A Theoretical Study of the Structures of Flavin in Different Oxidation and Protonation States

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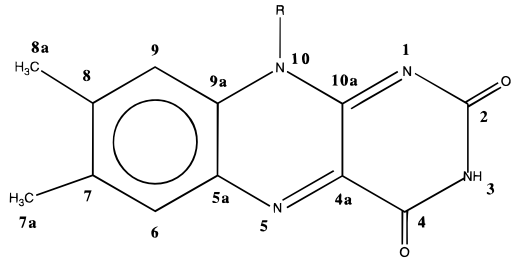
Abstract: Ab initio molecular orbital theory was used to investigate the structure of flavin in different oxidation and protonation states using lumiflavin as a model compound. According to our study, oxidized flavin is planar no matter what its protonation state or whether it participates in hydrogen bonding. The structures of flavin semiquinone radicals are planar or very close to planar, but the reduced flavin H_3Fl_{red} (**9**) is bent with a ring puckering angle of 27.3° along the N5 and N10 axis. The calculations indicate that N1 is the site of protonation for oxidized flavin, which is in agreement with several crystallographic studies. The calculated spin density distribution for flavin semiquinone radicals is also consistent with experimental results. Electrostatic potential derived charges at the RHF/6-31G* level of theory are also provided for both oxidized and reduced flavins.

Introduction

Flavins are very important biological redox reagents that can undergo both one-electron and two-electron redox processes.[1−7] Flavins can be incorporated into enzymes either covalently or noncovalently. In either case, the flavin is never a substrate but always a true coenzyme and remains tightly bound to the protein during reactions. The prosthetic groups of these flavoenzymes are riboflavin (vitamin B2), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). The active portion of the various flavins is a 7,8-dimethylisoalloxazine (Chart 1) substituted at nitrogen 10; the most widely used flavin model compound is lumiflavin, a 7,8-dimethylisoalloxazine with a methyl group at nitrogen 10. Flavins can exist in three different oxidation states: oxidized, flavin semiquinone radical, and reduced form. Depending on pH (or protonation state), there are several forms of flavin for each oxidation state. Since flavins act very efficiently in a wide variety of enzymatic reactions, it was proposed that specific interactions between flavin and apoflavoprotein play an important role in determining the pathway of flavin catalysis.[8,9] Factors such as mobility of flavin in the active site, microenvironment of the flavin binding pocket, and the planarity of the oxidized and reduced flavins were suggested to modulate the redox potential of flavoenzymes.[10,11] It has been demonstrated that the redox potential of flavin can be dramatically altered by the protein environment. For example, D-amino acid oxidase binds $FADH_2$ (the reduced form) about four orders of magnitude tighter than FAD (the oxidized form), changing the redox protein from −210 mV for the $FAD/FADH_2$ couple in solution to about 0 mV in the flavoenzyme (D-amino acid oxidase).[12] Flavoenzymes are involved in many important biological processes such as photosynthesis (ferredoxin reductase),[13] UV-damaged DNA repair (DNA photolyase),[14] and regulating the ratio of glutathione disulfide (GSSG) and its reduced form (GSH) (glutathione reductase).[6]

Chart 1



It was suggested that flavin changes shape during the redox process, from a planar oxidized form to a butterfly or bent shape reduced form.[1] Based on crystallographic models of several 10-alkylisoalloxazines, oxidized flavin appears to be planar.[15−17] Since most of these flavin analogues are cocrystallized with planar naphthalene-2,3-diol and in the crystal the isoalloxazines

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are sandwiched between naphthalene-2,3-diol molecules, this planarity could be a result of crystal packing. On the other hand, an analogue of the reduced flavin, 5-acetyl-9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine, is indeed not planar.[18] The ring is puckered along the N5 and N10 axis and the angle between the benzene and uracil planes is 144.5°. Replacement of the acetyl group at N5 by hydrogen changes the value to 159°. Whether this nonplanarity is due to steric effects is not clear.[10] Solution NMR studies suggest that reduced flavin is only slightly bent in nonpolar solvents and probably planar in polar solvents.[19] The intrinsic barrier for ring inversion is probably less than 20 kJ/mol (or 4.8 kcal/mol).[19] Due to difficulties in the interpretation of chemical shift data, there is significant ambiguity in the NMR studies. Thus, the reduced flavin has been proposed to be planar.[20] Although ESR and resonance Raman spectra have been reported for flavin radicals, their precise conformation is not known.[21,22]

In principle, molecular orbital theory can be used to address questions concerning the structures of flavins. However, due to the size of the molecule, previous theoretical studies on flavin related systems were based on either geometries derived directly from solid state or structures built from standard geometrical parameters or from related molecules; the level of theory used was probably not adequate.[23] In general, these calculations used fixed bond lengths and bond angles to make the calculations feasible. The semiempirical molecular orbital studies by Hall and co-workers,[24] using the MINDO/3 method,[25] were the first geometry-optimized theoretical studies. Depending on the level of theory, the calculated barrier to planarity from a bent reduced flavin ranged from about 2 kcal/mol to over 30 kcal/mol. The effect of geometrical constraints on the calculated results is unknown. Here we report a detailed investigation of the structure of flavin in different oxidation and protonation states using ab initio molecular orbital theory without the problems associated with the previous theoretical studies.

