Model Systems for Flavoenzyme Activity. Modulation of Flavin Redox Potentials through π-Stacking Interactions

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Received April 12, 1996
Revised Manuscript Received September 9, 1996

Redox-active organic molecules are important species in biological and man-made systems. In biological systems redox-active organics serve as cofactors, performing redox reactions and serving as electron shuttles. In materials, they serve such diverse functions as organic semiconductors,[1] light-harvesting devices,[2] and molecular magnets.[3] One area that has been generally neglected in the study of the redox chemistry of organic and bioorganic systems is the interplay between intermolecular forces (including hydrogen bonding and π-stacking) and redox behavior. To provide insight into these interactions of biological importance, we are currently investigating cofactor−receptor interactions using electrochemical and spectroscopic techniques.[4−6]

Enzyme−cofactor π-stacking interactions[7,8] are believed to play an important role in the modulation of flavin reactivities by the apoenzymes. An example is found in the flavodoxins. These proteins utilize a molecule of FMN as cofactor in a highly conserved binding site containing tryptophan and tyrosine residues (Figure 1).[9] Binding of this cofactor within the active sites alters the redox properties of the flavin, favoring formation of the semiquinone at low potentials. Recent literature[8b,10] proposes an active role for the neighboring aromatic side chains in modulating the redox properties of the flavin cofactor. Due to the complexity of the enzymatic system, however, the effects of π-stacking, hydrophobic effects, and electrostatic interactions are difficult to quantify individually.

To directly determine the effects of π-stacking on flavin redox chemistry, we have developed a model for studying this interaction. As shown in Figure 2, three-point receptor−flavin hydrogen bonding[4,12] by the diaminotriazine[13] orients the flavin over the aromatic surface. The modularity of the receptor design then allows the aromatic surface to be varied parametrically while other interactions are kept constant.

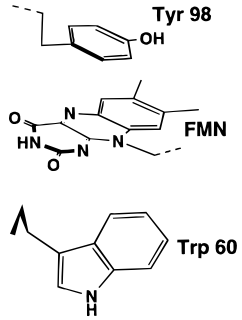

Figure 1. Flavin binding site of the flavodoxin isolated from *Desulfovibrio vulgaris*.[11]

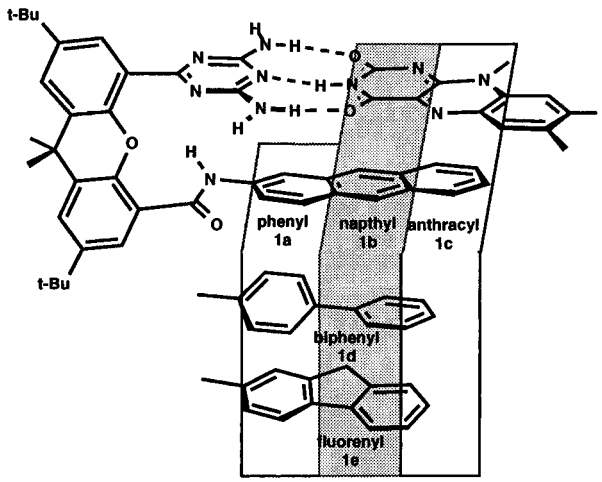

Figure 2. Binding of flavin **2** to receptor **1**, showing computationally predicted (AMBER force field[14]) $\pi-\pi$ overlap.

Stacking interactions between receptor **1** and flavin **2** were verified independently by fluorescence quenching and NMR titration. Addition of phenyl receptor **1a** resulted in moderate quenching of the fluorescence emission of flavin **2** at 511 nm due to hydrogen bonding (Figure 3). This was verified by comparison with the dipropyl amide of 2,6-diaminopyridine,[4] which provided comparable quenching. Addition of napthyl receptor **1b** provided a 3-fold decrease in fluorescence emission intensity over receptor **1a**, indicating moderate π-overlap. Anthracyl receptor **1c** provided almost complete quenching of fluorescence emission at 511 nm, indicating substantial overlap between the receptor and flavin fluorophore. In contrast, no quenching of the fluorescence of the *N*(3)-methylflavin **3** was observed upon addition of receptors **1a**−**e**, indicating that quenching of flavin **2** by receptors **1** occurs through the hydrogen-bound complex.

