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X-ray Crystal Structures of Conformationally Biased Flavin Models

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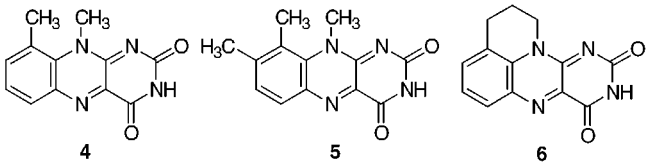
ABSTRACT



4 **5** **6**

X-ray crystal structures of the oxidized form of three conformationally biased flavin models are reported. Models 4 and 5 show significant distortion, which contributes to their bias for the fully reduced form.

The protein environment of the flavin cofactor defines the specific reactivity of a flavoenzyme.[1] These protein−flavin interactions include hydrogen bonding, conformation, $\pi-\pi$-interactions, solvent accessibility, and others. However, the precise mechanism and extent to which these interactions influence the redox and catalytic properties of flavin coenzyme is still poorly understood. Simplified model systems can provide useful information on these issues.[2,3]

There are three relevant oxidation states of flavin cofactor and various protonation states of each.[4] The preferred conformation of the oxidized and one-electron-reduced semiquinone states are planar, while the two-electron reduced flavin is believed to be bent along the N5,N10 axis. These predictions have been confirmed computationally[5] and by X-ray crystallographic analyses of flavin models.[6,7] To protect the fully reduced flavin from facile air oxidation, it was necessary to use a heavily substituted derivative (**3**) for the X-ray analysis (Figure 1).[7] The substituents significantly alter the electronic properties of the reduced flavin, and its relevance to the biological system can be questioned.

Massey and Hemmerich suggested that one mechanism by which the apoprotein could control the redox properties of flavin coenzyme was through conformational effects.[1a] Since that time, protein crystal structures of flavoenzymes

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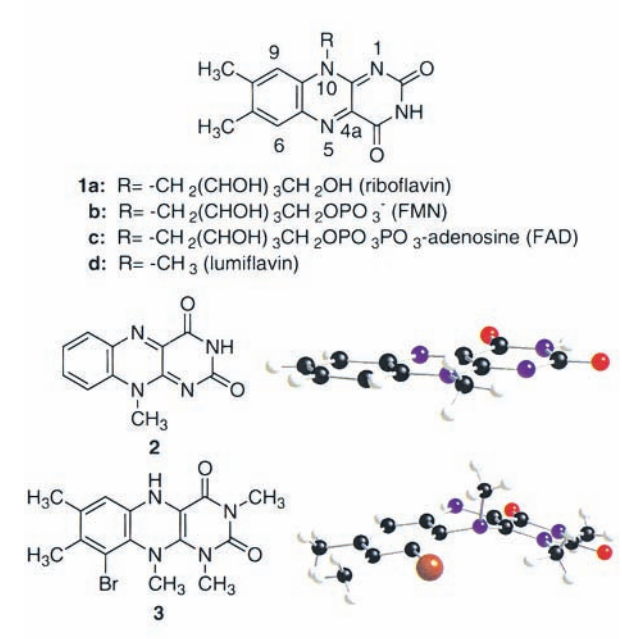
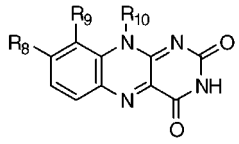


Figure 1. Structure of flavin cofactors and models.

indicate that conformation may indeed play a role in flavin reactivity. For instance, crystal structures of trimethylamine dehydrogenase and pyruvate oxidase (Figure 2) show the *oxidized* flavin cofactor to be significantly distorted from its preferred planar geometry and more closely resemble the conformation of fully reduced flavin.[8] Alternatively, the FMN cofactor of flavodoxin is roughly planar in all three oxidation states. [9]

We have been interested in developing chemical models to determine the role of conformation in flavin redox chemistry. We have prepared conformationally biased flavins **4**−**6** and measured their redox potentials by cyclic voltammetry (Figure 3).[3f,10] We predicted that the eclipsing interaction between the C9 and N10 methyl groups in the planar oxidized states of **4** and **5** would be alleviated upon reduction, as the N10 methyl shifts from an in-plane, pseudoequatorial to a pseudoaxial position, as shown in the X-ray structure of **3**. Thus, the eclipsing interactions serve as a conformational driving force for reduction. Model **6** possesses a three-carbon tether from C9 to N10, which prevents the N10 substituent from shifting to a pseudoaxial position upon reduction. As such, flavin **6** is predicted to be conformationally biased toward the oxidized form. Preliminary electrochemical and kinetic studies of these flavin models showed that they behave as predicted.[3f,10] We report herein, X-ray crystal structures of the oxidized forms of these models.



