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HYDROGEN BONDING OF FLAVOPROTEIN

I. EFFECT OF HYDROGEN BONDING ON ELECTRONIC SPECTRA OF FLAVOPROTEIN

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Summary

The effect of hydrogen bonding on the transition energy and the oscillator strength of the isoalloxazine nucleus of flavins was studied by the molecular orbital method. Among the possible hydrogen bondings examined, characteristic spectral shifts were found for the hydrogen bondings at N(1) and N(5) of the nucleus. The hydrogen bonding at N(1) resulted in the shift of the first absorption band towards blue and that of the second one towards red. On the other hand, the hydrogen bonding at N(5) resulted in the shifts of both the first and the second band towards red.

The spectral characteristics reported on *Clostridium* MP and *Desulfovibrio vulgaris* flavodoxin coincided with the calculated results. The application of the calculated results to D-amino acid oxidase (D-amino acid: oxygen oxidoreductase (deaminating), EC 1.4.3.3) led to the conclusion that hydrogen bonding occurs at O(12), N(3)H, O(14) and N(5) of the isoalloxazine nucleus. The occurrence of hydrogen bondings at O(12), N(3)H, and O(14) is favorable for N(5) of the isoalloxazine nucleus to accept electron from an electron donor.

Introduction

Upon complex formation of riboflavin with phenol, the first absorption band of the isoalloxazine shifted towards red, and the interaction energy of riboflavin · phenol complex was 6.1 Kcal/mol. The interaction was ascribed to the hydrogen bonding between the isoalloxazine nucleus of riboflavin and hydroxyl group of phenol [1]. Therefore, the shift of the first absorption band of the isoalloxazine towards red observed in the reconstruction of flavoproteins

Abbreviation: VSIP, valence state ionization potential.

such as the old yellow enzyme [2] and D-amino acid oxidase (D-amino acid: oxygen oxidoreductase (deaminating), EC 1.4.3.3) [3] is considered to be ascribed to the occurrence of hydrogen bonding between the isoalloxazine nucleus of the coenzyme and the apoenzyme. Theorell and Nygaard [4] proposed the occurrence of hydrogen bonding in the complex formation between the coenzyme, riboflavin 5'-phosphate, and the apoenzyme of the old yellow enzyme. Later, Kotaki et al. [5] studied the effect of hydrogen bonding occurring on the isoalloxazine on its absorption spectrum by using a model system.

These results led us to make a theoretical approach to the relation between the occurrence of hydrogen bonding on possible groups of the isoalloxazine and its absorption spectrum. The present paper deals with the results obtained along this line in order to gain a better understanding of the significance of hydrogen bonding in flavoproteins.

Methods

Molecular orbital calculation. In Fig. 1, possible hydrogen bondings at the hetero atoms (nitrogen and oxygen) of the isoalloxazine are shown. In order to examine the effect of such hydrogen bondings on the electronic spectra of the isoalloxazine, we carried out self-consistent field molecular orbital calculation including configuration interaction based on π-electron approximation, using both variable β approximation [6,7] for a two-center core integral and the Nishimoto-Mataga formula [8] for a two-center electron repulsion integral. The values of valence state ionization potential (VSIP) were approximately estimated in a way similar to those reported previously [9]. Hydrogen bonding can be expressed as a resonance hybrid of the following structure:

$$X\text{-}H\text{---}Y \leftrightarrow (X\text{-}H)^{-}\text{---}Y^{+} \leftrightarrow X^{-}\text{---}(H\text{-}Y)^{+}$$

Therefore, the hydrogen bond formation has an effect of decreasing the value of VSIP of electronegative atom X and an effect of increasing that of electronegative atom Y. Accordingly, upon formation of hydrogen bonding, the values of VSIP of carbonyl oxygen and of pyridine type nitrogen (=N-) should increase, while that of pyrrole type nitrogen (-NH-) should decrease. To check the effects of the different values of VSIP on the transition energy of the isoalloxazine nucleus, we varied the value of pyridine type nitrogen, N(1) or N(5), for a rather wide range, viz. from 14.12 eV (no hydrogen bond) to 17.12 eV. The results are shown in Fig. 2. From this figure, one can expect that the difference in the value does not change the characteristics in spectral shifts, though the magnitude of the shifts differed. Since the main purpose of this study is to clarify the characteristic change in the electronic spectra and electronic structure of flavin upon formation of hydrogen bondings, the VSIP values of the hetero atoms of the isoalloxazine without hydrogen bondings [7] were modified by adding or subtracting an arbitrary value, 1.0 eV. The values thus obtained are summarized in Table I. Another series of values was also obtained by adding or subtracting 0.5 eV.