Theoretical Method

Since lumiflavin is the most widely used model compound for flavin, we decided to use it in our study. A quantum mechanics approach is used to study the various structures of lumiflavin. The ab initio molecular orbital theory at the RHF/6-31G* level has been shown to give good results on molecular structures.[26] Since nitrogen centers N5 and N10 play a very important role in determining whether the flavin is planar or nonplanar, it is imperative to use a method that is capable of accurately treating these nitrogen centers. The closest model compound that contains these kinds of nitrogen centers is aniline ($PhNH_2$). It has been demonstrated that ab initio molecular orbital theory at the RHF/6-31G* level is able to reproduce the experimental inversion barrier and geometry for aniline.[27] For example, the calculated inversion barrier for aniline at the RHF/6-31G* level of theory is 1.67 kcal/mol, while the available experimental values are 1.3-1.6 kcal/mol.[27a] Chart 2 depicts the forms of lumiflavin investigated in this work; the naming convention of Kyte[20] was used throughout. The fully deprotonated form of oxidized flavin is designated as Fl_{ox}^- and the neutral oxidized form as HFl_{ox}. All ab initio molecular orbital theory calculations were carried out using the Gaussian 92 program using the 6-31G* basis set.[28] For close-shell systems (both the oxidized and reduced forms of flavin), the restricted Hartree-Fock (RHF)[29] method is used, while for open-shell systems (flavin radicals), the unrestricted Hartree−Fock (UHF)[30] method is used. The energy of each molecule was minimized with respect to all geometrical parameters. The geometry of each molecule was fully relaxed without any constraint except for the planar form of the reduced flavin. As a further test, we also did a geometry optimization of the reduced flavin H_3Fl_{red} (**9**) using the RHF/6-31+G* level of theory. The root mean square (rms) deviation between the structure at the RHF/6-31G* level and the structure at RHF/6-31+G* level is 0.01 Å, indicating that the inclusion of diffuse functions on heavy atoms has little effect on the calculated geometry. Due to the size and number of compounds that we wished to study, the use of a larger basis set with electron correlation is prohibitive; geometry optimization of one of these compounds at the RHF/6-31G* level of theory already takes more than 200 h of CPU time on a SGI R8000 computer.

Table 1. The Calculated Total Electronic Energies (au) for Various Species of Flavin at the 6-31G* Level of Theory

compd	energy (au)	compd	energy (au)
1	−866.848 511	**7**	−866.895 621 8
2	−867.225 931 1	**8**	−867.823 813 1
3	−867.195 946 4	**9**	−868.0096650
4	−866.262 970 5	**10**	−867.456 295 6
5	−867.432 413 7	**11**	−868.383 032 5
6	−867.434 382 4	**12**	−850.872 734 7

Results and Discussions

Ab initio molecular orbital calculations at the HF/6-31G* level of theory were used to study the structures of flavin in different oxidation and protonation states. Table 1 lists the calculated total electronic energy of each species involved in this study. In the following section, the theoretical results are presented and discussed according to the oxidation state.

Oxidized Flavin. It has been generally assumed that the oxidized form of flavin is planar because of electron delocalization among the three rings. Indeed, in the crystal structures of known flavoenzymes and model compounds (10-alkylisoalloxazines), the isoalloxazine ring is planar. However, the question remains as to whether this planarity is caused by either the enzyme environment or crystal packing forces. In order to address this issue, it is necessary to obtain the structure of free flavin in the oxidized form. Several theoretical studies have been reported;[23,24] however, due to the theoretical and/or

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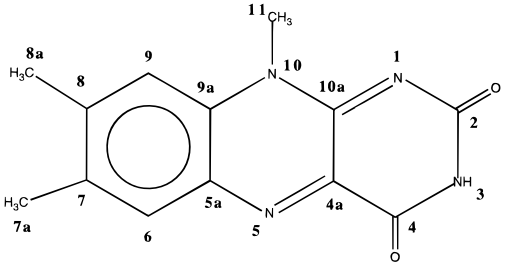
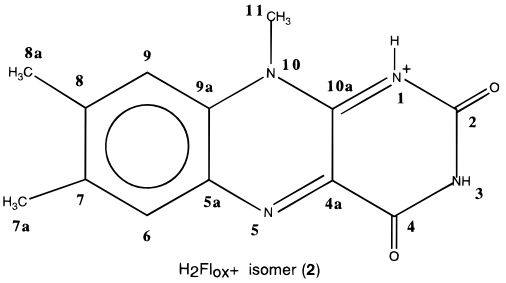
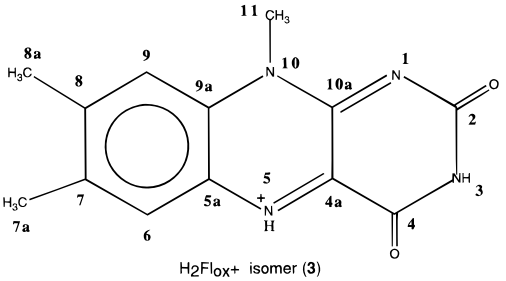
Chart 2