	R_8	R_9	R_{10}	E°′ (mV)
4	-H	$-CH_3$	$-CH_3$	-357
5	$-CH_3$	$-CH_3$	$-CH_3$	-368
6	-H	$-CH_2CH_2CH_2-$		-432

Figure 3. Structure of conformationally biased flavin models. Redox potential in 100 mM, pH 7.4 HEPES buffer vs Ag/AgCl (+198 mV vs SHE).

Flavins **4**−**6** were crystallized from formic acid and water by vapor diffusion, and solvent is present in each crystal structure. Crystallographic data is given in Table 1. A common feature of all three structures is that the tricyclic flavin ring system is packed into parallel sheets. Adjacent flavins are arranged so that the N3 proton and C2 carbonyl are within hydrogen-bonding distance. This arrangement is typified by **6**, which crystallized to give orange needles (Figure 4). For **6**, the oxygen−nitrogen distance is 2.86 Å and the N−H−O′ angle is 176.2°. Formic acid appears to be bridging the C2-carbonyl of one flavin with the C4-carbonyl of an adjacent flavin through H-bonding interactions. The carboxylate proton of formic acid is H-bonded to the C2-carbonyl oxygen with an O−O′ distance of 2.4 Å and an O−H−O′ angle of 176.4°. The orientation suggests that the formyl proton is H-bonded to the C4-carbonyl of the adjacent flavin. This is a much less polarized bond and

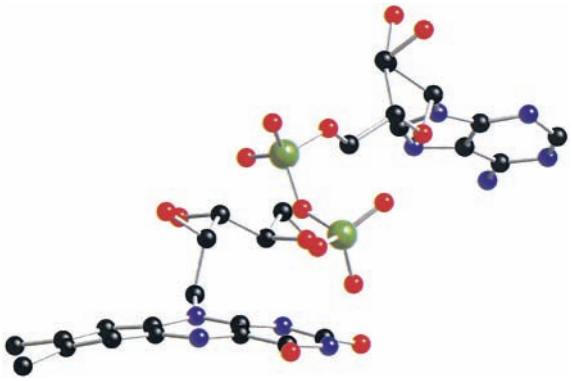


Figure 2. Distorted conformation of the oxidized FAD cofactor **1c** of pyruvate oxidase.

Table 1. Crystallographic Data for Flavin Models **4**−**6**

	flavin		
	4	**5**	**6**
crystal system	monoclinic	monoclinic	triclinic
space group	$P2_1/c$	C_2/c	$P1$
a	18.430 (12)	23.503 (5)	5.0378 (5)
b	18.341 (12)	6.7106 (10)	9.4623 (10)
c	7.691 (5)	17.435 (3)	14.2196 (15)
α	90	90	105.792 (2)
β	102.044 (9)	117.677 (3)	98.797 (2)
γ	90	90	92.324 (2)
R ($I > 2\sigma$)	0.0596	0.0689	0.0553
R_w^2	0.1526	0.1552	0.1549

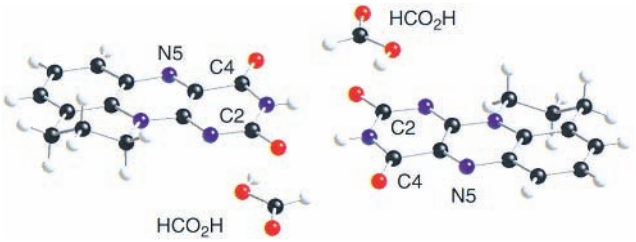


Figure 4. X-ray crystal structure of 9,10-propano-bridged flavin (**6**).

is expected to be a weaker interaction. Indeed, the distance between the C4-carbonyl oxygen and the carbon of formic acid is 3.56 Å which is a weak H-bond at best. The O′−H−C angle is 174°. The H-bonding between the pyrimidine units of 8,9,10-trimethylflavin **5** is nearly identical to that of **6**, with an N−O′ distance of 2.83 Å and an N−H−O′ angle of 167.35°. For 9,10-dimethylflavin (**4**), this H-bonding is unsymmetrical, with N−O′ distances of 2.85 and 2.92 Å and N−H−O′ angles of 176.34 and 168.53°, respectively. This hydrogen-bonding arrangement has been observed previously in the crystal structure of a charge-transfer complex of 10-propylflavin with naphthalenediol.[11,12] This hydrogen bonding may contribute to flavin reactivity in nonpolar solvents.