The excitation energies were calculated by the method of configuration interaction. All singly-excited configurations were allowed to interact.

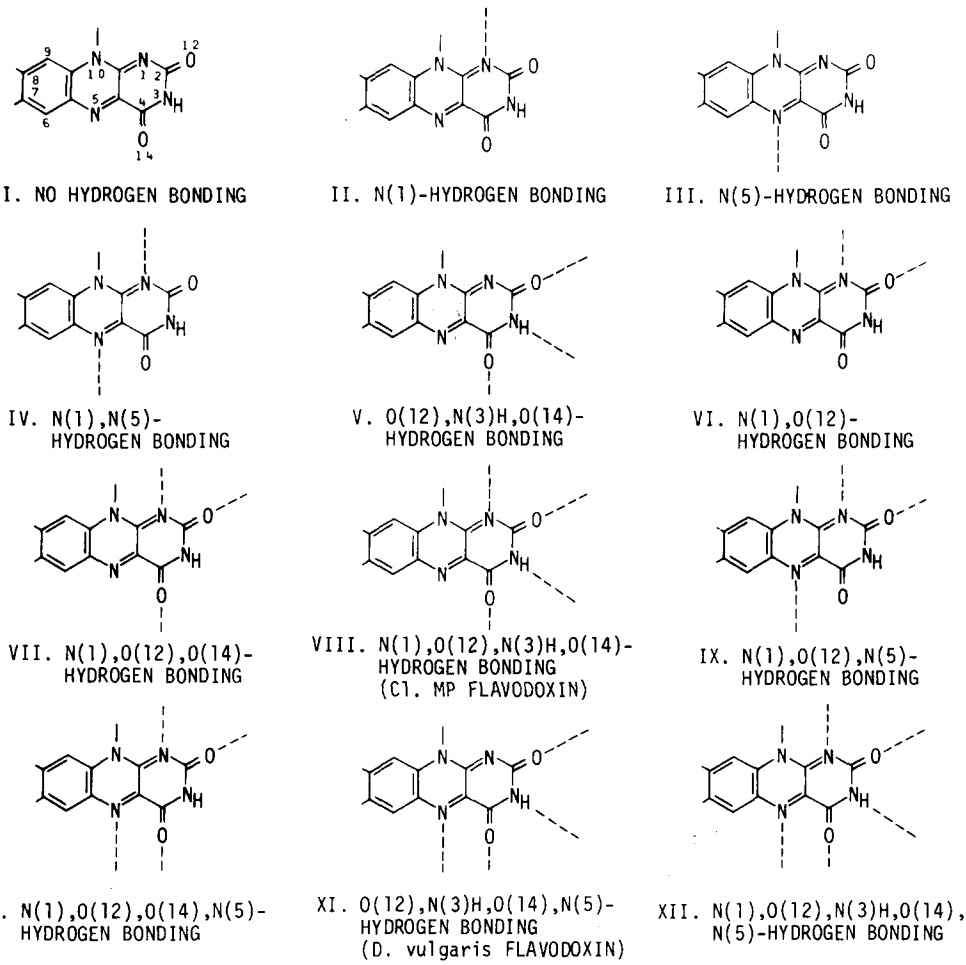

Fig. 1. Possible structures of hydrogen-bonded isoalloxazine.

TABLE I

PARAMETERS (VSIP) FOR THE MOLECULAR ORBITAL CALCULATION OF HYDROGEN BONDED ISOALLOXAZINE

VSIP values were indicated in eV. Numbering of atoms of the isoalloxazine is illustrated in Fig. 1, Type I. These parameters were estimated by considering a resonance hybrid of hydrogen bonding as described in text.

