Conformational Effects on Flavin Redox Chemistry

Justin J. Hasford, William Kemnitzer, and Carmelo J. Rizzo*

Department of Chemistry, Box 1822, Station B, Vanderbilt University, Nashville, Tennessee 37235

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Evans and Nelsen showed that the specific conformation of cyclic hydrazines and bianthrones had measurable effects on their electron-transfer chemistry.[1] Such conformational control of redox potentials could be particularly relevant in enzymatic electron-transfer reactions since the oxidized and reduced forms of redox cofactors often have different geometries; one dramatic example is riboflavin (**1a**, Figure 1).[2] The crystal structure of oxidized and reduced old yellow enzyme was solved at 2.0 Å resolution and showed the geometry of the oxidized FMN cofactor to be planar, while 1,5-dihydroFMN is bent along the N5, N10 axis (Figure 2).[3] These geometries are in accord with theoretical studies.[4]

Massey and Hemmerich proposed that the apoenzyme may "tune" the redox potential of the cofactor though control of conformation.[5] X-ray crystallographic analysis of some flavoenzymes suggest this may be a factor. For instance, the protein crystal structure of flavodoxin showed the cofactor's geometry to be nearly planar in all three oxidation states.[6] Alternatively, the oxidized FMN cofactor of trimethylamine dehydrogenase is bent along the N5−N10 axis and strongly resembles the conformation of 1,5-dihydroflavin.[7] We have synthesized a series of conformationally biased flavin models (**2**−**5**) to determine the role of conformation on the redox properties of flavins.

We used 10-methylisoalloxazine (**2**) as a reference since it will not be biased toward the oxidized or reduced form. When compared to **2**, 9,10-dimethylisoalloxazine (**3**) should show a preference for the reduced state, since the bent geometry of the corresponding 1,5-dihydroflavin will alleviate steric interaction between the two methyl substituents; this can be readily seen in the Chem 3-D representations in Figure 3. On the other hand, for the reduction of 9,10-bridged flavins **4** and **5**, the N10 substituent cannot shift from an equatorial to an axial position since this will impart torsional strain into the tethering carbon chain, and thus, the bridged flavins should show a preference for the oxidized state.

The model flavins were synthesized by the condensation of the required *N*-alkyl-*o*-phenylenediamine with alloxan[8] and the redox properties studied by cyclic voltammetry in 100 mM, pH 7.4 HEPES buffers using a standard three-electrode cell with a glassy carbon working electrode, Ag/AgCl reference electrode (+197 mV vs SHE), and platinum wire counter electrode. The electrochemistry of flavins is similar to that of quinones.[2,9] One-electron reduction gives a radical anion that in protic media is protonated to give the flavin semiquinone (Scheme 1). The second one-electron reduction is fast to give the reduced flavin anion; the pK_a of the N1 proton of 1,5-dihydroflavins is approximately 6.5 and will be largely ionized under aqueous conditions. In protic solvent, the entire ECE reaction is faster than the CV time scale and observed as a single wave and is electrochemically reversible. The results from the electrochemical studies are tabulated in Table 1 and show that the

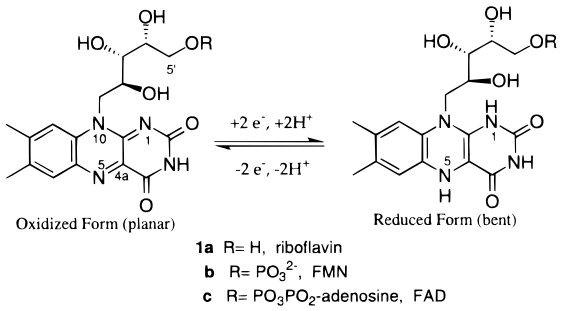

Figure 1.

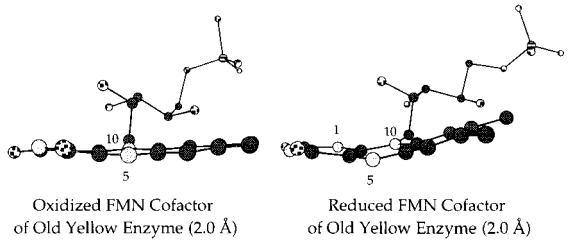

Figure 2.

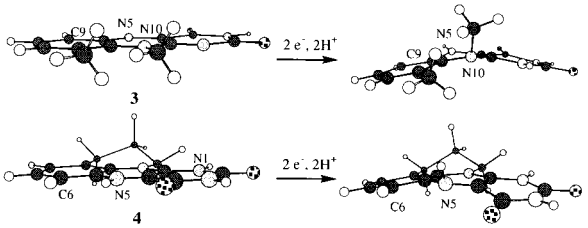

Figure 3.

