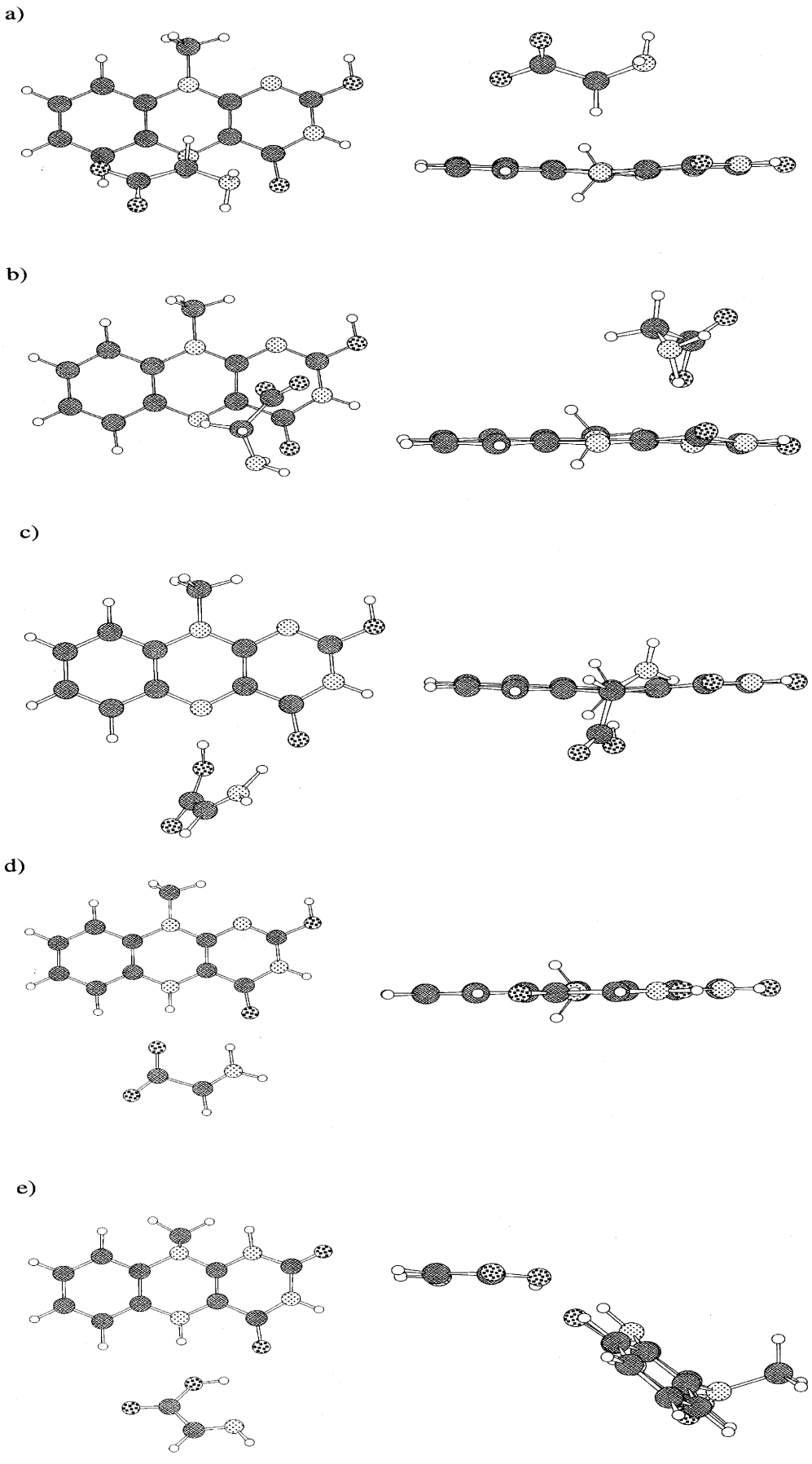

Fig. 1a–e. Optimized structures (full opt. or partial opt. at the RHF/3-21G level of theory) of reactant, intermediate, and product complexes. **a** Complex I (reactant), **b** complex II (C_α—H′ activated), **c** complex III [p^+ (intramolecular)-$2e^-$], **d** complex IV [p^+ (intermolecular)], **e** complex V (product)

2 Calculations

All calculations were carried out using the Gaussian 94 program [11]. The discussions in the present work are based on the results of RHF/6-31G*//6-31G* and RHF/6-31G*//3-21G calculations, because RHF/3-21G calculations usually overestimate hydrogen-bonding energies [12]. On the other hand, RHF/6-31G* calculations provide reasonable values for hydrogen-bonding energies and proton affinities [13]. As seen from Table 3, the relative energy calculated by the RHF/6-31G*//3-21G technique is almost the same as that calculated by the RHF/6-31G*//6-31G* technique. Due to the limitation of the computer facility, we cannot perform 6-31G*//6-31G* calculations for very flexible flavin-glycine complexes. We carried out the RHF/6-31G*//3-21G calculations for the study of the reaction pathway of FCDH and flavin-glycine complexes. The RHF/6-31G*//6-31G* technique was only used for the calculation of selected reaction intermediates.

In order to elucidate the reaction mechanism of the FCDH of amino acid, we used a simplified model system. The simplest amino acid, glycine, was chosen as a substrate.

Glycine in aqueous solution can, in the simplest way, be expressed as

Glycine: $H_2O\cdots H^+\cdots NH_2—CH_2—COO^-$,

where '$\cdots$' means hydrogen bonding.