Type of hydrogen bonding	Atom						
	N(1)	N(3)	N(5)	N(10)	O(12)	O(14)	C
I	14.12	26.70	14.12	26.70	17.70	17.70	11.16
II	15.12	26.70	14.12	26.70	17.70	17.70	11.16
III	14.12	26.70	15.12	26.70	17.70	17.70	11.16
IV	15.12	26.70	15.12	26.70	17.70	17.70	11.16
V	14.12	25.70	14.12	26.70	18.70	18.70	11.16
VI	15.12	26.70	14.12	26.70	18.70	17.70	11.16
VII	15.12	26.70	14.12	26.70	18.70	18.70	11.16
VIII	15.12	25.70	14.12	26.70	18.70	18.70	11.16
IX	15.12	26.70	15.12	26.70	18.70	17.70	11.16
X	15.12	26.70	15.12	26.70	18.70	18.70	11.16
XI	14.12	25.70	15.12	26.70	18.70	18.70	11.16
XII	15.12	25.70	15.12	26.70	18.70	18.70	11.16

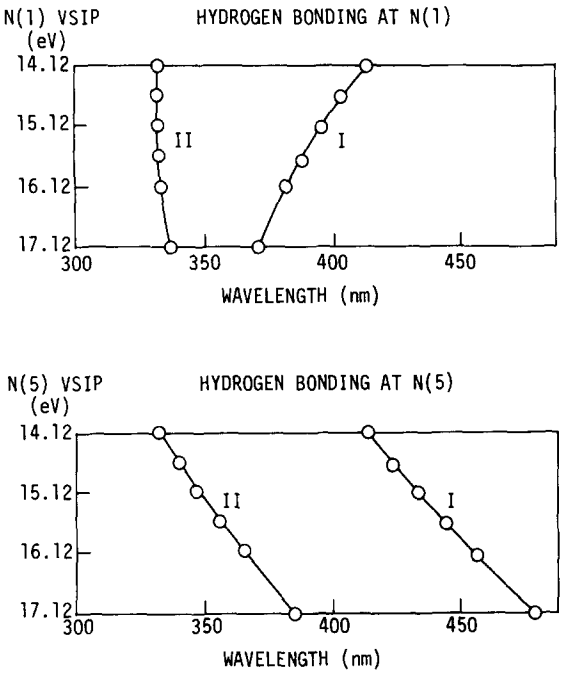

Fig. 2. Dependence of the wavelength of absorption bands on the values of VSIP upon formation of hydrogen bonding at N(1) or N(5). The calculated values of the first and the second absorption band in wavelength were plotted against the values of VSIP. Upper figure, for the case of hydrogen bonding at N(1); lower figure, for the case of hydrogen bonding at N(5). I, the first absorption band; II, the second absorption band.

Results

Calculated electronic spectra. Fig. 3 shows the calculated values of the transition energy and the oscillator strength (intensity of absorption) of the first and the second absorption band. The visible absorption bands obtained from the calculation without hydrogen bonding interactions locate at 414 and 332 nm. They are far shorter than those of the observed wavelengths, 445 and 350 nm. However, this disagreement is not important in considering the effect of hydrogen bonding interaction on the spectral characteristics [9]. As can be seen from the figure, upon hydrogen bonding at N(1) of the isoalloxazine the first absorption band shifts towards blue, while the second one does not shift (compare I with II in Fig. 3). Upon hydrogen bonding at N(5) of the isoalloxazine both absorption bands shift towards red (compare I with III in Fig. 3). Since the calculation was made by using VSIP values obtained by adding or subtracting an arbitrary value, 1.0 eV (see Methods), another calculation was made by using the values obtained by adding or subtracting 0.5 eV. The result showed the same characteristic shifts of the absorption bands except that the changes in transition energy were approximately half of the above data. Comparison of the data shown in Fig. 3 with each other indicates that the additional occurrence of hydrogen bondings at O(12), N(3)H and O(14) gives a minute effect on the shifts of the absorption bands caused by hydrogen bondings at

