Theor Chem Acc (1999) 103:109–116
DOI 10.1007/s002149900009

Theoretical
Chemistry Accounts

Regular article

Theoretical study on the lowest-frequency mode of the flavin ring

Sachiko Nakai[1,2], **Fumio Yoneda**[2], **Tokio Yamabe**[1,3]

[1] Institute for Fundamental Chemistry, 34-4 Takano-Nishihiraki-cho, Sakyo-ku, Kyoto 606-8103, Japan
[2] Fujimoto Pharmaceutical Corporation, 1-3-40 Nishi-Otsuka, Matsubara-shi, Osaka 580-8503, Japan
[3] Department of Molecular Engineering, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan

Received: 30 September 1998 / Accepted: 20 January 1999 / Published online: 7 June 1999

Abstract. The dynamic aspects along the normal vibrational motions of the lowest frequencies in the oxidized, radical, and reduced states of flavin (isoalloxazine) have been studied. In comparison with the twist motions in the oxidized state, the butterfly motions in the radical and reduced states turned out to bring more significant variations to the frontier molecular orbital energies and to the charge distributions on the atoms of the pyrazine ring in isoalloxazine. It can be considered that the electron transfers from and to the isoalloxazine ring can be adjusted or controlled by these variations. In the reduced states the electron release from the molecule, and in the radical states the electron release from or acceptance by the molecule, could be impelled by the butterfly motions, while in the oxidized state the electron acceptance by the molecule could be accelerated slightly by the twist motion.

Key words: Flavin ring – Isoalloxazine – Electron transfer – Twist motion and butterfly motion

1 Introduction

The flavin coenzymes, represented by flavin adenine dinucleotide or flavin mononucleotide, play essential roles in a wide variety of functions of the flavoproteins as oxidation–reduction catalysts in biological systems. These biocatalytic entities are the tricyclic flavin nuclei themselves, and, therefore, simple flavin derivatives have frequently been used as model compounds for investigations of flavin-catalyzed reactions. Although they have been studied extensively from chemical and biochemical points of view [1], it may be that theoretical studies [2] are still needed as a basis for deeper understanding of the physical and chemical properties as well as to provide insights into the function mechanisms of flavin.

The system used in this study is the simplest flavin ring, isoalloxazine, shown in Fig. 1. Isoalloxazine has three redox states, i.e. oxidized, radical, and reduced states, each of which includes anionic, neutral, and cationic species; however, the cationic species, which are not of biological relevance, were not treated here. Our preliminary studies, which are presented briefly in the Appendix have shown that the oxidized and radical species have planar conformations, while the reduced ones have butterfly conformations in the equilibrium states. These results were in good agreement with those of Zheng and Ornstein [3].

In this paper, we describe our theoretical investigation of unknown features of the flavin (isoalloxazine) ring system, focusing particularly on the relationship between the normal vibrational modes with the lowest frequencies and the frontier molecular orbitals which are directly relevant to the electron transfer from and to this molecule.

2 Method

The geometry optimizations were calculated by the ab initio method. We used the GAUSSIAN94 [4] program. All calculations were based on the 6-31G* basis set [5],using the restricted Hartree–Fock [6] method for the singlet states and the unrestricted Hartree–Fock [7] method for the radical states. The atomic charges were calculated by Mulliken population analysis [8]. The frequency analysis was computed in the GAUSSIAN94 program through the second derivatives of the energy with respect to the Cartesian nuclear coordinates.

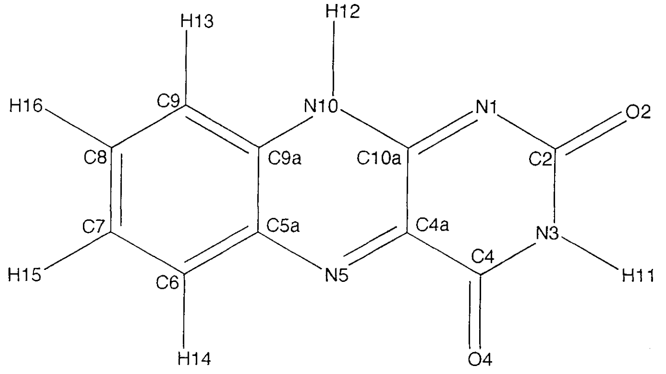
For the dynamic aspects along the vibrational motions of the molecule, we used the eigenvectors obtained from the frequency analysis to get the oscillating points of the molecule around the equilibrium positions and we executed a series of one-point ab initio calculations for fixed conformations of the molecule.

Since the normal coordinates are quantities of many values, we converted the normal coordinates to the angle representation for convenience, defining the bent angle α of the butterfly conformation of the molecule as indi-