Table 3. Calculated RHF/6-31G* and RHF/6-31G*//3-21G energies (a.u.) for the selected species (see Chart 2)

Species	Energy	
	RHF/6-31G*	RHF/631G*//3-21G
Flavin		
3. MIA	−788.773634	−788.771108
4. $MIAH_2$ (N1,N5)	−789.936579	−789.929545
5. $MIAH_2$ (O12,N5)	−789.918672	−789.916362
Protonated flavin		
6. $MIAH^+$(N1)	−789.145421	−789.142934
7. $MIAH^+$ (N5)	−789.115411	−789.113027
8. $MIAH^+$ (O12)	−789.150589	−789.147482
9. $MIAH^+$ (O14)	−789.126803	−789.123504
Flavin hydride		
10. $MIAH^-$ (N1)	−789.345547	−789.342902
11. $MIAH^-$ (N5)	−789.385235	−789.383442
12. $MIAH^-$ (O12)	−789.308976	−789.306466
13. $MIAH^-$ (O14)	−789.341404	−789.338791
Glycine		
14. GLH^- (O^-)	−282.253993	−282.251093
15. GLH^- (C_α^-)	−282.196285	−282.193649
16. GLH^- (N^-)	−282.191588	–
17. GLH^+ (C_α^+)	−281.990183	−281.987779
18. GL^{2-}	−281.382299	–
19. GL(Z)	−281.613129	−281.610187
20. GL(N)	−281.648158	−281.645820
Miscelleneous		
21. His	−225.196352	–
22. $HisH^+$	−224.814429	–
23. NAH	−454.642200	–
24. NA^+	−453.883957	–
25. H_2O	−76.010746	–
26. $[H_3O]^+$	−76.286564	–
27. NH_3	−56.184356	–
28. $[NH_4]^+$	−56.530770	–

The most widely used flavin model compound is lumiflavin, a 7,8-dimethyl isoalloxazine with a methyl group at N10 (Chart I). For the molecule (or complex) having a methyl group, the 6-31G* calculation sometimes met with serious difficulties at the geometry optimization, because the rotational barrier of the methyl group calculated by the 6-31G* basis set is very low [12]. In general, the optimization of the system having many low-frequency vibrational modes, which is a very flexible system, is rather difficult. For this reason, we used 10-MIA (Chart 1) instead of lumiflavin. The calculated total electronic energies and the optimized geometrical parameters for lumiflavins and MIAs (Chart 1) are summarized in Tables 4 and 5, respectively. As seen from Tables 4 and 5, the effect of the 7- and 8-methyls of lumiflavin on the electronic structure of the flavin framework is negligible. It should be noted that we cannot neglect the methyl group at N10 because, as shown in Fig. 2, the substituent at N10 plays an important role in the drastic geometrical change in the reaction pathway from complex IV to complex V, which seems to be responsible for the release of product. This is the reason why we use 10-MIA instead of the simpler isoalloxazine.

Table 4. Calculated RHF/6-31G* energies (total electronic energies) (a.u.) for lumiflavin, fully reduced lumiflavin, 10-MIA, and 10-$MIAH_2$

	RHF/6-31G* energy	ΔE^a
Lumiflavin	−866.848511	
Fully reduced lumiflavin	−868.009665	1.161154
10-MIA	−788.773634	
10-$MIAH_2$	−789.936579	1.162945

[a] Energy difference between the oxidized form and the fully reduced form

Table 5. The selected geometrical parameters (Å) for lumiflavin(1), fully reduced lumiflavin(2), 10-MIA(3), and 10-$MIAH_2$(4) at the RHF/6-31G* level of theory

	1	3[a]	2	4
Bond distance				
N1—C2	1.375	1.377	1.366	1.367
N1—C10a	1.283	1.282	1.382	1.382
C2—N3	1.398	1.397	1.375	1.376
C2—O12	1.190	1.190	1.195	1.194
C4—N3	1.364	1.364	1.383	1.383
C4—C4a	1.497	1.499	1.455	1.455
C4—O14	1.189	1.189	1.198	1.197
C4a—C10a	1.471	1.472	1.330	1.330
N5—C4a	1.266	1.264	1.404	1.403
N5—C5a	1.372	1.375	1.398	1.396
N10—C9a	1.387	1.388	1.439	1.438
N10—C10a	1.358	1.358	1.399	1.399
C5a—C9a	1.393	1.398	1.388	1.396
Bond angle				
∠ C(Me)—N10—N5	179.9	180.0	115.0	115.0
Dihedral angle				
∠ C4a—N5—N10—C9a	180.0	180.0	−151.9	−151.5

[a] For convenience of comparison, 3 is placed here

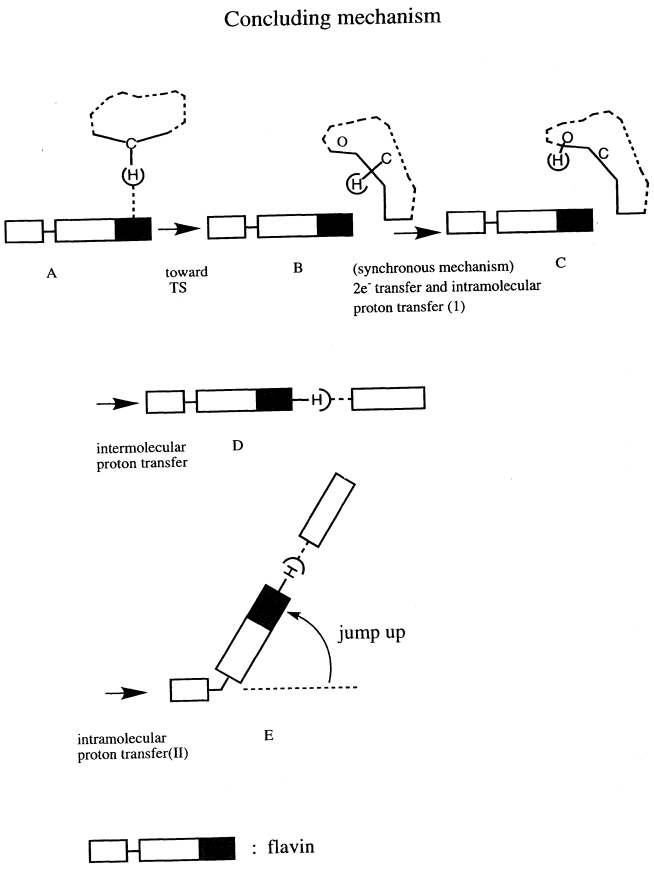

Fig. 2. Schematic description of the structural changes along the reaction pathway