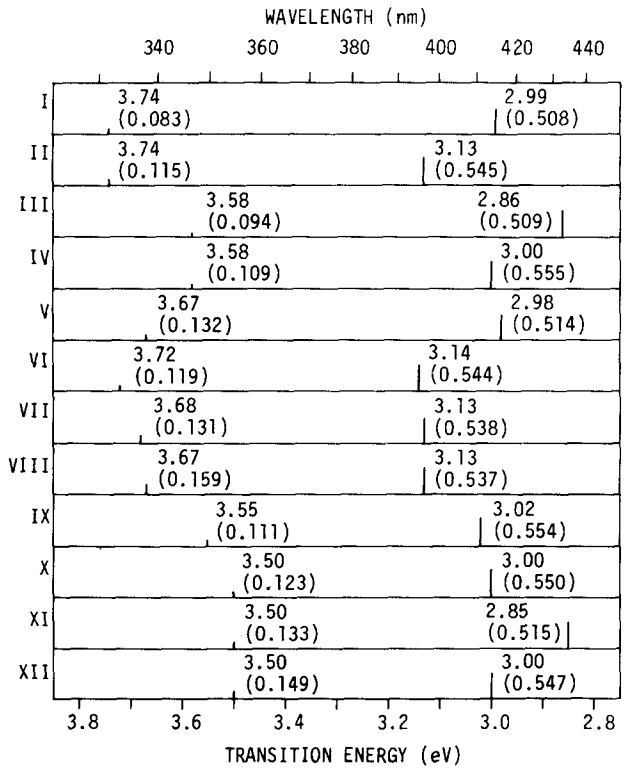

Fig. 3. Calculated values for the transition energy and the oscillator strength of various types of hydrogen-bonded isoalloxazine. The values of the transition energy for various types of hydrogen-bonded isoalloxazine shown in Fig. 1 were indicated by vertical bars both in eV and in wavelength scale. The height of the bars shows the relative intensity of the relevant absorption band. The numerical values in the figure indicate the transition energy and the oscillator strength (in parenthesis).

N(1) and/or N(5). Accordingly, when hydrogen bondings occur at all of the possible hetero atoms of the isoalloxazine, the shifts of both absorption bands (from I to XII in Fig. 3) are similar to those from I to IV in Fig. 3. In the former case, however, the red shift of the second absorption band is remarkable.

The changes in the oscillator strength upon formation of hydrogen bonding(s) also showed a characteristic behavior. The hydrogen bonding at N(1) increases the oscillator strength of both absorption bands remarkably (compare I with II in Fig. 3), and that at N(5) slightly increases the oscillator strength of the both (compare I with III in Fig. 3). When hydrogen bondings occur at O(12), N(3)H and O(14), change in oscillator strength of the first absorption band is minute, while that of the second one is remarkable (compare I with V in Fig. 3). When hydrogen bondings occur at all of the possible hetero atoms, oscillator strength of both the absorption bands increases remarkably (compare I with XII in Fig. 3).

Electron acceptability. It is also important to examine the influence of hydrogen bondings on the redox potential of the isoalloxazine. The redox potentials are related to the energy of the lowest unoccupied molecular orbital

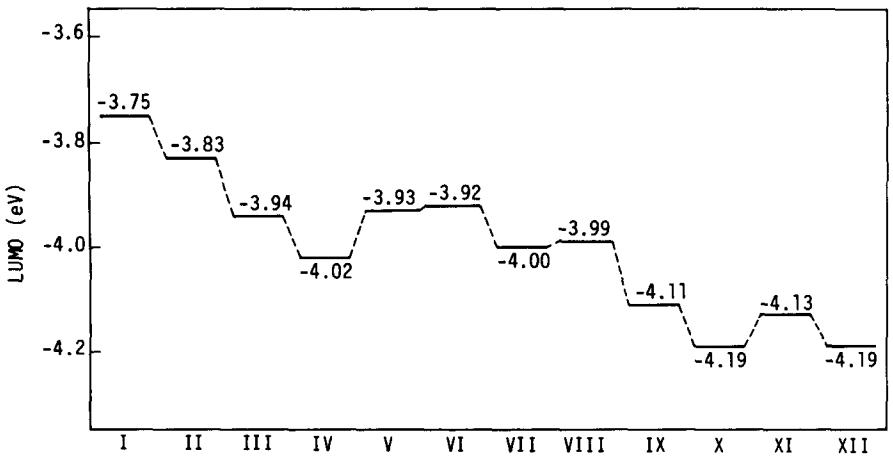

Fig. 4. Energy levels of the lowest unoccupied molecular orbital (LUMO) of various types of hydrogen-bonded isoalloxazine. The numerical values in the figure indicate the lowest unoccupied molecular orbital energy in eV.

[10,11]. As shown in Fig. 4, the hydrogen bonding interactions shown in Fig. 1 all enhance the electron affinity of the isoalloxazine. Namely, upon hydrogen bonding interactions, flavin could receive an electron from an electron donor more easily. Among the complexes studied, the effect is marked in the cases of type IX · XII complexes.