Further evidence for π-stacking in complexes of flavin **2** with hosts **1b**−**e** is provided by the enhanced association energy observed with these receptors relative to **1a**, as quantified through NMR titration.[15,16] As shown in Table 1, substantial increases in flavin binding were observed with increasing aromatic overlap, corresponding to an increase in the free energy

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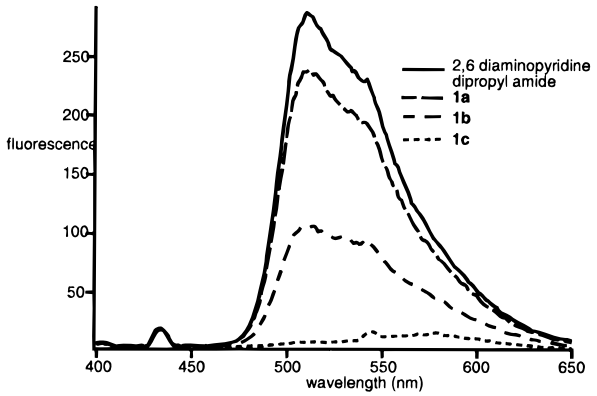


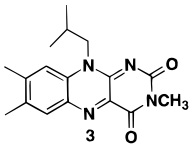
Figure 3. Fluorescence emission of flavin **2** in the presence of receptors in CH_2Cl_2. Excitation was at 445 nm, concentration of flavin **2**, 1×10^{-4}. Concentrations of receptors were chosen to provide 80% bound flavin: [2,6 diaminopyridine dipropyl amide] $= 7.5 \times 10^{-3}$; [**1a**] $= 1.05 \times 10^{-2}$; [**1b**] $= 4 \times 10^{-3}$; [**1c**] $= 6 \times 10^{-4}$ M.

Table 1. Binding Constants and Reduction Potentials[17] for Flavin **2**−Receptor **1** Complexes

receptor	K_a (M^{-1})[a]	ΔG_a (kcal/mol)	$E_{1/2}$ (mV)[b]	$\Delta E_{1/2}$ (mV)[b]	$\Delta\Delta G_{re}$ (kcal/mol)
none			−1290	0	0
1a	394	−3.52	−1272[c]	+18	−0.4
1b	1 080	−4.11	−1301[c]	−11	0.3
1c	11 520	−5.50	−1353[d]	−63	1.5
1d	2 800	−4.67	−1318[d]	−28	0.7
1e	17 600	−5.75	−1351[d]	−61	1.4

[a] $CDCl_3$, 23 °C, H(3) peak followed. [b] In CH_2Cl_2, tetrabutylammonium perchlorate carrier (0.1 M) , [**2**] $= 1 \times 10^{-3}$ M, 23 °C.[18] [c] [**1**] $= 1 \times 10^{-2}$M. [d] [**1**] $= 5 \times 10^{-3}$.

of complexation of anthracyl receptor **1c** of 2.1 kcal/mol over phenyl receptor **1a**.



With host−guest complexation verified, we next studied the effects of π-stacking on flavin reduction potentials. Cyclic voltammetry (CV) studies of flavin **2** (Figure 4, trace I) show a single reduction peak, but two separate oxidation couples. Peak **a** is the reversible oxidation couple of the reduction of **2** to the radical anion, while peak **b** represents the oxidation of fully reduced flavin.[18] Addition of phenyl receptor **1a** to flavin **2** provides a 18 mV less negative potential for the $E_{1/2}$ of the reversible redox couple (Figure 4, trace II).[19] This is due to the stabilization of the radical anion through receptor−flavin hydrogen bonding,[4] and corresponds to a 0.4 kcal/mol stabilization. No change was observed in the reduction potential of flavin **3** after the addition of receptors **1a**−**e**. For all receptors **1**, addition of relatively low concentrations of host provides near-limiting shifts in flavin potentials. This results from redox-enhanced hydrogen bonding, observed first by us with flavins,[4] and later by Smith with *o*-quinones and imides.[20]