---

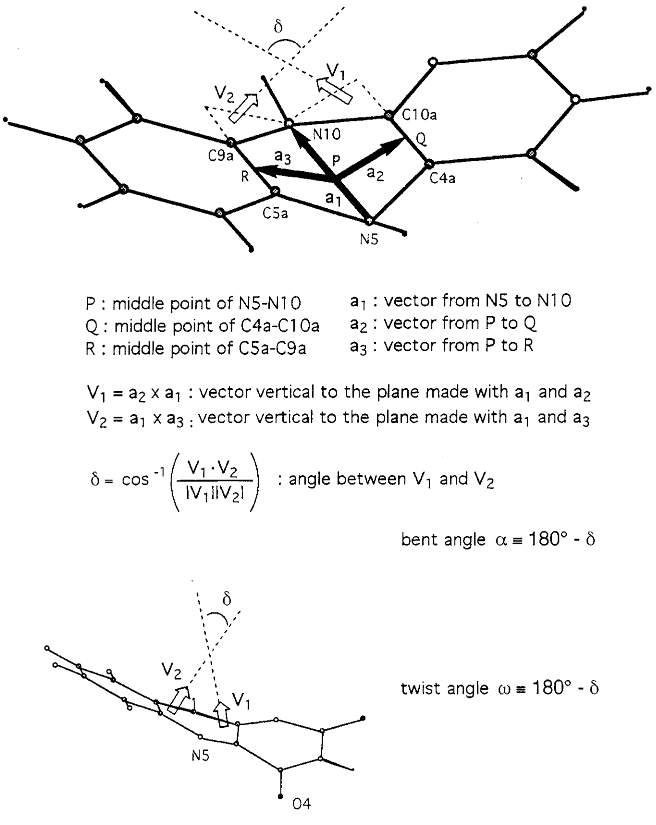
Correspondence to: S. Nakai

cated in Fig. 2. The same formula can be used for the twist angle ω of the twisted conformation of the molecule without problem.



states	code		electrons	charges
oxidized	F		110	0
radical	F^-		111	-1
	FH	: H17 on N5	111	0
reduced	FH^-	: H17 on N5	112	-1
	FH_2	: H17 on N5 and H18 on N1	112	0

Fig. 1. Isoalloxazines and notations



P : middle point of N5-N10 a_1 : vector from N5 to N10
Q : middle point of C4a-C10a a_2 : vector from P to Q
R : middle point of C5a-C9a a_3 : vector from P to R

$V_1 = a_2 \times a_1$: vector vertical to the plane made with a_1 and a_2
$V_2 = a_1 \times a_3$: vector vertical to the plane made with a_1 and a_3

$$\delta = \cos^{-1}\left(\frac{V_1 \cdot V_2}{|V_1||V_2|}\right)$$: angle between V_1 and V_2

bent angle $\alpha \equiv 180° - \delta$

twist angle $\omega \equiv 180° - \delta$

Fig. 2. Definition of the bent angle α and the twist angle ω

3 Normal vibrational modes of low frequencies

Figure 3 illustrates the normal vibrational modes of the five lowest frequencies of the oxidized (F), the radical (FH), and the reduced (FH_2) states of isoalloxazine. For the other species, F^- and FH^-, these were almost the same as the ones of FH and FH_2, respectively. It was observed that the lowest vibrational mode of the oxidized state was the "twist" motion, while the lowest ones of the radical and reduced states were the bent or "butterfly" motions. In all states, mode 2 was the twist motion. Taking the z-axis vertically to the isoalloxazine plane for the planer molecules, F and FH, modes 1–4 were all directed along the z-axis, i.e., motions perpendicular to the plane, while mode 5 had no z-component, i.e., motion in the plane. For the bent-form reduced state, it was not easy to check these directions, but except for mode 1 it can be seen from Fig. 3 that all states had quite similar modes 2–5. The frequencies of these five motions for all species studied are given in Table 1.

4 Dynamic variations along the lowest vibrational motions

When the nuclei are in oscillation, the molecular energies, orbital energies, and charge densities are known to vary according to the motion [9]. Although the GAUSSIAN94 program does not have the dynamic aspects, we have studied the variations in these values by calculating the static states of the molecule point by point along the eigenvectors of the normal modes, supposing that these values approximate the dynamic profiles of the molecule in motion. Thus, we examined the dynamic aspects of the lowest vibrational modes, i.e., the twist motion in the oxidized state and the butterfly motions in the radical and reduced states. The angle representations defined in Fig. 2 corresponded well to the normal coordinates of these vibrational modes. Figures 4–6 represent the results.

4.1 Hartree–Fock energies

The top graphs in Figs. 4–6 show the variations of the total Hartree–Fock energies along the twist and bent angles for the oxidized, radical, and reduced isoalloxazines, respectively. These curves seem to represent well the harmonic oscillators around the equilibrium positions. The equilibrium angles were 0° for oxidized and radical states, and those for FH_2 and FH^- of the reduced state were 16.1 and 27.1°, respectively: the equilibrium state of FH^- is more bent than that of FH_2. The positive and negative signs of the angle were just taken conventionally for the opposite sides of the flat plane (0°), and the reduced states actually had two equilibrium positions on both sides of the flat plane, representing the double-well potential. These two so-called convex and concave conformations had no difference energetically or in charge distributions, and saddle transition states which had totally flat conforma-