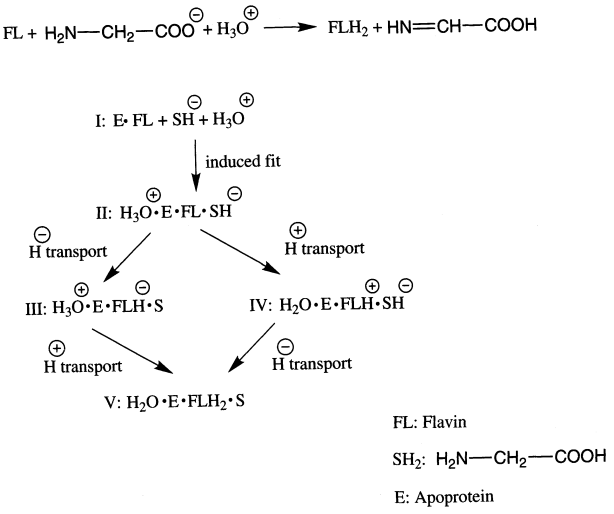

Fig. 3. Possible reaction schemes of the flavin-catalyzed dehydrogenation (*FCDH*) of amino acid: *FL*; flavin *SH*2; substrate *E*; apoprotein

There are two possible schemes for the reaction corresponding to Eq. (2). These are shown in Fig. 3. As we reported in previous work [6], the approach of the α-hydrogen (H′) of the substrate C_α—H′ bond toward N5 of flavin from any direction was very repulsive due to the exchange repulsion between the closed shells (Pauli exclusion principle). Therefore, we chose the pathway in which at the first stage of reaction, the protonated flavin is generated.

3 Results and discussion

In the FCDH reaction of amino acid, the α-hydrogen (H′) of the C_α—H′ bond of amino acid moves to N5 of the isoalloxazine framework of the flavin cofactor by a hydride-transfer mechanism. However, as we reported earlier [6], the approach of H′ to N5 of 10-MIA from any direction was always very repulsive, and no proton or hydride transfer was obtained. This means that the neutral flavin is a poor hydride acceptor, which is confirmed by ab initio MO calculations (Table 2). In order to solve this important problem, we investigated possible reaction pathways using the calculated RHF/6-31G* energies of selected species listed in Chart 2. Their energies are summarized in Table 3.

3.1 Proton affinity of 10-MIA

It is useful to know the effect of protonation on the reactivity of flavin and the protonation site. Of course, the site having the largest proton affinity (PA) is the protonation site. There are four possible protonation sites in flavin, i.e., N1, N5, O12, and O14. The calculated PAs are summarized in Table 1.

As seen from Table 1, the order of the PAs is O12 > N1 > O14 > N5. Thus our calculation identified that O12 is the protonation site for flavin. This is a rather surprising result, because usually the PA of nitrogen is larger than that of oxygen [12, 13].

Our result might be explained as follows. Flavin has a merocyanin partial structure, which is described by the resonance structures shown in Fig. 4. When O12 is protonated, the contribution of structure II should be enhanced, and the central ring of isoalloxazine takes a NAD^+-like structure. The calculated 6-31G* geometries of 10-MIA and O12-protonated 10-IMA shown in Figs. 5 and 6 confirm this expectation.

Since NAD^+ is a good hydride acceptor, it presides over the hydride-transfer reaction [8]. Therefore, it is

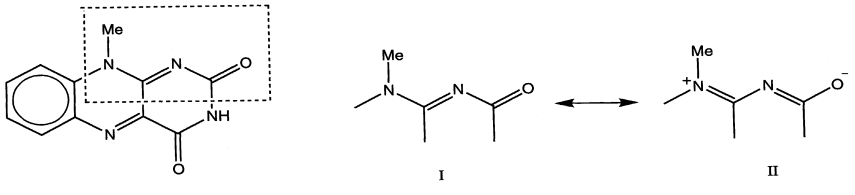

Fig. 4. The resonance of the merocyanine partial structure in the flavin

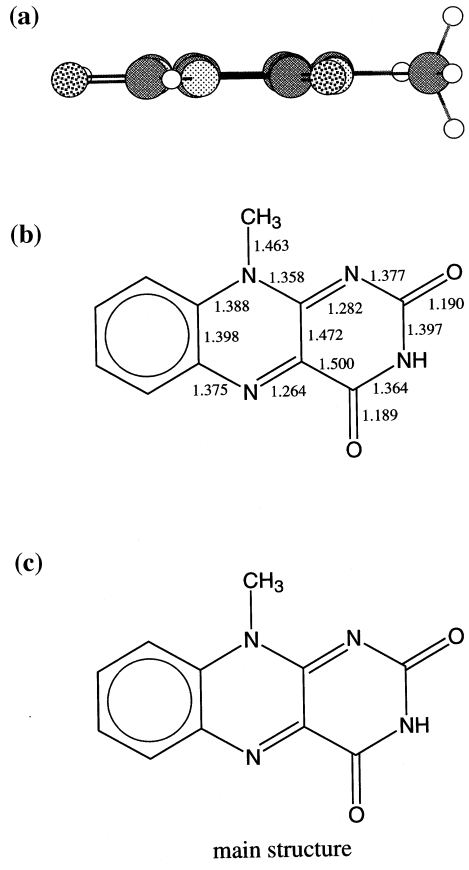

Fig. 5a–c. The optimized geometry of methyl isoalloxazine (*MIA*) at the RHF/6-31G* level of theory and the main structure