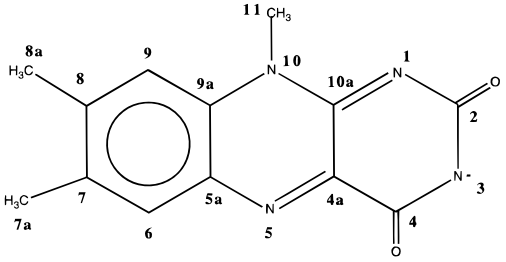
HF_{ox} (**1**)



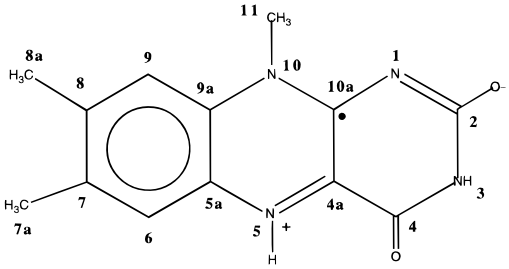
$H_2Fl_{ox}^+$ isomer (**2**)



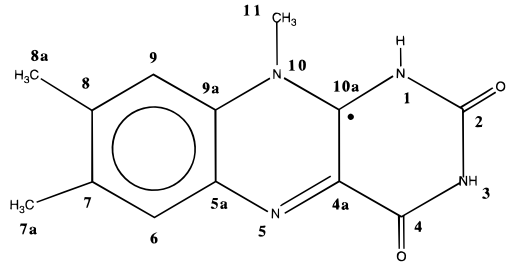
$H_2Fl_{ox}^+$ isomer (**3**)



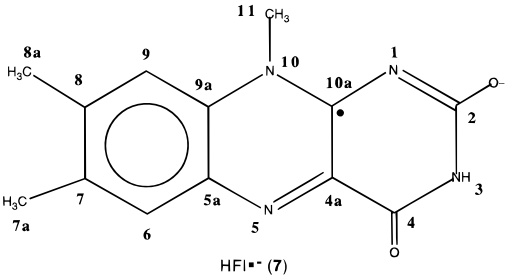
Fl_{ox}^- (**4**)



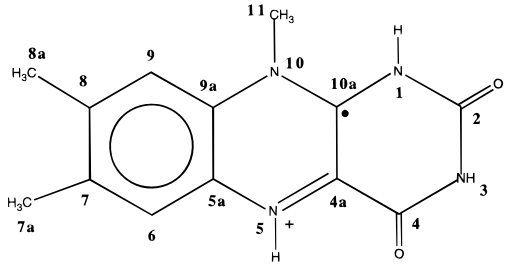
$H_2Fl^{\bullet}$ (**5**)



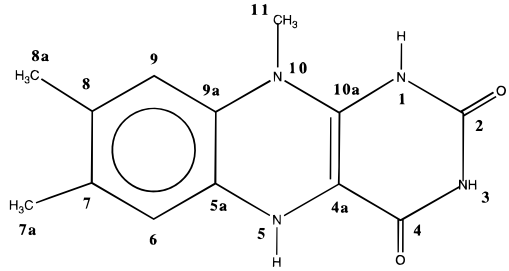
isomer of $H_2Fl^{\bullet}$ (**6**)



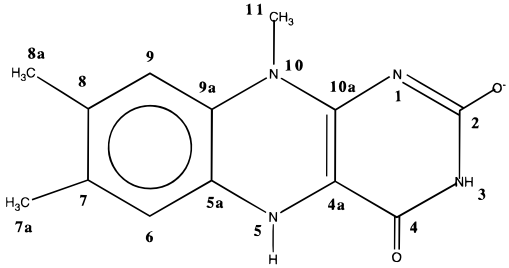
$HFl^{\bullet -}$ (**7**)



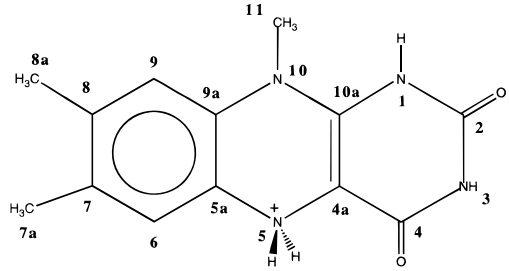
$H3Fl^{\bullet +}$ (**8**)



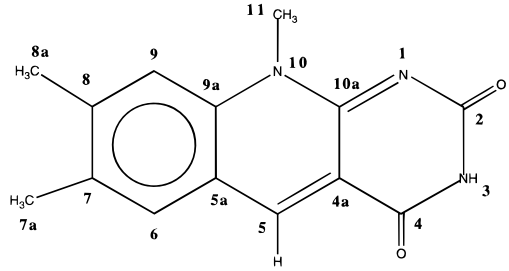
H_3Fl_{red} (**9**)