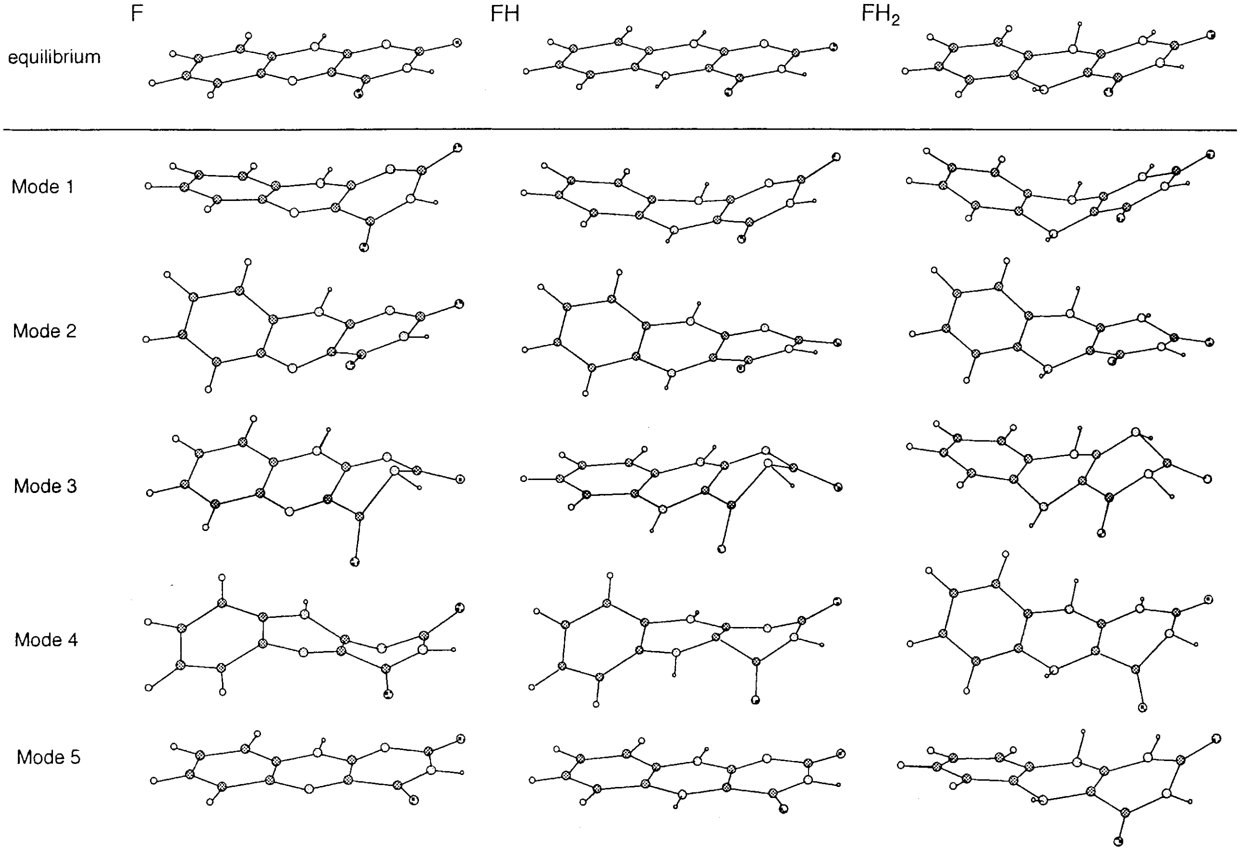


Fig. 3. The five normal modes of the lowest frequencies of neutral species of oxidized *F*, radical *FH*, and reduced states *FH*$_2$. The *top figures* are the equilibrium conformations

Table 1. The frequencies of the normal modes of the five lowest vibrations (cm^{-1}). The values are scaled by 0.89 of the GAUSSIAN94 output frequencies

	F	F$^-$	FH	FH$^-$	FH$_2$
Mode 1	55.43	54.08	56.12	49.56	29.46
Mode 2	90.14	80.47	79.36	87.10	87.36
Mode 3	124.46	148.48	142.84	149.09	138.79
Mode 4	168.97	177.63	170.76	157.97	158.56
Mode 5	190.18	187.56	179.82	204.31	171.79

tions were found between them. The depths of the double well were about 2 kcal/mol for both FH$_2$ and FH$^-$ species, which were regarded as the energy differences between the transition states with flat conformations and the equilibrium states with butterfly conformations (see Appendix).

Although the maximum value attained by the vibration depends on the temperature of the molecule, we deduced the maximum angle allowed for an oscillation around the equilibrium positions from the angle corresponding to the energy variation of this double-well depth. These angles were about 10 or 15° from the equilibrium positions of FH$^-$ or for FH$_2$, respectively. We applied this criterion to the other species for convenience and found from their respective equilibrium positions that about 5° for the twist motion in the oxi-

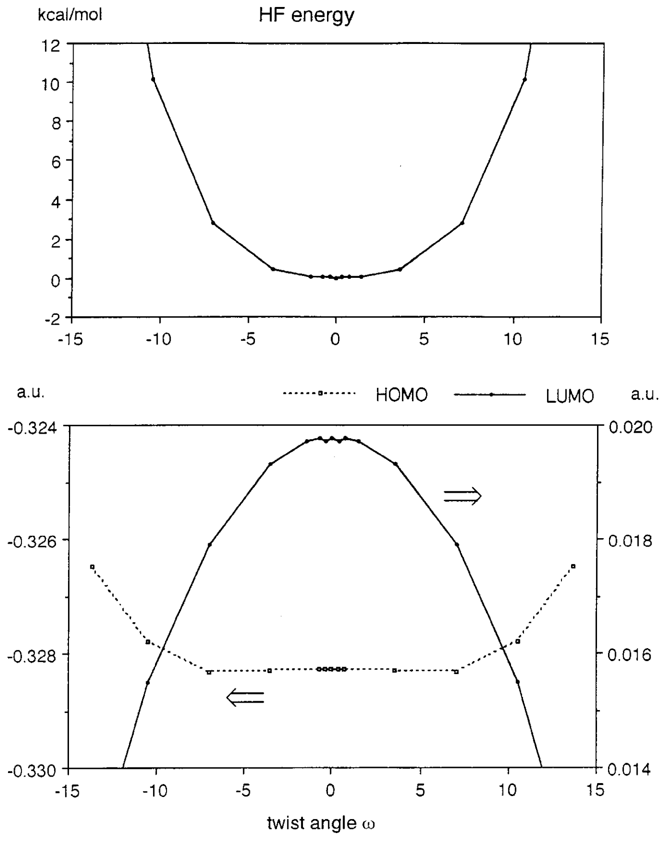


Fig. 4. The variations in the Hartree–Fock (HF) energies, the LUMO energies, and the HOMO energies of the oxidized species F. The abscissa is the twist angle ω in degrees