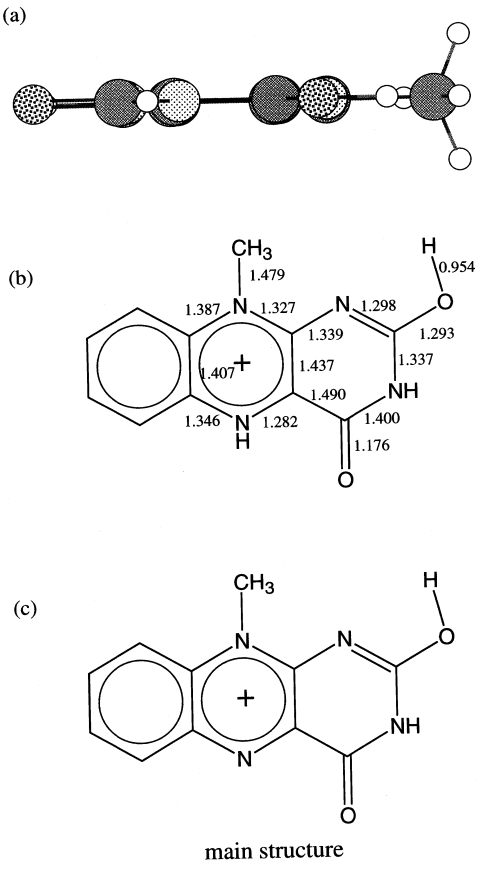

Fig. 6a–c. The optimized geometry of O12-protonated MIA at the RHF/6-31G* level of theory and the main structure

expected that 10-MIAH$^+$(O12) plays an important role in the hydride transfer of H′ in the course of the FCDH reaction.

3.2 Hydride affinity of flavin

From many experiments, it has been concluded that the fist stage of the FCDH reaction is the hydride transfer from the substrate C_α—H′ bond to N5 of flavin [1]. Therefore, in order to discuss the hydride-transfer mechanism, it is necessary to know the hydride affinity (HA) of flavin and the hydride-accepting site. There are four possible hydride-accepting sites in flavin, i.e., N1, N5, O12, and O14. The calculated HAs are summarized in Table 2.

For comparison, the HAs of protonated 10-MIAs and nicotinamid cation (NA^+), which is a model compound of $NAD^+$are also given in Table 2. Comparison of the HAs of 10-MIA and NA^+ makes it clear that the hydride-accepting ability of flavin is much less than that of NAD^+, thus flavin is not a good hydride acceptor. On the other hand, flavin protonated at O12 or N1 is expected to act as a good hydride acceptor, because the HAs of 10-MIAH$^+$(O12) and 10-MIAH$^+$(N1) are larger than that of NA^+. Thus, neutral flavin is a poor hydride acceptor, but the protonated flavin is a better hydride acceptor than NAD^+. As we reported in previous work [6], when the FCDH reaction starts from the O12-protonated flavin species, the energy barrier (activation energy) of reaction decreases drastically. Furthermore, when we moved H′ of glycine toward N(5) of 10-MIAH$^+$(O12) keeping the $N(5)\cdots C_\alpha$ distance constant (3 Å), two electrons were suddenly transferred to flavin from glycine at the middle region where the $N(5)\cdots H'$ distance was 1.5 Å (0.4 Å move of H′), and subsequently proton transfer followed.

The problem is how the protonated flavin is generated by a low-energy. According to our theoretical study of proton transport along a nonlinearly interacting hydrogen-bonded chain [15], an active proton (solitonic proton) is transported by an energy of only 0.3 eV = 7 kcal/mol. Therefore, if we can assume such a proton transport channel (PTC) in apoprotein, the PTC will be switched on by the substrate anion and the protonated flavin is generated in the following way:

$$\mathrm{E\cdot FL + H_3O^+\cdot NH_2—CH_2—COO^-}$$

$$\downarrow \quad \text{induced fit}$$

$$\mathrm{H_3O^+\cdot E\cdot FL\cdot NH_2—CH_2—COO^-}$$

$$\downarrow \quad \text{PTC is switched on}$$

$$\mathrm{H_2O\cdot E\cdot FLH^+\cdot NH_2—CH_2—COO^-}$$

where E and FL mean apoprotein and flavin, respectively. This is the first stage of reaction. In the present work, the substrate-enzyme complex (complex I; $FLH^+ \cdot NH_2—CH_2—COO^-$) of Scheme 1 is the starting point of the ab initio calculations of the reaction mechanism of the FCDH of glycine.

3.3 Flavin-glycine complex I (reactant)

We assume that the substrate is taken into the enzyme by the mechanism of the induced-fit theory [14]. DAO will recognize the substrate, D-amino acid, by three-points recognition (probably two hydrogen bonds and one CH-π interaction) generating the flavin-glycine complex. Kobayashi et al. [16] mentioned in their work that $CH \cdots \pi$ interaction acts as an important driving force for host-guest complexation.

Due to the limitation of the computer facility, we used RHF/6-31G*//3-21G calculations for the structural changes of the reaction intermediate. Why we used the 6-31G* basis set instead of the 3-21G basis set for the calculation of the total electronic energy is as follows. The hydrogen-bonding energy is overestimated in general at the RHF/3-21G level of theory. In contrast, RHF/6-31G*//3-21G calculations give reasonable values for both hydrogen-bonding energies and proton affinities [12]. It should be noted that E(RHF/6-31G*//6-31G*)$-$$E$(6-31G*//3-21G) gives the same order of energy (Tables 1, 3).