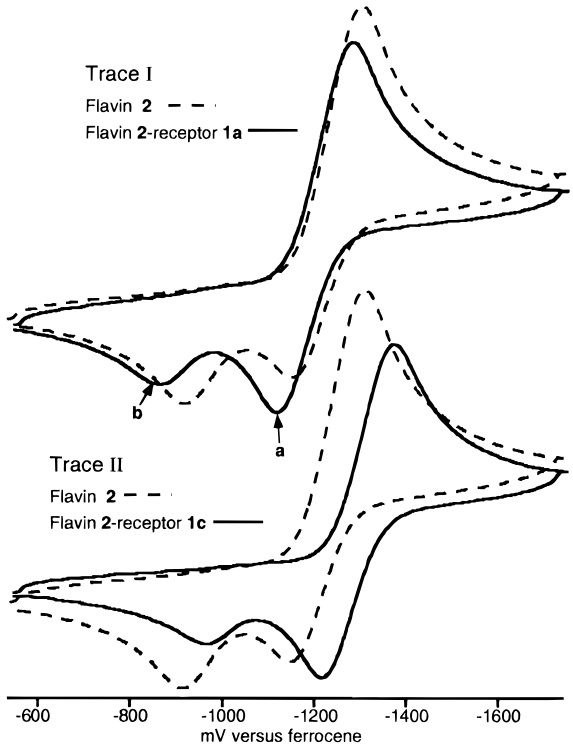


Figure 4. Cyclic voltammetry of 10-isobutylflavin **2** and the complexes of the flavin **2** with receptors **1a** (trace I) and **1c** (trace II). CH_2Cl_2 was used as solvent, with tetrabutylammonium perchlorate carrier (0.1 M). Concentration of flavin **2**, 1×10^{-3} M and receptors **1a**, 1×10^{-2}, and **1c**, 5×10^{-3} M; scan rate 500 mV/s; $T = 23$ °C.

Increasing receptor−flavin stacking interactions resulted in the flavin reduction potential moving to more negative potentials. Addition of anthracyl receptor **1c** shifted the reduction potential of flavin **2** to 63 mV more negative than flavin **2** alone. Using phenyl receptor **1a** (with identical hydrogen bonding but no $\pi-\pi$ overlap) as a control, we can conclude that stacking makes the flavin more difficult to reduce by 91 mV, representing a free energy change for the reduction process ($\Delta\Delta G$) of 2.1 kcal/mol.

The increasingly negative reduction potentials observed as π-overlap increases indicate that receptor−flavin stacking interactions are more favorable than receptor−flavin radical anion interactions. This is a direct result of the transformation of the electron-poor oxidized flavin to the electron-rich radical anion. This would be expected to diminish the favorable electrostatic interaction between flavin **2** and the relatively electron-rich aromatics of the receptors.[21]

In summary, we have used a series of receptors to examine the effects of π-stacking on flavin recognition and redox potentials. We have established that aromatic stacking interactions between these receptors and their flavin guests effectively modulate redox potential over a 91 mV (2.1 kcal/mol) range.

Acknowledgment. This research was supported by the National Science Foundation (Grant CHE-9528099) and the Petroleum Research Fund of The American Chemical Society (Grant 30199-G4). V.M.R. thanks Research Corporation for a Cottrell Fellowship.

Supporting Information Available: ^{1}H NMR titration data, cyclic voltammetry plots, and plots of flavin $E_{1/2}$ vs receptor concentration for receptor **1a**−**e**-flavin **2** complexes, and the Job plot for the receptor **1c**−flavin **2** complex (6 pages). See any current masthead page for ordering and Internet access instructions.

JA9612110

(15) Performed via addition of aliquots of hosts **1a**−**e** to a solution of flavin **2**. The plot of the chemical shifts of H(3) as a function of receptor concentration provided a titration curve. Association constants were determined through nonlinear least squares curve fitting. All curves provided a good fit to the 1:1 binding isotherm. The Job plot of the receptor **1c**−flavin **2** complex was likewise consistant with 1:1 binding stoichiometry.

(16) Fluorescence titrations using receptors **1a**−**c** provided essentially identical binding constants.

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