Scheme 1

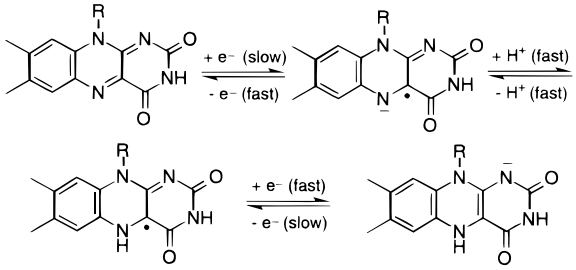

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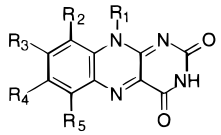
Table 1. Reduction Potentials of Flavins 1−9 in 100 mM, pH 7.4 HEPES Buffer vs Ag/AgCl (+197 mV vs SHE)



flavin	R_1	R_2	R_3	R_4	R_5	$E_p{}^a$	$E_p{}^c$	$E^{\circ\prime}$	$\Delta E^{\circ\prime}$
1a	ribityl	−H	$-CH_3$	$-CH_3$	−H	−462	−414	−438	−31
1b	FMN	−H	$-CH_3$	$-CH_3$	−H	−470	−416	−443	−36
1c	FAD	−H	$-CH_3$	$-CH_3$	−H	−470	−416	−443	−36
2	$-CH_3$	−H	−H	−H	−H	−428	−386	−407	0
3	$-CH_3$	$-CH_3$	−H	−H	−H	−378	−336	−357	+50
4	$-CH_2CH_2CH_2-$		−H	−H	−H	−450	−414	−432	−25
5	$-CH_2CH_2-$		−H	−H	−H	−428	−392	−410	−3
6	$-CH_3$	−H	−H	−H	$-CH_3$	−436	−392	−414	−7
7	$-CH_3$	−H	−H	$-CH_3$	−H	−438	−400	−419	−12
8	$-CH_3$	−H	$-CH_3$	−H	−H	−460	−418	−439	−32
9	$-CH_3$	−H	$-CH_3$	$-CH_3$	−H	−480	−432	−456	−49

[a] The formal standard potential of each substrate was determined by the relationship $E^{\circ\prime} = (E_p{}^c + E_p{}^a)/2$.[11]

redox properties of conformationally biased flavins **3**−**5** behave as predicted. The reference flavin **2** has a reduction potential of −407 mV. Reduction of **3** is more favorable by +50 mV (1.20 kcal/mol). Tethered flavins **4** and **5** are more difficult to reduce by −25 and −3 mV, respectively.

Substituents on the benzene subnucleus of flavins are expected to have an electronic effect on the redox properties. The electron-donating alkyl groups at C9 of **3**−**5** makes reduction more difficult; reference flavin **2** is unsubstituted. To determine the magnitude of these electronic effects, flavins **6**−**9** were synthesized, and their reduction potentials are listed in Table 1. The magnitude of the electronic effect depended on the location of the methyl group. The initial reduction of flavins give a radical anion (Scheme 1) that is coupled to the benzene moiety through the N5 position. The C8-methyl group of **8** is para to N5 and has the largest electronic effect ($\Delta E^{\circ\prime} = -32$ mV). Surprisingly, the effect of a C6-methyl group (**6**) that is ortho to N5 is small ($\Delta E^{\circ\prime} = -7$ mV); however, this is in accord with previously reported work.[10] Given its position, there may be a steric component to this value. Since alkyl substituents at C9 of flavins **3**−**5** are meta to N5, we estimate the electronic effect to be similar to flavin **7** ($\Delta E^{\circ\prime} = -12$ mV) in which the C7-methyl group is also meta to N5. The electronic component of N9 alkylation on redox properties is estimated to be small.

The change in reduction potential for tethered flavins **4** and **5** is smaller than that of **3**. Simple modeling (Figure 3) showed very little conformational change in the three-carbon bridge of **4** upon reduction since the N5 hydrogen can adopt a pseudoaxial position instead of the N10 substituent. In free flavin, the N10 substituent shifts from an in-plane equatorial position in the planar oxidized form to a pseudoaxial position above the flavin ring system in the bent reduced state. The N10 ribityl group found in FMN and FAD, however, is the main binding domain of the enzyme−coenzyme complex. As such, the N10 ribityl group does not have the same conformational degrees of freedom as free flavins and is locked in the in-plane position, even in the reduced form. This can be seen in the Chem 3D representation of reduced FMN from old yellow enzyme (Figure 2) where the N5 hydrogen adopts a pseudoaxial position in the bent conformation. Tethered models **4** and **5** best mimic the conformation of the protein-bound cofactor.

The conformation of the cofactor is thought to play a role in modulation of flavin redox potential by flavoenzymes. We present here the first experimental evidence that the redox properties of flavins can be driven by conformational effects. The redox potential for our conformationally biased flavins is modulated over a 75 mV range (1.7 kcals/mol), which is much smaller than that seen in flavoenzymes. It must be recognized that redox modulation by the apoenzyme can occur by a number of mechanisms including hydrogen bonding, π-stacking, and desolvation in addition to conformation. Schultz had previously studied the role of conformation on flavin redox potential using an antibody that selectively binds one of the oxidation states.[12] Much like the enzymatic systems, the redox properties of the antibody-bound flavin model may have these other effects superimposed on the results. In comparison with other flavin models, the conformational component of flavin redox properties is smaller than recent results on the effect of hydrogen bonding to the pyrimidine subnucleus of flavins (155 mV range, 3.6 kcals/mol)[13a] and is in the same range as π-stacking interactions (91 mV range, 2.1 kcals/mol).[13b] The combination of these effects observed in flavin models approaches those of enzymatic systems.

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Supporting Information Available: Typical experimental procedures for the preparation of flavins **3**−**8** and copies of their ^1H and ^{13}C NMR spectral. Copies of cyclic voltammograms of compounds **1**−**9** (22 pages).

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