We constructed the structure of the flavin-glycine complex I shown in Fig. 1a, satisfying the three-points recognition model and the X-ray structure of the active center of DAO [3]. The structure of complex I is partially optimized by the RHF/3-21 G technique, freezing the structures of glycine and $MIAH^+$(O12) at their RHF/3-21G structures. The $N5 \cdots H'$ distance in the partially optimized structure is 2.1 Å. Since the van der Waals contact for the corresponding atomic pair is 2.75 Å [17], there is obviously $CH \cdots \pi$ interaction between N5 and the $H'—C_\alpha$ bond. The total electronic energy of complex I calculated by RHF/6-31 G*//3-21 G (partial) is -1071.505710 a.u. (Table 6), which is more stable by -0.107135 a.u. (67.2 kcal/mol) than the simple sum of the RHF/6-31G*//3-21G energies of the composite species.

This amount of stabilization came mainly from electrostatic interaction and partly from $CH \cdots \pi$ interaction. The structure of complex I was rather similar to that proposed by Miura et al. [5].

3.4 Flavin-glycine complex III (Michael complex ?)

As mentioned earlier, in our previous model of the hydrogen-bonded flavin-glycine complex [6], two-electron transfer took place suddenly and proton transfer followed at a $N5 \cdots H'$ distance of 1.5 Å. This result can be explained as follows. There are two kinds of possible potential energy surfaces (PES): one describes the PES of the dissociation reaction expressed by

$$MIAH_2(O12, N5) \rightarrow MIAH^-(O12) + H^+ \ ; \tag{3}$$

the other is the PES for the dissociation reaction

$$NH_2—CH_2—COO^- \rightarrow NH_2^+—CH—COO^- + H^- \ . \tag{4}$$

When these PESs cross each other at the middle region of $N5 \cdots C_\alpha$ (about 0.4 Å move of H′), two-electron transfer suddenly takes place followed by proton transfer. Our previous model, however, assumed a "T-state" in enzyme chemistry [8].

We performed the partial optimization at the RHF/3-21 G level for the geometry of the $MIAH^+(O12) \cdot NH_2—CH_2—COO^-$ complex, keeping the N5-H′ distance constant (1.5 Å). Then an unexpected complex III [$MIAH^-(O12) \cdot NH_2^+$=CH—COOH] shown in Fig. 1c is obtained, in which the following two kinds of hydrogen bonds are formed: one is $O14 \cdots H^+—NH =$ (approximately in-plane) and the other is $N5 \cdots H'—O—CO—$ (approximately vertical).

This complex should be generated by the following schemes.

The intramolecular proton-transfer reaction

$$NH_2—CH_2—COO^- \rightarrow NH_2—CH^-—COOH \tag{5}$$

takes place first, and is followed by two-electron transfer

$$MIAH^+(O12) \ + \ NH_2—CH^-—COOH \rightarrow MIAH^-(O12) \ + \ NH_2^+=CH—COOH \ . \tag{6}$$

Table 6. Calculated RHF/6-31G*//3-21G energies (a.u.), stabilization energies ΔE (a.u.), HOMO and LUMO energies and charge transfers for the flavin-glycine complexes

	RHF/6-31G*//3-21G energy		ΔE	HOMO	LUMO	ΔQ[b]	Notes
		Simple sum[a]					
Complex I	−1071.505710	−1071.398575	−0.1071	−0.28006	−0.02137	−0.9577	Partial opt
Complex II[c]	−1071.504126	–	–	−0.16938	0.08810	0.8010	Partial opt
Complex III	−1071.494165	−1071.294245	−0.199920	−0.19018	0.04651	0.7330	Partial opt
Complex IV	−1071.552149	−1071.526549	−0.025600	−0.21858	0.07363	−0.0035	Full opt
Complex V	−1071.584363	−1071.575365	−0.008998	−0.26532	0.10844	0.0041	Full opt

[a] Sum of RHF/6-31G*//3-21G energies of composite species (See Table 3)
[b] Net charge on the glycine part
[c] Structure of the glycine part is very deformed

Surprisingly, complex III is more stable than the previous transition state by about 60 kcal/mol. In complex III, a charge of 1.733*e* (Table 6) is moved from NH_2—CH^-—COOH to the flavin ring system. This means that back charge transfer ($\Delta q = 0.267e$ from $MIAH^-$ to NH_2^+=CH—COOH) takes place. Although, the geometry optimization of complex III at the RHF/3-21 G or the RHF/6-31 G* level of theory has not yet succeeded, the full optimized structure is obtained by the RHF/PM3 method. The N5$\cdots$H′ distance is 1.724 Å. The HOMO-LUMO gap of complex III is obviously smaller than those of complexes I, IV, and V (Table 6). Thus, complex III seems to be the "Michael complex" or the "purple complex" of the DAO · amino acid adduct suggested by Yagi and Osawa [18].

3.5 From complex III to complex IV

When we use the geometry of complex III with an N5$\cdots$H′ distance of 1.5 Å as the initial guess of the geometry optimization at the RHF/3-21 G level of theory, the optimization proceeds monotonously to the planar geometry of complex IV [$MIAH_2$(O12, N5) · NH_2^+=CH—COO^-, Fig. 1d] because the reaction pathway is down the hill of the PES. In the reaction pathway from complex III to complex IV the following intermolecular proton-transfer reaction takes place:

$$MIAH^-(O12) \cdot NH_2^+{=}CH{-}COOH \rightarrow MIAH_2(O12, N5) \cdot NH_2^+{=}CH{-}COO^- \qquad (7)$$

In this complex, two kinds of strong hydrogen bonds are formed; one is

$$O14\cdots H^+{-}NH{=}, \qquad [r(O14\cdots H^+) = 1.648 \text{ Å}] \ ;$$

the other is

$$N5 - H'\cdots O^-{-}CO{-} \qquad [r(H'\cdots O^-) = 1.732 \text{ Å}] \ .$$

The RHF/3-21 G geometry of complex IV shown in Fig. 7 is completely planar (Fig. 1d).

