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Reduction of Hg(II)•EDTA by Conformationally Biased Flavins

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Abstract: The rates of Hg(II)•EDTA reduction by conformationally biased flavins are reported. The rates of the reduction correlate to the redox potential of the flavin model. In the case of 8,9,10-trimethylflavin (**6**), the rate was slower than expected from the redox potential.

Flavoenzymes are versatile biological redox catalysts which utilize derivatives of riboflavin (vitamin B_2) to mediate electron-transfer.[1] The conformation of flavin coenzyme is dependent on its oxidation state. While the oxidized and the semiquinone forms are planar, the fully reduced state has a bent conformation; these geometries have been confirmed crystallographically and computationally.[2,3] Massey and Hemmerich suggested that a possible mechanism by which the apoenzyme could modulate the redox properties of the cofactor was through control of conformation.[4] Protein crystallographic studies of flavoenzymes suggest this may be a factor.[5,6] In order to determine the role of conformation on flavin redox chemistry, we have recently reported the synthesis of conformationally biased flavin models **4** and **5** and studied their redox properties by cyclic voltammetry.[7] This work provided the first experimental evidence that the redox chemistry of flavins could be driven in a predictable way though conformational effects. Upon reduction, the N10 substituent of free flavin shifts from an in-plane, pseudoequitorial position to a pseudoaxial position above the isoalloxazine ring system (Figure 1). When a methyl group was placed at C9 as in flavin **5**, a better steric arrangement was achieved

Figure 1

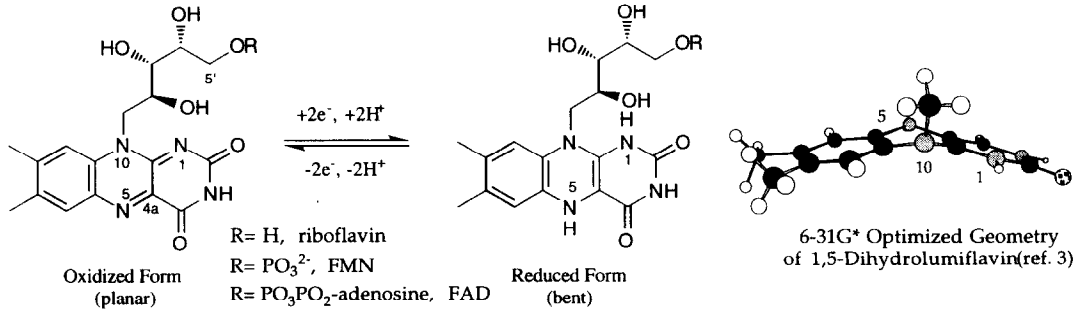

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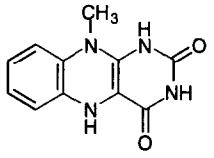
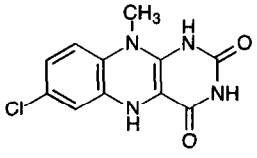
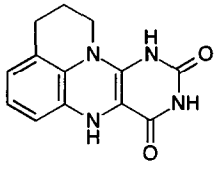
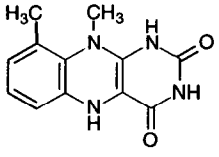
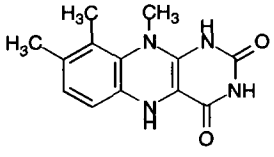
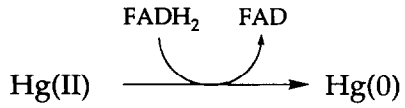
Table 1.[10] Reduction of Hg(II)•EDTA by flavin models under pseudo first order conditions in 100 mM, pH 7.4 HEPES buffer at 30 °C. Rates are an average of 5 runs with the standard deviation as the error. Reactions were carried out to at least five half-lives.

	Flavin	k' (min^{-1})	k_{rel}	Redox Potential [7] (mV vs Ag.AgCl)
1	1,5-Dihydroriboflavin	0.420 ± 0.016	1.6	-438
2		0.260 ± 0.005	1.0	-407
3		0.096 ± 0.009	0.37	-371
4		0.445 ± 0.017	1.7	-432
5		0.077 ± 0.004	0.30	-357
6		0.056 ± 0.004	0.22	-368

upon reduction and a shift in the reduction potential to a less negative value was observed. When the 9,10-positions were tethered as in **4**, the N10 substituent was held in-plane even in the reduced state, making reduction less favorable. To complement the electrochemical studies, we wished to determine if the observed conformational preferences translated into a measurable kinetic effect. We report here the two-electron reduction of mercuric ion by free 1,5-dihydroflavin models.