$H_2Fl_{red}^-$ (**10**)



$H_4Fl^+_{red}$ (**11**)



(**12**)

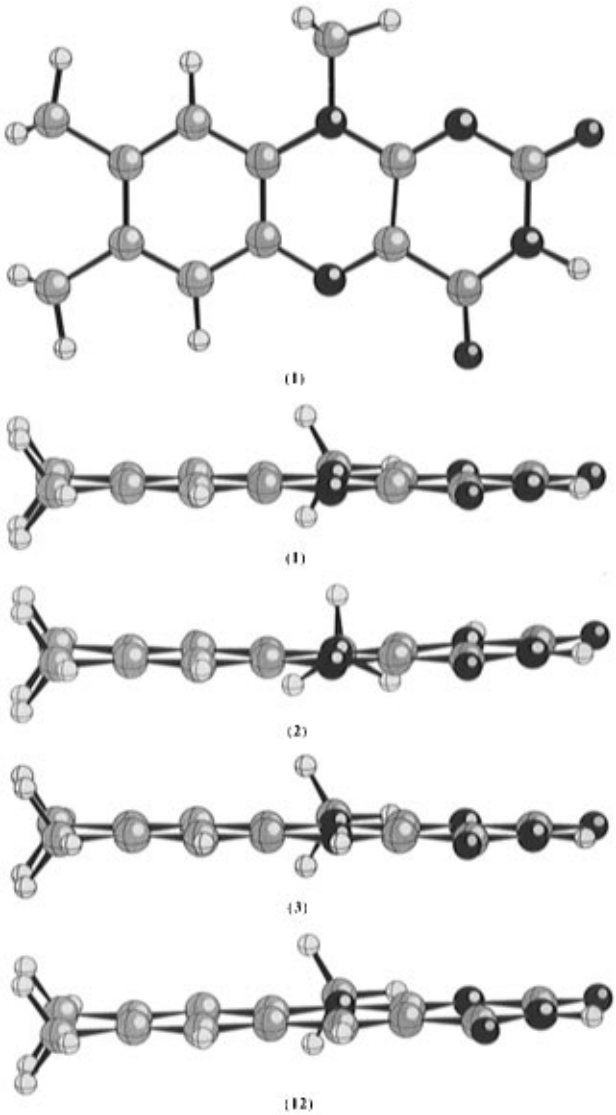


Figure 1. The optimized geometries for the oxidized form of flavin at the RHF/6-31G* level.

computational approximations used, the reliability of these results are questionable. We, therefore, performed an ab initio molecular orbital calculation at the RHF/6-31G* level of theory on the neutral oxidized flavin (HFl_{ox}). The optimized geometry and some geometrical parameters are shown in Figure 1 and Table 2, respectively. According to our calculation, it is clear that the free oxidized neutral flavin is indeed planar, indicating that the planarity observed previously in the 10-alkylisoalloxazines is not forced by the enzyme environment or crystal packing forces. The planarity of oxidized flavin is due to electron delocalization (or conjugation). The C−C bond length between C4 and C4a is about 1.50 Å, indicating a single bond. Therefore, the bond at C4a-N is imine-like and C4a could act as an electrophilic site. On the other hand, with respect to the two carbonyl groups, N5 could also be an electrophilic site. Furthermore, since N5 has one lone pair of electrons, it could also act as a nucleophilic site and can be protonated or form a hydrogen bond with donor groups of a protein or solvent (such as water). Previous experimental studies did demonstrate that both C4a and N5 can act as electrophilic sites.[20]

For the protonated species, there are two isomers: protonation at either N1 (**2**) or N5 (**3**). Again, both protonated isomers (**2** and **3**) are planar. In **2**, the N1 center is slightly pyramidal and the N10 center is essentially planar; while in **3**, all nitrogen centers are planar. It should be pointed out that even in aniline the nitrogen center is pyramidal despite electron delocalization.[31] Accordingly, the nitrogen centers in flavins may easily deform in the presence of unfavorable steric interactions. Therefore, in some of the flavin model compounds, the planarity of nitrogen centers may change from compound to compound depending on the substituent groups on the nitrogen centers or on the carbon atoms nearby, which makes interpretation of the experimental data difficult. Protonation at N1 is favored by 18.8 kcal/mol. This conclusion is in agreement with the crystal structures of several flavin model compounds;[32] it is also in agreement with the MINDO/3 calculations, which predicted that protonation of N1 is favored by 10−12 kcal/mol.[24b] The crystal structures of riboflavin hydrobromide hydrate, 10-methylisoalloxazine hydrobromide dihydrate, oxidized lumiflavine hydrochloride hydrate, and 7,8,10-methylisoalloxazinium nitrate all demonstrate that N1 is the site of protonation.[32]