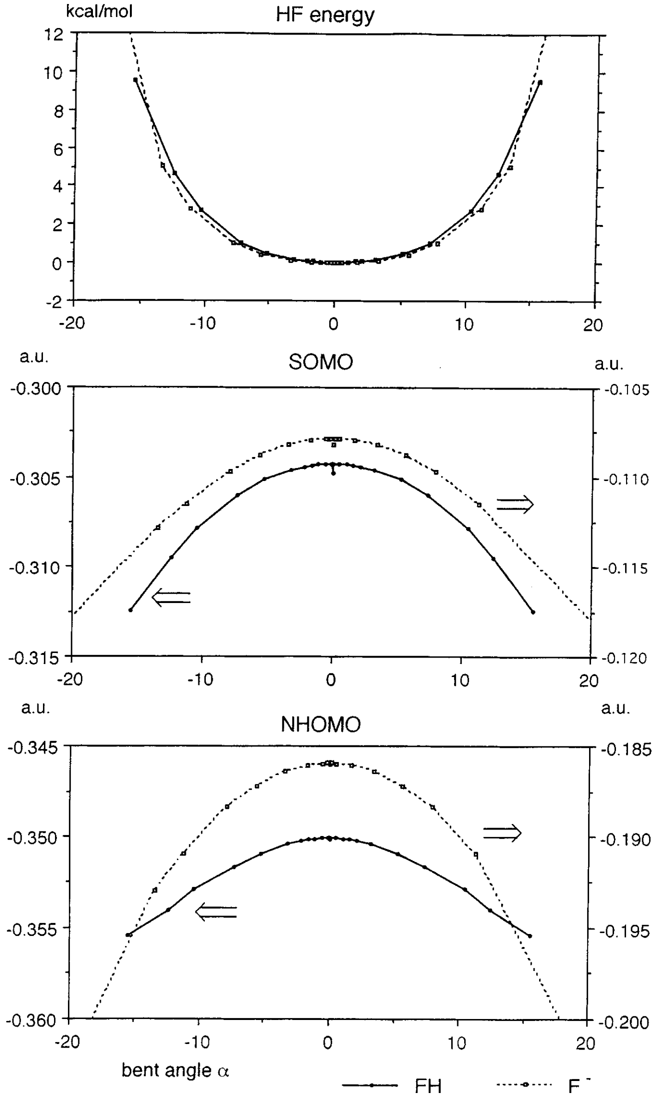


Fig. 5. The variations in the HF energies, the SOMO energies, and the NHOMO energies of the radical species FH and F^-. The abscissa is the bent angle α in degrees

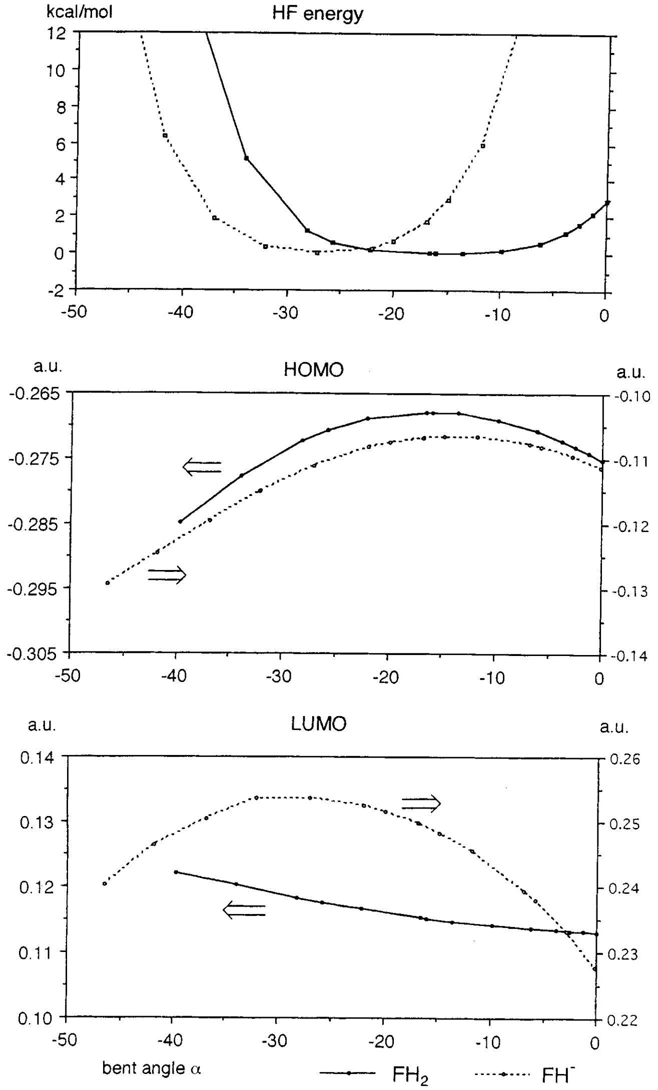


Fig. 6. The variations in the HF energies, the HOMO energies, and the LUMO energies of the reduced species FH^- and FH_2. The abscissa is the bent angle α in degrees, the sign of which is chosen optionally. The curves presented here are only approaches from one equilibrium position on the $\alpha < 0$ side and naturally curves on the $\alpha > 0$ side can be produced similarly