3.6 From complex IV to complex V (product)

When we perform the geometry optimization of the $MIAH_2$(N1, N5) · NH_2^+=CH—COO^- complex at the RHF/3-21G level of theory, we obtain the more stable complex V [$MIAH_2$(N1, N5) · NH=CH—COOH] shown in Fig. 8 which has a bent and jump-up structure shown in Fig. 1e. In the pathway from complex IV to complex V, two kinds of intramolecular hydrogen-transfer reactions take place by the concerted mechanism;

$$MIAH_2(O12, N5) \rightarrow MIAH_2(N1, N5) \qquad (8)$$

$$\text{and} \quad NH_2^+{=}CH{-}COO^- \rightarrow NH{=}CH{-}COOH \ . \qquad (9)$$

The energy barrier of the reaction in Eq. (8) is less than 10 kcal/mol when one H_2O molecule participates in this reaction [7]. This energy barrier will be overcome by the supply of the stabilization energy released (20.2 kcal/mol, Table 6) in the isomerization reaction from complex IV to complex V. It is interesting to note that complex V has a bent and jump-up structure (Fig. 1e). By this jump-up, the product (imino acid) can jump up by more than 7 Å from the planar position. Therefore this motion will help the release of product.

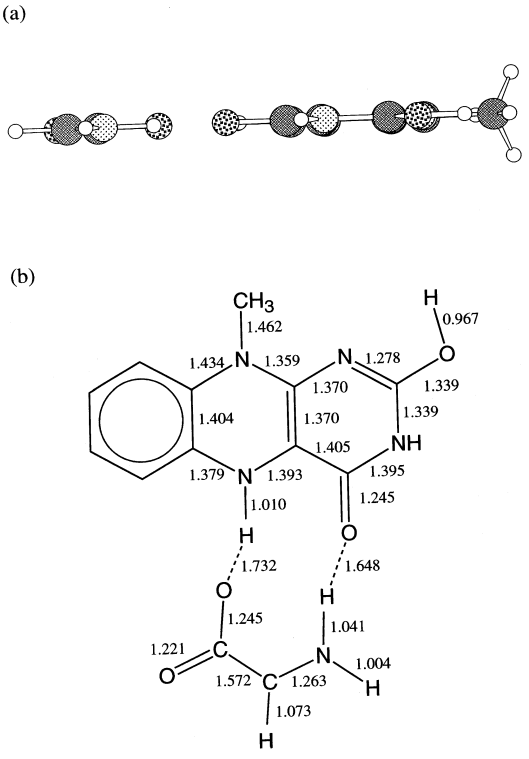

Fig. 7a, b. RHF/3-21G structure of complex IV. The complex takes the completely planar structure as shown in Fig. 1

3.7 Complex II (C_α—H′ bond activation)

A serious problem in the present study is that we cannot yet obtain the transition-state structure for the isomerization reaction from complex I to complex III. However, it is obvious that the isomerization reaction (Eq. 5) takes place by the activation of the C_α—H′ bond (lengthening of bond by the excitation of the vibrational state of the C_α—H′ bond). The reaction proceeds via a transient structure with $r(C_\alpha{-}H') = r(H'{-}O)$ of glycine (NH_2—CH_2—COO^-). When we perform the geometry optimization of this transient species in the free NH_2—CH_2—COO^- at the RHF/6-31 G* level of theory, we obtain the optimized value of $r(C_\alpha{-}H' = r(H'{-}O) = 1.3634$ Å. The total electronic energy of the transient activated complex II [$MIAH^+$(O12) · transient species] [O14$\cdots$H—NH— of transient species; r(O14$\cdots$H) = 1.80 Å] was −1071.3996 a.u. This is 66.6 kcal/mol higher than for complex I. A reasonable energy for complex II should be much lower. When we use an option of opt = (TS, noeig), another geometry is obtained. The total electronic energy of this transient species was −1071.504126 a.u., which is only 1.0 kcal/mol higher than for complex I. The N5$\cdots$H′ distance was 1.803 Å, which is only 0.079 Å longer than that of