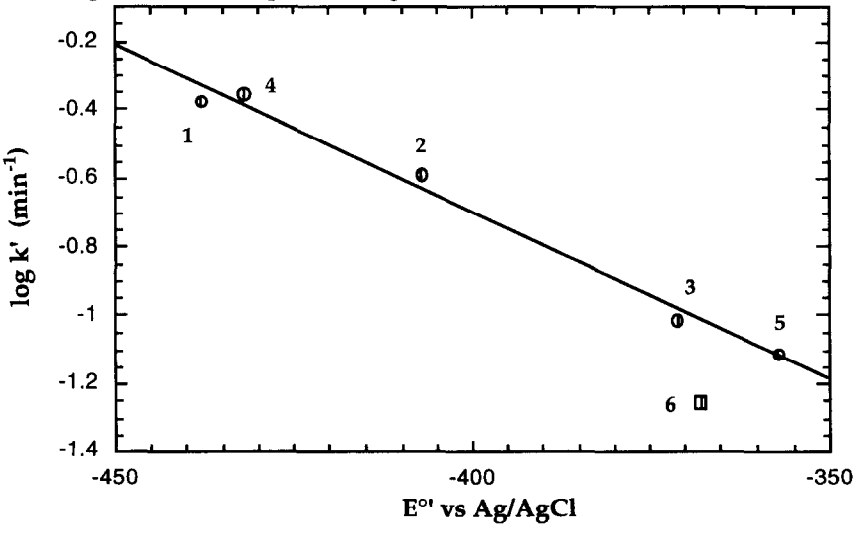
The one- and two-electron reduction of metal ions with flavin semiquinone and 1,5-dihydroflavins has been previously studied.[8] Much of this work was in connection with mechanistic studies on the bacterial heavy metal detoxification enzyme mercuric ion reductase in which an active site FAD is used as an electron-transfer conduit from NADPH for the reduction of Hg(II) to Hg(0); the mercuric ion is ligated to up to four active site



cysteines.[9] It has been shown that the rate of reduction of mercuric ion by free dihydroflavin is dependent on the ligand.[8a,b] We examined the reduction of Hg(II)•EDTA with dihydroflavins **1-6** in 100 mM, pH 7.4 HEPES buffer at 30 °C under pseudo first-order conditions as previously described by Walsh.[8a] The flavins were reduced with sodium dithionite and their rate of re-oxidation upon addition of excess Hg(II) was monitored by UV spectroscopy at 450 nm under anaerobic conditions;[10] rate constants were determined by non-linear least-squares fits. Since flavin **5** is biased towards the reduced state, we expected its re-oxidation to be retarded, whereas flavin **4** is biased towards the oxidized form and its re-oxidation is expected to be accelerated; our results are summarized in Table 1. The re-oxidation of bridged dihydroflavin **4** is approximately six-fold faster than **5**, which is consistent with our predictions. We have also examined 8,9,10-trimethylflavin **6**. We reasoned that the steric buttressing between the C8 and C9 methyl groups should enhance the conformational bias towards the reduced state. The re-oxidation of **6** by Hg(II)•EDTA was slightly slower than **5**.

It is well documented that the rates of free flavin mediated reactions are linearly correlated with the redox potential or polaragraphic half-wave potential of the flavin;[11] this linear relationship holds for flavins **1-5** (Figure 2). For conformationally biased flavins **4** and **5**, the rates of re-oxidation by Hg(II)•EDTA were reflected in the redox potential however, dihydroflavin **6** showed an off-linear kinetic effect. The rate of re-oxidation of **6** was approximately half that predicted from its redox potential. We attribute this off-linear behavior to an enhanced conformational bias toward the reduced state due to the steric crowding of the methyl groups. This result shows that the rate of flavin mediated reactions can be influenced by conformational considerations.

Figure 2. Plot of log k' versus redox potential. log k'= -0.0098 E°' - 4.6. (R= 0.993)



Through the synthesis of flavins **4** and **5** we had previously shown that the conformational effects could provide a thermodynamic driving force for flavin redox chemistry.[7] In the present study, we have established a linear relationship between the rate of mercuric ion reduction and the redox potential of the flavin. For flavin **6**, an off linear effect was observed. While the magnitude of the effect was relatively modest, it provides evidence that the rates of flavin mediated reactions can be influenced by the conformation of the flavin.

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