dized state and about 10° for the butterfly motions in the radical states were permissible. This criterion was not rationalized physically and methodologically but was quite reasonable from the viewpoint of the reliability of the calculated results, because it was observed that the Hartree–Fock energies varied largely suddenly after passing these maximum angles, as seen in Figs. 4–6.

4.2 Molecular orbital energies

The middle and bottom graphs of Figs. 4–6 represent the variations of the frontier molecular orbital energies. We also examined the variations of the other orbital energies and found that some did not change at all, some varied like the frontier orbitals, and some varied inversely to them, depending on the magnitude of the coupling [10] between the orbital and the normal mode concerned. Also, some energy-level exchanges were found around the extreme end of the oscillation. Even in the core orbitals large energy variations were found because of their being close to the nuclei in motion. As a consequence, the variations of the frontier orbital energies are considered to be representative and also to be important for influencing the electronic properties of the molecule. Thus, the largest variations of the frontier orbitals along the lowest vibrational motions were found at about 0.01 a.u. (6.3 kcal/mol), 0.005 a.u. (3.1 kcal/mol), and 0.001 a.u. (0.6 kcal/mol), in the HOMOs of the reduced states, in the SOMOs of the radical states and in the LUMO of the oxidized state, respectively, when their Hartree–Fock energies varied by about 2 kcal/mol. It was noticed that the variations accompanied with the butterfly motions were especially large in comparison to the one due to the twist motion. In other words, the HOMOs of the reduced states and the SOMOs of the radical states were coupled strongly with the butterfly motions.

In the reduced states, the energies of the HOMOs in both FH^{-} and FH_2 curiously have maxima at the bent angle of about 15°, in spite of the fact that the energy of the LUMO of FH^{-} has a maximum around the same angle as that of the equilibrium position, 27°, and that the energy of the LUMO of FH_2 changes little. This means that in the reduced states the electron leaves more easily in the butterfly conformation with a bent angle of about 15° than in the flat or more bent conformation. On the other hand, in the radical states, the energies of the SOMOs have maxima at the flat conformations and minima at their allowed extreme bent conformations of about 10°. In the radical states, the electron leaves the SOMOs easily in the flat conformations and comes into them easily in the butterfly conformations; thus, the butterfly conformations with a bent angle of 10–15° can be regarded as a special conformation for the couplings between the electron transfers and the vibrations.

4.3 Variations of charge distributions

An interesting feature regarding the charge distributions on the isoalloxazine ring under the butterfly vibrational motions was found. The variations of the Mulliken charges on the pyrazine ring atoms along the bent angles of the butterfly motions are shown in Fig. 7: the variations on the other atoms were much smaller compared to those are presented here. It is seen that on the nitrogen atoms, N5 and N10, the electron densities increase when the molecule approaches the flat conformation, while on some of the carbons forming the pyrazine ring, C5a, C9a, and C10a in FH^{-}, C4a and C5a in FH_2, and C9a and C10a in the radical species, the electron densities decrease as the conformations become nearly flat. Thus, the total charge displacements on the pyrazine ring by the butterfly motions, which we defined as the sum of absolute values of the charge differences on the six pyrazine ring atoms between the two conformations (here the nearly flat conformations and the most bent conformations in the limits of the oscillations defined previously) were about 0.6 in FH^{-}, 0.36 in FH_2, and 0.2 in the radical species.

We also found differences in the charge distributions between the two equilibrium states, the butterfly conformations of the energy minimum and the totally flat conformations of the saddle point (transition state) of the reduced states of the isoalloxazines. It is noted that the flat conformations attained dynamically by the oscillations are not exactly flat, namely “quasiflat”, in which the hydrogens on N5 and N10 are out of plane, while the transition states are totally flat. Figure 8 represents the differences in the charge distributions on all atoms of the reduced isoalloxazines, FH_2 and FH^{-}’ subtracting the ones of the butterfly equilibrium states from those of the flat transition states. The results show more clearly than in the dynamic studies the tendency for the electrons to move toward nitrogens N5 and N10 from carbons C4a, C5a, C9a, and C10a of the pyrazine ring with the change from the butterfly conformation to the flat one.

Consequently it can be said that the butterfly vibrational motion brings about very significant changes in the charge distribution of the pyrazine ring, particularly in the reduced states. No such large change can be seen in the twist motion.

5 Discussion

The present study is based on the supposition that the dynamics of the molecule can be approximated by a series of static molecular situations. At present there is no method for the dynamics treated with the vibronic system of the molecule, so we have used the static approach based on the Born–Oppenheimer approximation which separates the motion of the nuclei from the electronic states. Thus a series of electronic states were calculated for the series of static molecular conformations modified step by step along a reasonable direction: here the direction of the normal vibrational modes. In the limit of a certain neighborhood of the equilibrium position, the results obtained in a way such as for the molecular vibrational dynamics of the lowest-frequency mode have been found to be reliable enough to propose interesting and meaningful results.