The electron acceptability of the particular atom in the isoalloxazine nucleus can be measured by the square of the atomic orbital coefficients in the lowest unoccupied molecular orbital (frontier electron density) [12]. Frontier electron density of non-hydrogen bonded isoalloxazine is essentially similar to the reported result [13,14]. Namely, without hydrogen bonding, N(5) of the nucleus is the most dominant atom to accept an electron. The change of the frontier electron density at N(5) upon formation of hydrogen bondings is shown in Table II. Hydrogen bondings at O(12), N(3)H and O(14) remarkably increase the frontier electron density at N(5). The frontier electron density at N(5) decreases due to the hydrogen bonding at N(5). However, this is restored in part by the formation of the additional hydrogen bondings at O(12), N(3)H and O(14).

TABLE II

THE FRONTIER ELECTRON DENSITY AT N(5)

The frontier electron density was calculated from the atomic orbital coefficient at N(5) in the lowest unoccupied molecular orbital, as reported by Fukui et al. [12].

No hydrogen bonding	0.236
Hydrogen bonding at N(5)	0.223
Hydrogen bondings at O(12), N(3)H and O(14)	0.242
Hydrogen bondings at O(12), N(3)H O(14) and N(5)	0.227

Discussion

The present molecular orbital calculation indicates that the occurrence of hydrogen bonding at N(1) or N(5) markedly modifies the absorption spectrum of the isoalloxazine and that the hydrogen bondings at the other atoms of the isoalloxazine give little influence on its absorption spectrum. To check the validity of these results, flavodoxins are the best instances, since the occurrence of hydrogen bondings on the isoalloxazine nucleus was reported by X-ray crystallographic analysis. In the flavodoxin crystal obtained from *Clostridium* MP, hydrogen bondings occur as represented in Fig. 1, VIII [15,16]. Another flavodoxin obtained from *Desulfovibrio vulgaris* was reported to possess hydrogen bondings as shown in Fig. 1, XI [17,18]. The results of the molecular orbital calculation in the relevant cases were illustrated in Fig. 3. Although the conformation of flavodoxins in solution may not be the same with that in crystals, the theoretical data reported in this paper are in good accord with the data reported by Mayhew and Ludwig [19].

From the above argument, it seems reasonable to correlate the characteristic change in the absorption spectrum of the isoalloxazine nucleus with the occurrence of hydrogen bondings. On the basis of this assignment, D-amino acid oxidase was examined for the hydrogen bondings involved in the formation of the complex of FAD and the apoenzyme. The absorption spectrum of FAD in aqueous media possesses the peaks of the first and the second absorption band at 450 and 375 nm, respectively. Upon complex formation with D-amino acid oxidase apoenzyme, the shift towards red with a slight decrease in absorbance is found in the first absorption band, while appreciable shift is not found in the second absorption band [3]. This spectral change accords with that from Fig. 3, XII–XI obtained theoretically. This implies that the hydrogen bonding at N(1) of FAD in aqueous solution disappears upon its complex formation with the apoenzyme, while N(5) still interacts with the apoenzyme or remains to interact with water molecule via hydrogen bonding.

The hydrogen bonding at N(5) increases the electron affinity of the isoalloxazine nucleus, while it slightly decreases the magnitude of the frontier electron density at N(5). However, if additional hydrogen bondings occur at O(12), N(3)H and O(14), the electron affinity of the isoalloxazine nucleus increases and N(5) still possesses relatively high electron acceptability. This seems to be the case for both *D. vulgaris* flavodoxin and D-amino acid oxidase. When substrate combines with D-amino acid oxidase, amino nitrogen seems to interact with N(5) of the isoalloxazine nucleus of the coenzyme [20,21]. In this case, if the hydrogen bonding at N(5) is still remaining, the amino nitrogen should interact with N(5) in a sterically possible way. Otherwise, the hydrogen bonding at N(5) disappears prior to the interaction between N(5) and the amino nitrogen of the substrate. In the latter case, the frontier electron density of N(5) increases, which accelerates the electron flow from the substrate to the coenzyme. In any way, hydrogen bondings occurring at O(12), N(3)H and O(14) are considered to be one of the important factors for the catalytic activity of flavoproteins.

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