Our main interest in these flavin models was to examine their conformation. Eclipsing interactions were expected to distort the preferred planar structure of the oxidized forms of models **4** and **5** and conformationally bias them toward the reduced state. Space-filling models of **4** and **5** suggested that the 9- and 10-methyl groups were within van der Waals contact. Indeed, in both crystal structures out-of-plane bending of these substituents is observed.

Analysis of orange-brown needles of **4**, showed a disordered N10-methyl (Figure 5). This indicated that this group was "breathing," moving from above the plane of the isoalloxazine ring system to below. The amplitude of this motion, however, was asymmetric. Interestingly, the crystal contained two forms of **4**, which differed by the amplitude of the N10 breathing motion. Using the CrystalMaker 4.04 software package,[13] a plane was fit through the 14 atoms making up the isoalloxazine ring system. In the first form

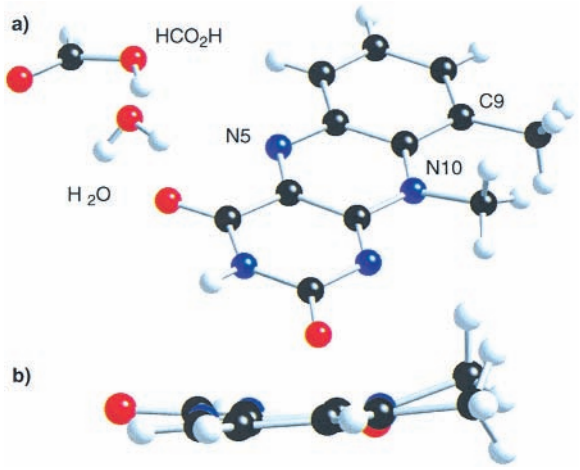


Figure 5. (a) Crystal structure of 9,10-dimethylflavin (**4**). (b) Side view.

of **4**, the N10-methyl group was displaced 0.468 and −0.360 Å from this plane, while the same displacement was 0.510 and −0.209 Å in the second form. In both cases, the C9-methyl group appears to be static and is displaced 0.028 and 0.124 Å from the plane, respectively. The tricyclic isoalloxazine ring system for the first form is relatively planar, with the 14 ring atoms generally lying within ±0.050 Å from the best plane. The largest deviation is the N10 atom, which is displaced 0.054 Å. The C2 and C4 carbonyl oxygens are 0.121 and −0.029 Å off the plane, respectively. These distortions cause the pyrimidine portion of the molecule to be slightly twisted. The second form within the crystal is more distorted with the C2 and C4 carbonyl oxygens −0.214 and 0.188 Å from the plane, giving this form a more pronounced twist. We believe the two observed forms of **4** result from crystal packing forces. In both forms, the hydrogen bonding to solvent and to each other through the pyrimidine portion are identical. A water molecule is situated to suggest a bifurcated hydrogen bond to both N5 and the C4 carbonyl group. Bifurcated hydrogen bonds to these positions are common in flavoproteins. A molecule of formic acid is hydrogen bonded to the water.

Analysis of orange needles of 8,9,10-trimethylflavin (**5**) showed an ordered structure (Figure 6). The addition of the C8-methyl group appears to lock the conformation of the N10-methyl. The planarity of the isoalloxazine system is somewhat more distorted than that of **4** or **6**. Several atoms were displaced from the best plane fit through the 14 ring atoms. The aryl carbons C6−C9 deviate from the plane by 0.078, 0.058, −0.050, and −0.115 Å, and several of the dihedral angles involving the aromatic carbons significantly deviate from zero, the largest being C8−C9−C10a−C5a which is 7.06°, indicating a distorted aromatic unit. The N5 atom is displaced 0.104 Å from the plane while the C2 and C4 carbonyl oxygens lie 0.110 and −0.152 Å off the plane, giving this flavin a pronounced twist along the long axis of the molecule. We predicted that the addition of a methyl group at C8 would enhance eclipsing interactions between

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(13) (a) CrystalMaker Software; P.O. Box 183; Bicester, Oxfordshire, OX6 7BS, U.K. (b) + and − designations are arbitrary and used to indicate the relative direction from the plane.