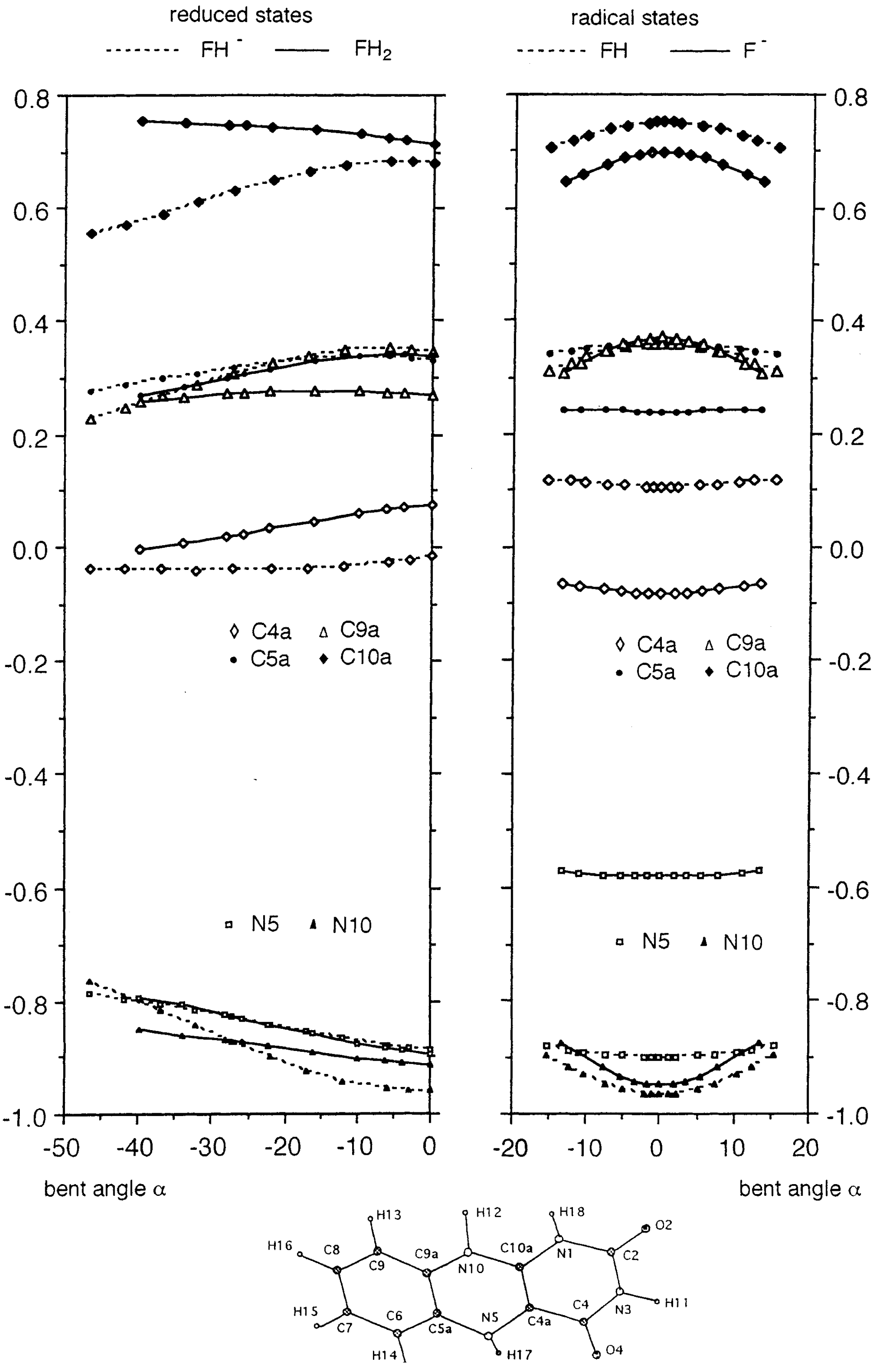
The bent and twist angles used instead of the normal coordinates, as defined in Fig. 2, represented well the normal coordinates and corresponded well to the actual torsion angles; for example, the torsion angles C9a-C5a-N5-C4a and C6-C5a-N5-C4a in the equilibrium state of FH_2 are 17.1 and 14.7°, respectively, and for FH^{-} these are 27.6 and 25.0°, respectively, while by our definition, the equilibrium bent angles of FH_2 and FH^{-} were $\alpha = 16.1$ and 27.1°, respectively.

The appreciable discontinuities at 0° in the curves of the SOMOs of FH and F^{-} in Fig. 5 originated from the insufficient precision of the input data for the calculations which were given numerically instead of the modification of the checkpoint file because of technical problems. For the other cases, these discontinuities at the equilibrium points were negligible; thus, we neglected these discontinuities in order to get the quantitative characteristics.

The HOMO energies of the flat conformations of the transition states in the reduced states, FH_2 and FH^{-}, were much higher (more than 10 kcal/mol) than those of the dynamically attained quasiflat conformations. In principle, the flat transition states for the inversions of the convex and concave equilibrium conformations have no relation to these quasiflat conformations, even though their conformations are very similar. The molecule could not reach the transition states simply by the normal vibrational motions in the ground states.