Table 2. The Calculated Geometrical Parameters for the Oxidized Form of Flavin at the RHF/6-31G* Level of Theory

bond	**1**	**2**	**3**	**4**	**12**[a]
C_2-O	1.1902	1.1793	1.1769	1.2057	1.1921
C_2-N_3	1.3974	1.3630	1.4196	1.3498	1.4002
N_3-C_4	1.3643	1.3847	1.3398	1.3266	1.3597
C_4-O	1.1893	1.1783	1.1901	1.2111	1.1984
C_4-C_{4a}	1.4974	1.4975	1.4999	1.5296	1.4763
C_2-N_1	1.3755	1.4094	1.3862	1.4397	1.3712
N_1-C_{10a}	1.2829	1.3426	1.2715	1.2590	1.2886
$C_{4a}-N_5$[a]	1.2655	1.2816	1.2731	1.2638	1.3416
N_5-C_{5a}[a]	1.3719	1.3450	1.3798	1.3884	1.4277
$C_{4a}-C_{10a}$	1.4706	1.4301	1.4592	1.4793	1.4486
$C_{10a}-N_{10}$	1.3583	1.3227	1.3586	1.3862	1.3691
$N_{10}-C_{11}$	1.4630	1.4775	1.4749	1.4498	1.4642
$N_{10}-C_{9a}$	1.3871	1.3967	1.3874	1.3815	1.3877
$C_{9a}-C_{5a}$	1.3926	1.4017	1.3951	1.3950	1.3972
$C_{5a}-C_6$	1.3985	1.4096	1.3996	1.3892	1.4025
C_6-C_7	1.3721	1.3638	1.3662	1.3811	1.3694
C_7-C_{7a}	1.5108	1.5095	1.5093	1.5118	1.5107
C_7-C_8	1.4135	1.4255	1.4238	1.4018	1.4137
C_8-C_{8a}	1.5098	1.5069	1.5074	1.5118	1.5098
C_8-C_9	1.3798	1.3800	1.3792	1.3845	1.3782
C_9-C_{9a}	1.3981	1.3951	1.3976	1.3965	1.4017

[a] In 5-deazalumiflavin **12**, the N_5 is replaced by CH. The $C_{4a}-N_5$ and N_5-C_{5a} are in fact $C_{4a}-C_5$ and C_5-C_{5a}.

If N5 is replaced by a CH group as in 5-deazalumiflavin (**12**), only site 5 could be electrophilic. Again, this is indeed what was observed experimentally; 5-deazalumiflavin only participates in hydride transfer processes.[2] The calculated geometrical parameters and structure for **12** are also shown in Table 2 and Figure 1. Compound **12** is planar and the bond length between C4 and C4a is about 0.02 Å shorter than in the oxidized flavin.

The proton at N3 is relatively acidic and can be deprotonated (the pK_a being about 10). The structure of this deprotonated species ($Flox^-$) is also planar. Thus, it is clear from our calculations that oxidized flavin is planar no matter what its protonation state or hydrogen bonding environment.

Flavin Semiquinone Radicals. The structures of flavin radical in three protonation states are investigated: one proton ($HFl^{\bullet -}$, **7**), two protons ($H_2Fl^{\bullet}$, **5** and **6**), and three protons ($H_3Fl^{\bullet +}$, **8**). For $H_2Fl^{\bullet}$, two isomers were examined. Since flavin radicals are open-shell systems, the UHF method was used in

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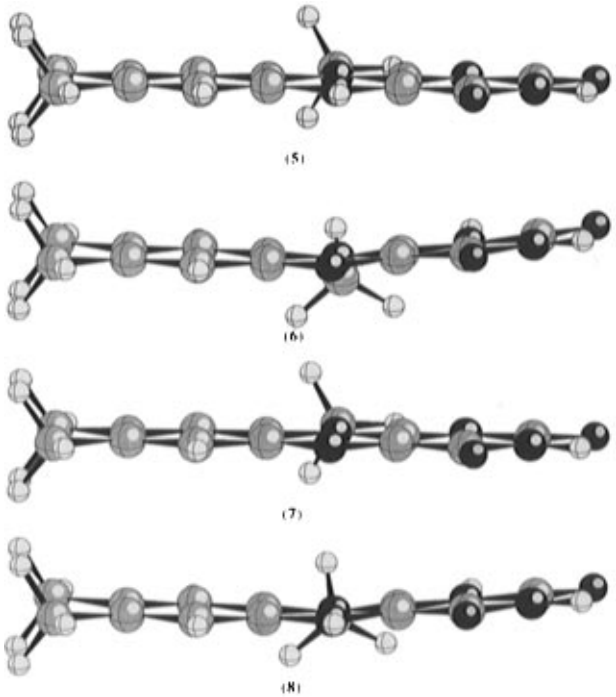

Figure 2. The optimized geometries for flavin semiquinone radicals at the UHF/6-31G* level.