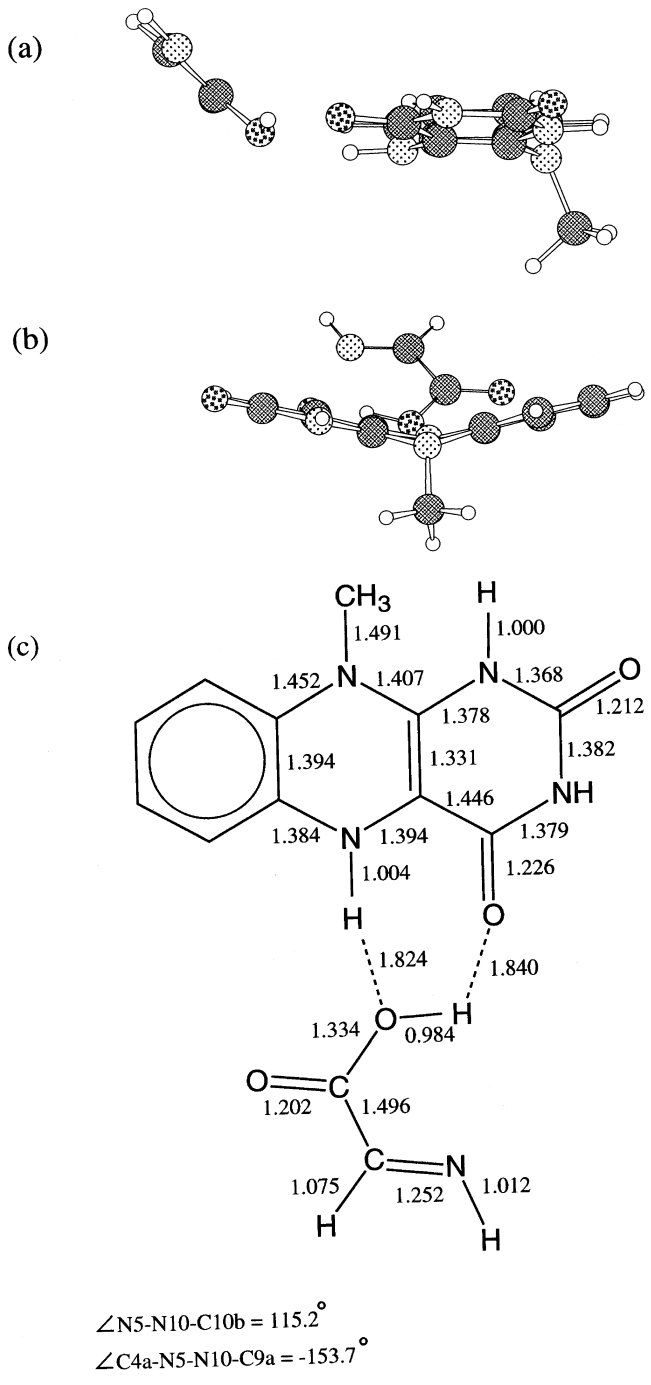

Fig. 8a–c. RHF/3-21G structure of complex V. The complex takes a bent and jump-up structure as shown in Fig. 1

the PM3-optimized geometry of complex III. Therefore, in this paper we refer to this intermediate as complex II.

3.8 Examination of other representative mechanisms

The reaction mechanism which came first to general attention is the "carbanion mechanism (p^+-$2e^-$)" proposed by Massey and Ghisla [9]. Their mechanism assumed that the first step of reaction is the proton abstraction of the substrate C_α—H′ bond by a protein base, such as histidine. Our calculation at the RHF 6-31G* level of theory shows that the calculated proton affinity of imidazole (the model compound of histidine) is 239.7 kcal/mol, (Table 1), while the deprotonation energy of NH_2—CH_2—COO^- is 547.0 kcal/mol (Table 3). Thus, the reaction system would become very unstable due to the instantaneous generation of a double-minus anion of amino acid. In order to overcome this instability, special interaction with apoprotein such as the neutralization of excess negative charge is necessary; however, at present, this problem remains unsolved.

The "ionic mechanism" proposed by Miura et al. [5] is also interesting because the model for this mechanism was constructed on the basis of their X-ray data of the active center of DAO [3]. We have calculated the energies of some flavin-glycine adducts shown in Chart 3, which are concerned with the first step of the ionic mechanism. The results are summarized in Table 7. As seen from Table 7, this mechanism seems to be energetically possible. However, the problem is that adducts 2 and 3 (see Chart 3) are more stable than adduct 1 (Miura's adduct) by 8.3 and 18.9 kcal/mol, respectively. Thus, if the mechanism via adduct 1 is possible, another pathway via adduct 3 is expected to proceed more easily. In a future paper, we will discuss this mechanism in detail.

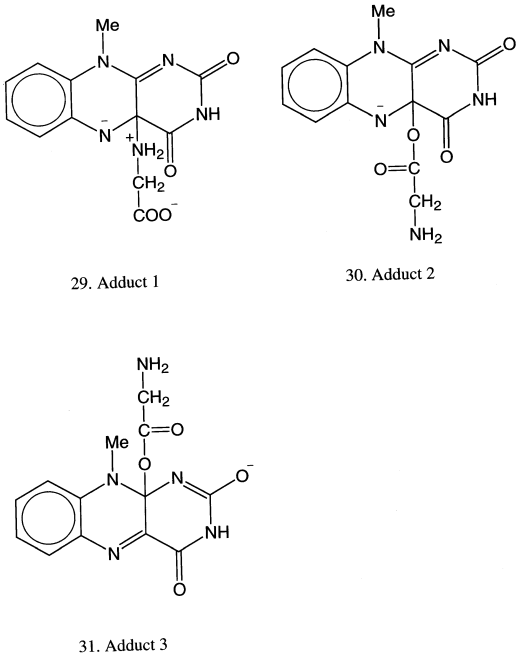

Chart 3

Table 7. Calculated RHF/6-31G*//3-21G energies (a.u.) for some possible reaction intermediates (see Chart 3)

	RHF/6-31G*//3-21G energy[a]
29. Adduct 1	−1071.000612
30. Adduct 2	−1071.013800
40. Adduct 3	−1071.030665
Sum of *E*s of 3 and 14 (Chart 2)	−1071.022201

[a] 1 a.u. = 627.51 kcal/mol

4 Concluding remarks

The structural, electronic and mechanistic aspects of the FCDH of amino acid for the model reaction

$$\text{10-MIA} + \text{glycine} \rightarrow \text{10-MIAH}_2 + \text{imino acid}$$

have been investigated by ab initio MO calculations at the RHF/6-31G* level of theory (full opt or opt with constraints). The conclusive mechanism obtained from the results of the present work can be summarized as follows.