The normal modes with the negative frequencies of these transition states, which correspond to the reaction coordinates for the inversions of the equilibrium conformations, were not butterfly motions but just movements of the two hydrogens on N5 and N10, for both FH_2 and FH^{-}, with much higher frequencies than the butterfly motions; thus, the molecule needs further flip-

Fig. 7. The variations in the charge distributions on the pyrazine ring atoms under the butterfly motions of the radical and the reduced species



ping movement of these hydrogens to climb up to the saddle transition point of the flat conformation. Besides, as was discussed previously, the charge distributions in the dynamical quasiflat conformations were quite similar to those of the flat conformations of the transition states.

It must be noted that the vibrational modes 1–5 in Fig. 3 are in the most extreme points of the vibrations, quite exaggerated for the purpose of good recognition of the modes. For example, the picture of mode 1 of FH_2 corresponds to the state with a bent angle of about $\alpha = 40°$.

6 Conclusion

The study presented here gives a new profile of the isoalloxazine (flavin) ring system. The dynamic aspects

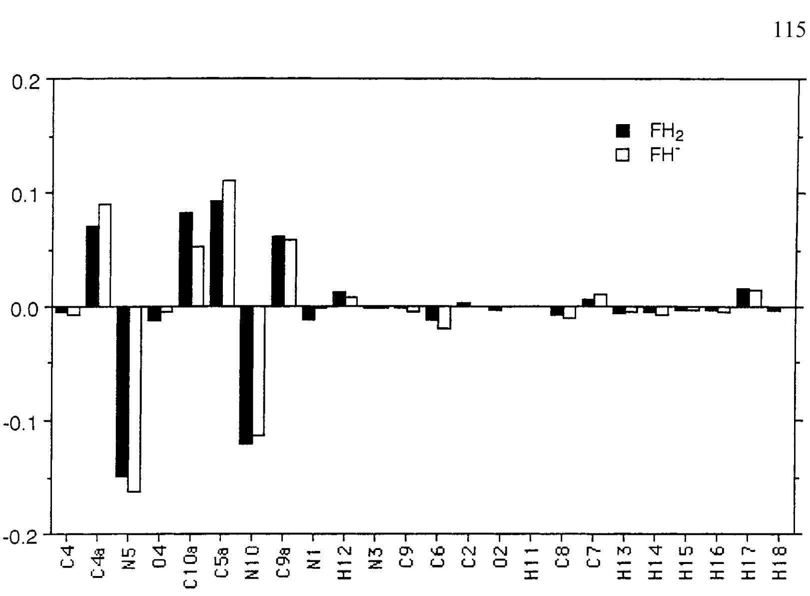
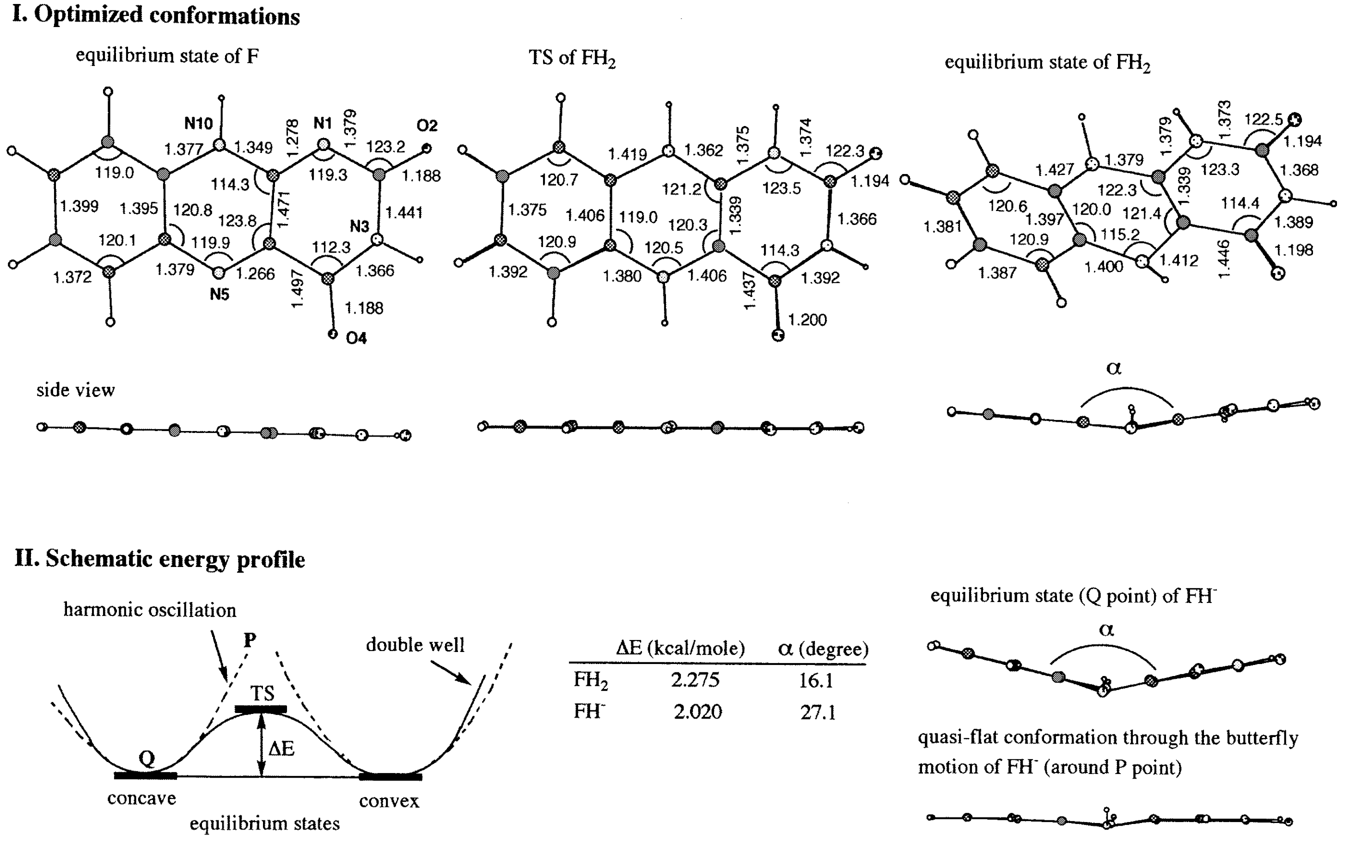