Table 3. The Calculated Geometrical Parameters for the Radical Form of Flavin at the UHF/6-31G* Level of Theory

bond	**5**	**6**	**7**	**8**
C_2-O	1.1922	1.1942	1.2121	1.1807
C_2-N_3	1.4005	1.3620	1.3865	1.3706
N_3-C_4	1.3661	1.3955	1.3822	1.3750
C_4-O	1.2046	1.1899	1.2106	1.1921
C_4-C_{4a}	1.4467	1.4691	1.4423	1.4557
C_2-N_1	1.3741	1.3760	1.3507	1.4012
N_1-C_{10a}	1.2895	1.3774	1.3182	1.3531
$C_{4a}-N_5$	1.3435	1.3679	1.3700	1.3578
N_5-C_{5a}	1.3741	1.3473	1.3392	1.3528
$C_{4a}-C_{10a}$	1.4316	1.3634	1.4023	1.3794
$C_{10a}-N_{10}$	1.3648	1.3675	1.3871	1.3389
$N_{10}-C_{11}$	1.4569	1.4540	1.4456	1.4681
$N_{10}-C_{9a}$	1.4108	1.4143	1.3963	1.4231
$C_{9a}-C_{5a}$	1.4060	1.4218	1.4265	1.4098
$C_{5a}-C_6$	1.3985	1.4167	1.4184	1.4079
C_6-C_7	1.3914	1.3895	1.3896	1.3826
C_7-C_{7a}	1.5102	1.5107	1.5117	1.5090
C_7-C_8	1.4113	1.4174	1.4136	1.4219
C_8-C_{8a}	1.5109	1.5104	1.5113	1.5094
C_8-C_9	1.4002	1.4048	1.4070	1.3993
C_9-C_{9a}	1.3966	1.3917	1.3926	1.3947

these calculations. Although the use of the UHF method can introduce some spin contamination, it should not significantly affect the structure. The UHF approach can also give spin density distributions. Figure 2 and Table 3 show the optimized geometry and some geometrical parameters. Radical anion ($HFl^{\bullet -}$, **7**) and **5** are essentially planar. On the other hand, in flavin radicals **6** and **8** the nitrogen centers tend to be pyramidal, and the overall structures of **6** and **8** are slightly puckered. Compound **6** is predicted to be slightly lower in energy than **5**, the energy difference being 1.2 kcal/mol. Overall, the isoalloxazine ring is similar in both **5** and **6** except for some significant differences in bond lengths around the two protonation sites (N1 and N5).

We also calculated the spin density distribution in each flavin radical. Even though the calculated spin density may not be very accurate due to spin contamination, since we are only interested in the relative spin density distribution, the results should be qualitatively useful. Table 4 lists the calculated spin density distributions for flavin semiquinone radicals **5** to **8**. In **5**, the positions that have the high spin density are N1, C4a, N5, C6, C8, and C9a; positions at C5a, C7, and C9 have large negative spin density. In **6**, C10a, N5, C6, C8, and C9a have the high spin density and C4a, C5a, C7, and C9 have high negative spin density. The spin density in **7** is mostly distributed among the benzene ring and N5, thus the ESR spectrum of **7** would be similar to that of the Ph−NR radical. It has been demonstrated experimentally that the spin bearing part of the flavin radicals (**5**−**8**) is the 6,7-dimethylquinoxaline.[21] Our calculated results are clearly in agreement with these experimental observations. It is also clear from Table 4 that protonation of flavin radicals perturbs spin density distribution.

Table 4. The Calculated Spin Density Distribution at the UHF/6-31G* Level for Flavin Radicals (5−8)

atom	**5**	**6**	**7**	**8**
N1	0.132	−0.002	0.013	0.011
C10a	−0.137	0.335	0.070	0.006
C2	−0.055	−0.005	0.000	0.013
N3	−0.001	−0.011	−0.014	−0.004
C4	−0.095	0.061	0.016	−0.037
C4a	0.560	−0.337	−0.042	0.162
N5	0.343	0.818	0.733	0.571
C5a	−0.654	−0.738	−0.712	−0.540
C6	0.699	0.798	0.792	0.647
C7	−0.694	−0.760	−0.743	−0.652
C7a	0.076	0.082	0.082	0.068
C8	0.729	0.821	0.807	0.754
C8a	−0.082	−0.094	−0.096	−0.083
C9	−0.687	−0.746	−0.694	−0.698
C9a	0.633	0.728	0.656	0.640
N10	0.055	0.062	0.059	0.126