1. Assumed mechanism before the reaction:

$$\mathrm{E \cdot FL + H_3O^+ \cdot NH_2{-}CH_2{-}COO^-}$$
$$\downarrow \quad \text{induced fit}$$
$$\mathrm{H_3O^+ \cdot E \cdot FL \cdot NH_2{-}CH_2{-}COO^-}$$
$$\downarrow \quad \text{PTC is switched on}$$
$$\mathrm{H_2O \cdot E \cdot FLH^+ \cdot NH_2{-}CH_2{-}COO^-}$$

2. RHF/6-31G* calculations for the model reaction:

$$\text{Complex I} \quad \mathrm{FLH^+(012) \cdot NH_2{-}CH_2{-}COO^-}$$
$$\downarrow \quad \text{two-electron transfer}$$
$$\text{Complex II} \quad \mathrm{[FLH^-(012) \cdot (NH_2{-}CH_2{-}COO)^+]}$$
$$\text{(front of TS)} \quad \text{(activation of } \mathrm{C_\alpha{-}H'} \text{ and C—C bonds)}$$
$$\downarrow \quad \text{intramolecular proton}$$
$$\text{TS(I)} \quad \text{transfer}$$
$$\downarrow$$
$$\text{Complex III} \quad \mathrm{[FLH^-(012) \cdot NH_2^+ = CH{-}COOH]^-}$$
$$\text{(almost TS)} \quad \text{(Michael complex ?)}$$
$$\downarrow \quad \text{intermolecular proton transfer}$$
$$\text{Complex IV} \quad \mathrm{FLH_2(012,N5) \cdot NH_2^+ = CH{-}COO^-}$$
$$\text{(planar structure)} \quad \downarrow \quad \text{intramolecular hydrogen}$$
$$\text{TS(II)} \quad \text{transfer and drastic}$$
$$\downarrow \quad \text{geometrical change}$$
$$\text{Complex V} \quad \mathrm{[FLH_2(N1,N5) \cdot NH{=}CH{-}COOH]}$$
$$\text{(bent and jump-up structure)}$$

The schematic description of the structural changes along the reaction pathway, the reaction pathway, and the energy diagram for the present model reaction are shown in Fig. 2, 9, and 10, respectively.

The rate-determining step for the model reaction used in the present study would be the step from complex I to complex III. The activation energy for TS(II) is less than 10 kcal/mol, but this energy may be

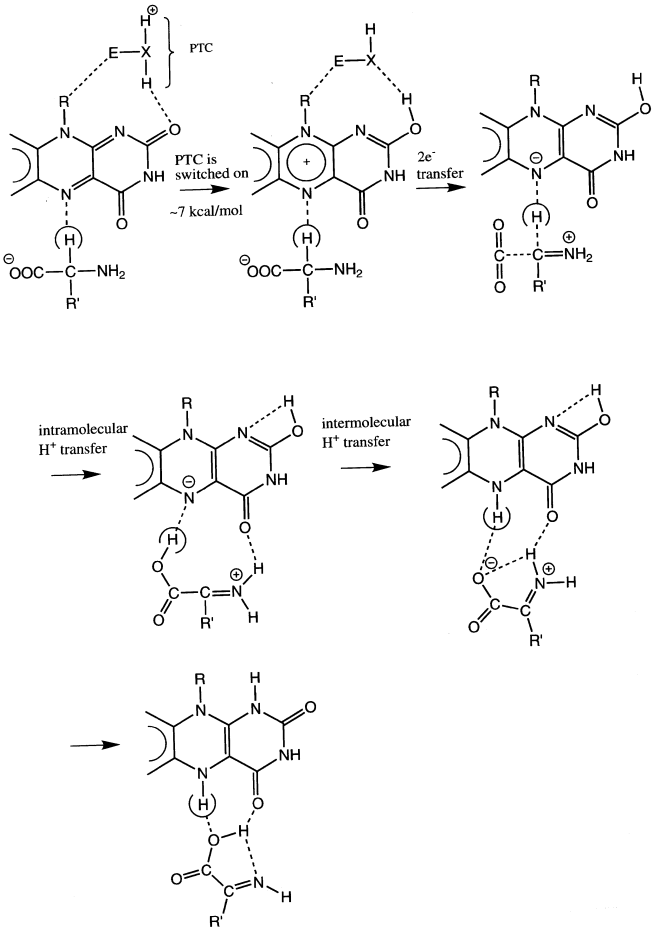

Fig. 9. FCDH mechanism obtained by the present study – proton transport channel (*PTC*) driving hydride transfer

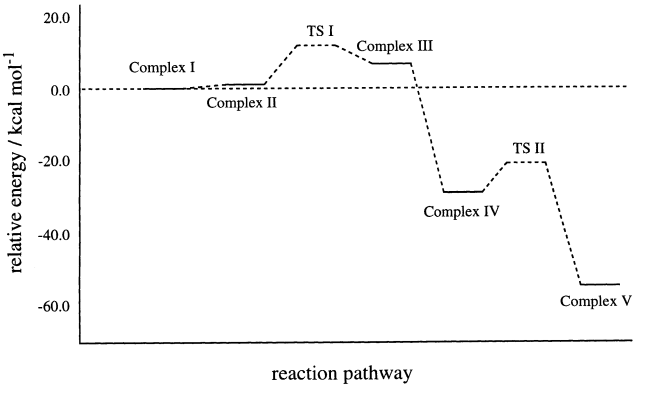

Fig. 10. Energy diagram for the present model reaction: *solid line*; calculated, *broken line*; estimated (see text)

supplied by the energy released on the pathway of the downhill reaction.

The present work was carried out using a simplified model system. Furthermore, because of the HF calculation, the effect of electron correlation cannot be taken into consideration. Apoprotein of DAO plays the decisive role in the FCDH reaction of amino acid, such as the energy supply/release, regulating the geometrical change of the flavin-substrate complex in the intermediate states etc. In the present study, we consider only the PTC in the apoprotein. Although we have not yet succeeded in determining the transition-state structure, there is a possibility of proton tunneling on the pathway from complex II to complex III. If this is possible, the effective activation energy will be lowered considerably. In spite of these simplifications, the present work may provide a reasonable reaction mechanism for the FCDH of amino acid.

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