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Geometrical Change of a Flavoenzyme Model through Hydrogen Bonding to the Pyrimidine Ring

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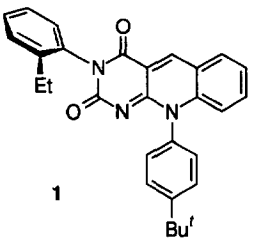
Abstract: 10-(4-*tert*-Butylphenyl)-3-(2-ethylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione affords a 1:1:1 crystalline inclusion complex with urea and ethanol. It is observed that the lengths of the conjugated bonds, N(1)-C(10a)-C(4a)-C(5), which participate in redox reactions are changed significantly through hydrogen bonding at the pyrimidine ring. Copyright © 1996 Elsevier Science Ltd

Flavoenzymes, which require flavin coenzymes such as flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), play significant roles in biological systems.[1] Flavin coenzymes are bound covalently or held tightly to the amino acid residues and the peptides of apoproteins at the active site leading to enhancement of the reactivities of flavin coenzymes.[2] Taking into consideration the fact that flavin coenzymes are located in chiral environments, a variety of optically active flavin derivatives have been synthesized and investigated so far.[3-12] The results elucidate that a chiral face of a flavin, especially its pyrimidine ring moiety, significantly influences the stereochemistry of the reaction with a substrate.

We have synthesized atropisomeric flavoenzyme models and revealed not only the influence of the functional groups of apoproteins on the stereochemistry in (net) hydride transfer reactions with an NAD(P)H analog but also the molecular arrangement at the transition state of the reactions by investigating the stereochemical reactivities of these model compounds.[13,14] Moreover, we have carried out X-ray crystallographic analyses of these compounds and found that some of them form a crystalline inclusion complex[15,16] with a solvent molecule (methanol or ethanol) through hydrogen bonding at the carbonyl group of the pyrimidine ring.[17,18] The analyses indicate that the included solvent molecule brings a geometrical change of the flavin skeleton such as the lengths of conjugated bonds, N(1)-C(10a)-C(4a)-C(5), participating in redox reactions.[18,19] Therefore, by comparing the geometry of a flavin molecule which includes a hydrogen bond in a crystal with that of the same flavin which does not, it is possible to simulate geometrical change observed when an oxidized flavin coenzyme is activated through hydrogen bonding with apoproteins at the active site.

In the course of our X-ray crystallographic studies, it has been confirmed that the crystal of 10-(4-*tert*-butylphenyl)-3-(2-ethylphenyl)-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (**1**) by recrystallization from ethanol include no hydrogen bonding.[20] Thus, we tried to prepare an inclusion complexof **1** with urea, expecting a formation of hydrogen bond as well as of more interesting host-guest inclusion complex. Fortunately, we could obtain a crystalline inclusion complex of **1**•urea•ethanol[21] from a solution of **1** and urea (2 mol equivalent to **1**) in ethanol and succeeded in the X-ray crystallographic analysis enough to discuss the difference in the length of the conjugated bonds participating in redox reactions.[22]



1

The ORTEP drawing of the inclusion complex illustrated in Figure 1 shows that there exist two hydrogen bonds between the pyrimidine ring of **1** and the urea molecule. In addition, the urea molecule forms hydrogen bonds with an ethanol molecule and adjacent urea molecule in the inclusion complex. The lengths of these hydrogen bonds are listed in Table 1.

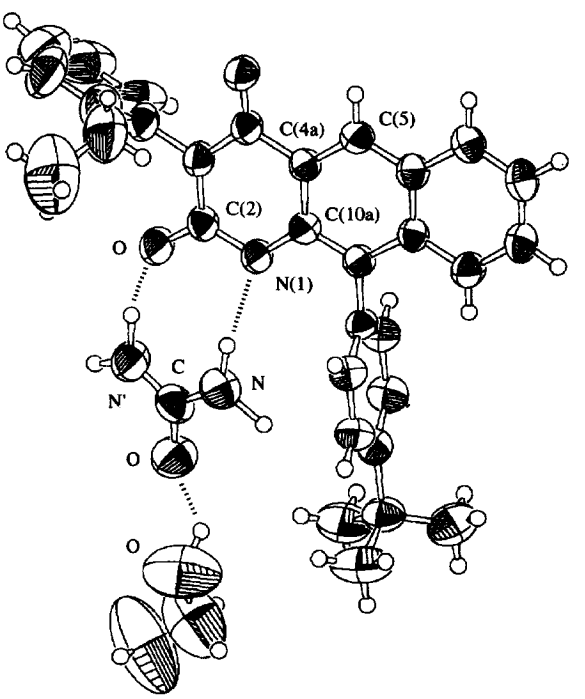


Figure 1. The ORTEP drawing of **1**•urea•ethanol with displacement ellipsoids at 50% probability level. The (*S*)-enantiomer of **1** only is illustrated here. The hydrogen bonds are indicated by broken lines.