Table 5. The Calculated Geometrical Parameters for the Reduced Form of Flavin at the RHF/6-31G* Level of Theory

bond	**9**	**10**	**11**
C_2-O	1.1946	1.2149	1.1819
C_2-N_3	1.3755	1.3919	1.3720
N_3-C_4	1.3826	1.3770	1.3756
C_4-O	1.1976	1.2252	1.1984
C_4-C_{4a}	1.4554	1.4079	1.4368
C_2-N_1	1.3664	1.3472	1.3985
N_1-C_{10a}	1.3821	1.3260	1.3575
$C_{4a}-N_5$	1.4036	1.4169	1.4520
N_5-C_{5a}	1.3978	1.3559	1.4710
$C_{4a}-C_{10a}$	1.3301	1.3724	1.3562
$C_{10a}-N_{10}$	1.3989	1.3964	1.3490
$N_{10}-C_{11}$	1.4685	1.4419	1.4645
$N_{10}-C_{9a}$	1.4389	1.4111	1.4160
$C_{9a}-C_{5a}$	1.3883	1.4170	1.3809
$C_{5a}-C_6$	1.3894	1.3848	1.3821
C_6-C_7	1.3840	1.3965	1.3811
C_7-C_{7a}	1.5112	1.5124	1.5099
C_7-C_8	1.4015	1.3808	1.4048
C_8-C_{8a}	1.5117	1.5127	1.5098
C_8-C_9	1.3845	1.4041	1.3859
C_9-C_{9a}	1.3849	1.3776	1.3906

Reduced Flavin. Since there are 20 π-electrons over 16 π-orbitals, it was speculated that H_3Fl_{red} (**9**) would be too unstable to be planar, due to electron repulsion. This electron repulsion can be relieved by puckering of the ring along the N5 and N10 axis. The left-hand part of the molecule is like a 1,2-diamino-4,5-dimethylbenzene and the right hand part of the molecule resembles a 5,6-diaminouracil. A crystallographic structure of an analogue of reduced flavin was found to be in a bent shape, the angle between the 1,2-diamino-4,5-dimethylbenzene and 5,6-diaminouracil planes being 144.5° (puckering

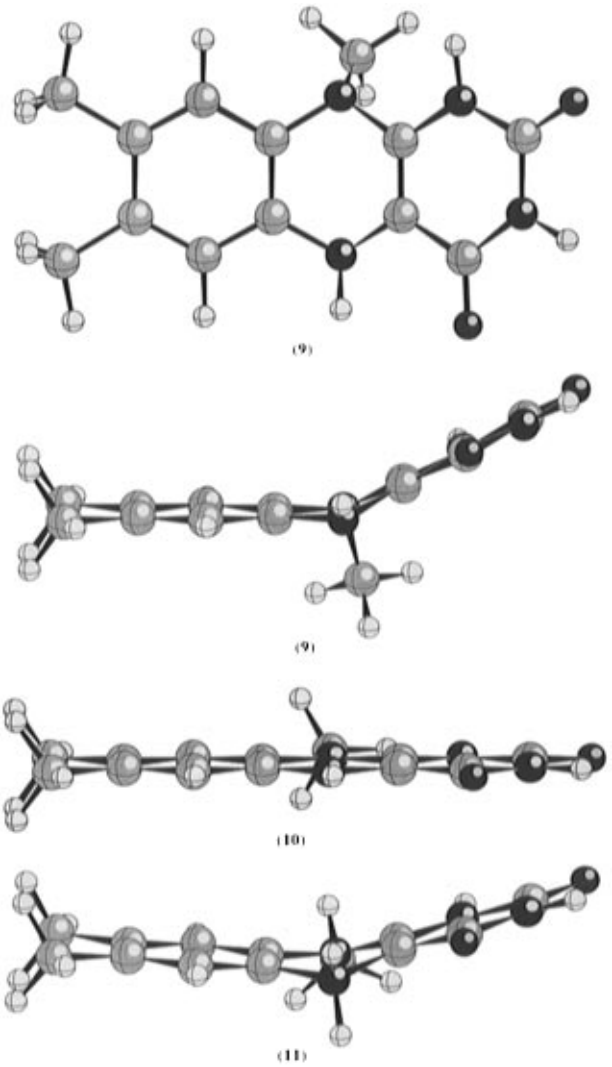

Figure 3. The optimized geometries for the reduced flavin at the RHF/6-31G* level.

angle of 35.5°).[18] However, because of the bulky groups present, it is not clear whether the puckering of the ring is caused by the presence of these bulky substituent groups or is caused by electron repulsion. On the other hand, earlier theoretical calculations indicate that the reduced flavin is bent, but as noted above, the reliability of these results is questionable. Therefore, based on pK_a arguments and earlier NMR observations, Kyte recently suggested that the reduced flavin is probably planar.[20] To clarify this issue, the geometry of H_3Fl_{red} (**9**) was optimized using the ab initio molecular orbital method at the RHF/6-31G* and RHF/6-31+G* levels. Two different views of the optimized structure at the RHF/6-31G* level are shown in Figure 3 and some geometrical parameters are given in Table 5; since the calculated structure at the RHF/6-31+G* level is essentially the same as the RHF/6-31G* geometry, no further discussion about the RHF/6-31+G* results is given. Surprisingly, the calculated structure is bent and the angle between the two parts is 152.4° (or puckering angle of 27.6°). The calculated puckering angle is about 8° smaller than in the bulky analogous compound; this difference is probably in part due to the presence of the bulky groups. Previous calculations using the PRDDO (partial retention of diatomic differential overlap) method with fixed bond lengths and angles yield a puckering angle of about 15°;[23a] for the MINDO/3 geometry-optimized structure, the puckering angle is about 27°.[24] In **9**, both N5 and N10 centers are pyramidal, although they are less pyramidal than a