Fig. 8. The differences in the charges distributions between the flat conformations of the transition states and the butterfly conformations of the equilibrium states of the reduced states, FH^- and FH_2. The values are obtained by subtracting the charges of the butterfly conformation from those of the flat conformation



	ΔE (kcal/mole)	α (degree)
FH_2	2.275	16.1
FH^-	2.020	27.1

along the normal vibrational motions with the lowest frequencies have been studied. It was found that the butterfly motions in the reduced and the radical states brought significant variations to the frontier molecular orbital energies and charge distributions. In the reduced states, the HOMO energy has a maximum in the butterfly conformations with a bent angle of about 15°, from which the electron leaves more easily than in other conformations. For the radical states, when the molecule is bent at an angle of 10°, the SOMO energy is at its minimum at the extremity of the vibration and the electron gets into this orbital more easily than in other conformations. In addition, when the molecule is flat, the SOMO energy is at its maximum and the electron leaves this orbital more easily than in the butterfly conformations.

On the other hand, in both reduced and radical states, when the molecule comes into the planar conformation through the butterfly motions, the electron moves toward the nitrogens from the carbons in the pyrazine ring, while the charges on the other atoms are almost unchanged. In comparison with these results, the twist motion of the oxidized state results in considerable changes neither in the molecular orbital energies nor in the charge distributions. Consequently, the dynamic variations due to the butterfly motions are quantitatively large enough to adjust or control the electronic situations of the flavin ring system and thus these motions can accelerate the electron transfers in the system. In connection with these results, it is interesting to cite the fact that in a certain protein [11], very low frequency vibrational motions play important roles for reactions such as electron transfer.

Acknowledgement. This paper is dedicated to the late Professor Kenichi Fukui. The calculations of this work were done on the NEC/SX-4 supercomputer in the Institute for Fundamental Chemistry. While this work was in progress, Professor Fukui, who died on 9 January 1998, was still head of the Institute. S.N., who was a visiting research fellow in the Institute at that time, appreciates his advice very much.

Appendix

I The results from our preliminary studies and **II** a schematic profile of the dynamic simulation along the harmonic oscillation (*dotted line*) and the true feature of the energy variation (*solid line*) concerned with the butterfly motion.

References

1. (a) Müller F (1991) Chemistry and biochemistry of flavoenzymes, vol 1. CRC Press, Boca Raton, pp 1–71; (b) Walsh C (1979) Enzymatic reaction mechanisms. Freeman, San Francisco, pp 358–431; (c) Walsh C (1980) Acc Chem Res 13: 148; (d) Bruice TC (1980) Acc Chem Res 13: 256
2. (a) Hall LH, Mohney BK (1993) Quant Struct-Act Relat 12: 44; (b) Wouters J, Durant F, Champagne B, Andre J-M (1997) Int J Quantum Chem 64: 721
3. Zheng Y-J, Ornstein RL (1996) J Am Chem Soc 118: 9402
4. Frisch MJ, Trucks GW, Schlegel HB, Gill PMW, Johnson BG, Robb MA, Cheeseman JR, Keith TA, Petersson GA, Montgomery JA, Raghavachari K, Al-Laham MA, Zakrzewski VG, Ortiz JV, Foresman JB, Cioslowski J, Stefanov BB, Nanayakkara A, Challacombe M, Peng CY, Ayala PY, Chen W, Wong MW, Andres JL, Replogle ES, Gomperts R, Martin RL, Fox DJ, Binkley JS, Defrees DJ, Baker J, Stewart JP, Head-Gordon M, Gonzalez C, Pople JA, Gaussian 94. Gaussian, Pittsburgh, Pa
5. Hariharan PC, Pople JA (1973) Theor Chim Acta 28: 213
6. Rothan CC (1951) Rev Mol Phys 23: 69
7. Pople JA, Nesbet RK (1954) J Chem Phys 22: 571
8. Mulliken RS (1955) J Chem Phys 23: 1833
9. (a) Salem L (1969) Chem Phys Lett 3: 99; (b) Alper JS, Silbey R (1969) J Chem Phys 52: 569; (c) Pearson RG (1972) J Am Chem Soc 94: 8287
10. Lipari NO, Duke CB, Pietronero L (1976) J Chem Phys 65: 1165
11. Vos MH, Jones MR, Breton J, Lambry J-C, Martin J-L (1996) Biochemistry, 35: 2687