Table 1. The Lengths of Hydrogen Bonds in the Inclusion Complex

Atom		Bond Length, Å		
A	B	A–H	H–B	A–B
N(urea)	N(1)	0.97	2.17	3.133(4)
N'(urea)	O(=C(2))	1.10	1.92	2.976(4)
O(ethanol)	O(urea)	0.97	1.98	2.719(6)
N'(urea)	O(urea)	0.98	2.02	2.923(5)

The lengths of the conjugated bonds that participate in redox reactions are listed in Table 2. Evidently, the bond lengths of the N(1)-C(10a)-C(4a)-C(5) conjugated system are changed when the hydrogen bonds with urea exist: both bond lengths of N(1)-C(10a) and C(4a)-C(5) (which are represented formally by a double bond) become longer by 0.023 Å, whereas that of C(10a)-C(4a) (which is represented formally by a single bond) becomes shorter by 0.021 Å than those in free **1**, respectively. The distortion of 0.02 Å is significant: both flavin derivatives subjected to X-ray analyses are in the oxidized form and the difference in length between (formal) single and double bonds in **1** is about 0.1 Å. Thus, 0.02 Å amounts to 20% of the full difference. The result indicates that the hydrogen bonding at the pyrimidine ring affects the electronic structure of the flavin greatly: the π-electrons in the conjugated system are shifted to the N(1) position through hydrogen bonding with urea so that the geometry of the oxidized flavin can approach to that of its reduced form and that the electron density at the C(5) position is expected to become low.

Table 2. The Selected Bond Lengths of **1** and **1**•urea•ethanol

	Length, Å		
Bond	**1**	**1**•urea•ethanol	Δ^a
O=C(2)	1.209(3)	1.211(4)	0.002
C(2)–N(1)	1.363(4)	1.362(4)	–0.001
N(1)–C(10a)	1.306(4)	1.329(4)	0.023
C(10a)–C(4a)	1.435(4)	1.414(5)	–0.021
C(4a)–C(5)	1.337(4)	1.360(5)	0.023

[a] The chenge of bond length induced though hydrogen bonding.

Thus, the geometry of a flavin compound in the oxidized form are brought close to that of its reduced form through hydrogen bonding at the pyrimidine ring. This observation strongly supports the proposal that the geometry of an oxidized flavin coenzyme at the active site is distorted into an activated form through hydrogen bonding with the functional groups of apoproteins, which is considerably different from that undistorted.[23,24] Furthermore, it is expected that flavin molecules can be utilized as a host compound in molecular recognition through hydrogen bonding network and that a variety of molecular recognitions will be possible by modifying a flavin molecule with various functional groups.

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18. Kawai, Y.; Kunitomo, J.; Ohno, A. submitted for publication.
19. The positional numbers of **1** in the present paper is shown according to the IUPAC numbering system.
20. Ohno, A.; Kunitomo, J.; Kawai, Y. unpublished results, which will be published elsewhere in near future.
21. Crystal data: formula, $C_{32}H_{37}N_5O_4$; formula weight, 555.68; crystal dimensions, 0.60 × 0.50 × 0.40 mm; color, yellow; habit, prismatic; crystal system, monoclinic; space group, $P2_1/n$ (No. 14); a = 16.084(2), b = 17.304(3), c = 11.691(2) Å, β = 110.23(1)°, V = 3035(1) Å^3; Z = 4; D_{calc} = 1.209 g/cm^3; μ = 6.54 cm^{-1}; R = 0.087, R_w = 0.128.
    Data collection and structure analysis: diffractometer, Rigaku AFC7R diffractometer; $\omega/2\theta$ mode; $2\theta_{max}$ = 120.2°; radiation, Cu-Kα (λ = 1.54178 Å); temperature, 20.0 °C; 4984 measured reflections, 4728 independent reflections, 3604 reflections included in the measurement; 371 parameters; direct method; full-matrix least-square refinement with all non-hydrogen atoms refined anisotropically and hydrogen atoms not refined.
22. Although the R factor is relatively large because the temperature factors of atoms of the enclosed ethanol are large, those of **1** and the urea molecule are small and the bond lengths and angles of these molecules are expected to be sufficiently reliable.
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