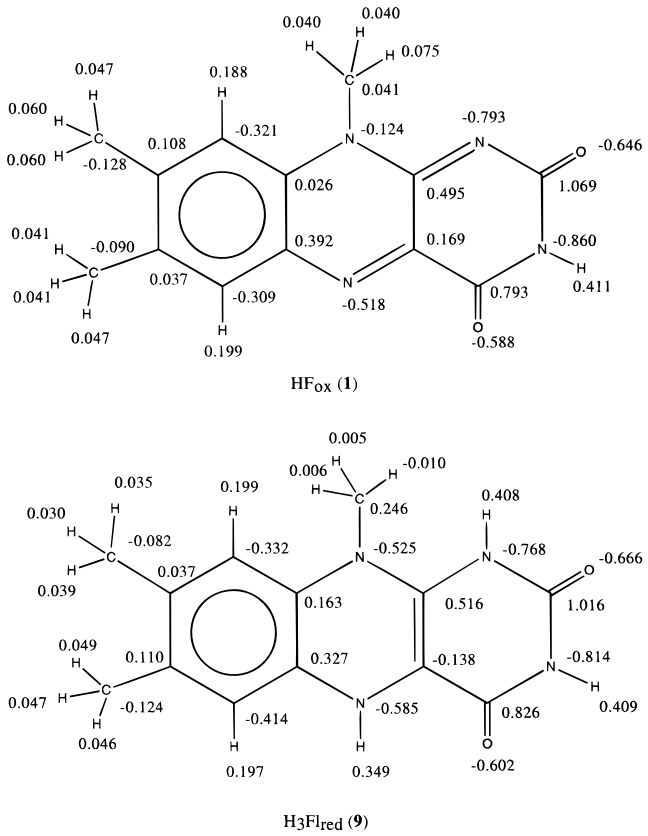

Figure 4. The electrostatic potential derived atomic charges for oxidized and reduced flavins.

tetrahedral nitrogen center; the hydrogen on N5 and the methyl on N10 point in opposite directions. The hybridization of N5 and N10 is probably somewhere between sp^2 and sp^3.

If **9** is bent, how do we explain the NMR observation? One possibility is that the barrier for the interconversion between two bent structures is relatively small and the NMR studies are unable to tell whether it is planar or it is in fast equilibrium. To address this question, we also calculated the planar form of H_3-Fl_{red} (**9**) by forcing it to be planar; the calculated energy difference between **9** and the planar form is estimated to be about 6.4 kcal/mol. Since ring flattening and nitrogen center inversion could take place in separate steps, the activation barrier should be smaller than 6.4 kcal/mol. Furthermore, this barrier could also be further reduced in solution since the puckered form has a smaller dipole moment than the planar form (4.9 D vs 5.4 D). Clearly, in the gas phase, the reduced flavin **9** in the free form is bent. Previous solution NMR investigations suggested that the intrinsic ring inversion barrier is less than 20 kJ/mol (or 4.8 kcal/mol).[19] The calculated barrier was about 2 kcal/mol using the MINDO/3,[24] about 4 kcal/mol using PRDDO method,[23a] and about 30 kcal/mol using a low level ab initio molecular orbital theory.[23d] Our calculation seems to support the NMR studies, i.e., the free reduced flavin is bent with a low intrinsic barrier of inversion. As far as the structure of reduced flavin in flavoenzymes is concerned, the presence of a protein environment may be able to force it to be planar.[33]

The calculated results for **10** and **11** are also included in Figure 3 and Table 5. As expected, **11** is puckered, however compound **10** is planar. The calculated puckering angle in **11** is about 22°. Again the most significant differences in bond length occur around the two protonation sites (N1 and N5). The

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bond between C5a and N5 is a double bond in **9** and **10**, but a single bond in **11**.

Conclusions

The structures of lumiflavin in different oxidation and protonation states were investigated using ab initio molecular orbital theory at the HF/6-31G* level. Based on our theoretical study, it is clear that the oxidized flavin and flavin radicals are planar or very close to planar, but the reduced flavin adapts a bent structure. In the reduced flavin H_3Fl_{red} (**9**), the isoalloxazine ring is puckered along the N5 and N10 axis with a puckering angle of 27.3°. Our calculations also identified that N1 is the site of protonation for the oxidized flavin, which is in agreement with previous experimental observations. For flavin semiquinone radicals, the calculated spin density distributions are also consistent with experimental results. Now with a better understanding of the free flavin structure, one may be able to understand the effects of solvent and protein environment on the structure and reactivity of flavins; to aid such future studies, Figure 4 depicts the electrostatic potential derived atomic point charges based on RHF/6-31G* wave functions, for oxidized and reduced forms of flavin within the CHELPG[34] scheme.

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