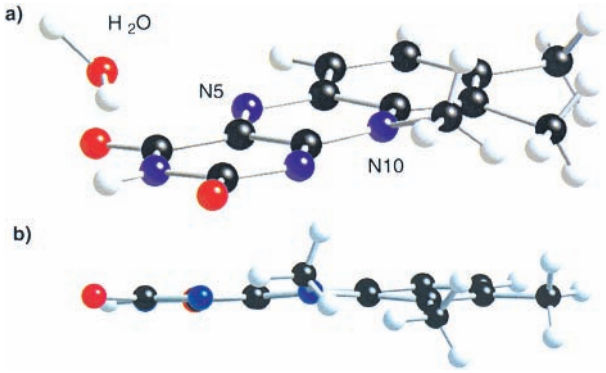

Figure 6. (a) Crystal structure of 8,9,10-trimethylflavin (**5**). (b) Side view.

the C9 and N10 methyl groups. The displacements of the C8, C9, and N10 methyl groups are −0.091, −0.379, and 0.500 Å, respectively. It is interesting to note that the adjacent C8 and C9 methyl groups are on the same side of the plane. A water molecule is situated to suggested hydrogen bonding to the C4 carbonyl and N5. However, this water is disordered and conclusions about its involvement in hydrogen bonding cannot be confirmed.

The three-carbon tether of flavin **6** was designed to restrict the flavin nucleus in a planar conformation. Most of the 14 ring atoms are within 0.050 Å from the best plane fit through these atoms. The largest displacements are N10, C2, and C4, which are 0.083, −0.085, and 0.072 Å from the plane. As expected, the six-atom ring making up the tether is in a half-chair conformation. The carbons directly bonded to C9 and N10 are displaced 0.087 and 0.198 Å from the plane of the isoalloxazine ring system.

The results from our structures should be compared to that of the unperturbed structure of 10-methylflavin.[6c] Nearly all heavy atoms are within 0.05 Å of the best plane fit through the isoalloxazine ring system; the only exception was the C4 carbonyl oxygen which was 0.09 Å off the plane. It is interesting to note that these crystals, which were grown by slow evaporation from DMSO, did not show the hydrogen bonding between pyrimidine units observed in our structures. Two other previously reported flavin models have been shown to have significantly perturbed geometries (Figure 7). Flavinium cation **7** possesses methyl−methyl eclipsing interactions similar to those of **4** and **5**.[6a] The distortions in the isoalloxazine nucleus of **7** is similar to that of **5**, except for N10 and N1, which show a larger displacement (−0.127 and +0.162 Å, respectively) from the best plane defined by

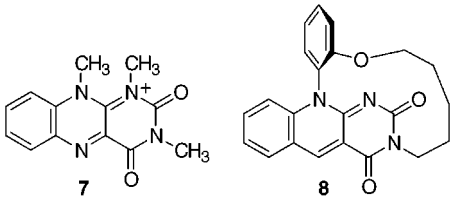

Figure 7. Other conformationally perturbed flavin models.

the 14 ring atoms. Consequently, the N10- and N1-methyl groups are displaced −0.601 and 0.709 Å, which is significantly larger than that for **4** or **5**. The cationic nature of **7**, however, significantly changes the electronic properties and reactivity of this model.

Shinkai prepared a series of conformationally biased flavins and 5-dezaflavins tethered from N10 to N3.[3b] A 5-deazaflavin (**8**) with the shortest tether and presumably most strained was examined crystallographically. The authors noted that **8** possessed a relatively large dihedral angle between the pyrimidine and pyrazine rings of 9.5°, which was attributed to strain caused by the tether. The N3 bridgehead position deviated from the plane of the isoalloxazine ring system by 0.234 Å. Rates of flavin-mediated reaction were correlated with the length of the tethering chain; however, the redox potentials of these models were only modestly correlated.

In conclusion, we have determined the conformation of oxidized flavin models **4**−**6** by X-ray crystallography. Flavins **4** and **5** show significant distortions due to eclipsing interactions of the methyl groups. These interactions serve as a conformational driving force for their reduction. These predictions on reactivity have been confirmed by cyclic voltammetry.[3f,10] It should also be noted that molecular mechanics calculations using Macromodel 6.5 and the MM3 force field did not accurately predict the geometry of our models, instead giving largely planar conformations of the isoalloxazine ring system with much smaller out-of-plane distortions of the C9 and N10 substitutents. We are currently examining how the conformational biases of flavins **4**-**6** affects their chemistry, and these results will appear in the future.

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Supporting Information Available: Crystallographic data and fractional coordinates for flavins **4**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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