

## Forum Original Research Communication

# Modulating the Redox Property of a Flavin Analog Through Adjustment of Its Microenvironment in a Self-Assembled Monolayer

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### ABSTRACT

Self-assembled monolayers (SAMs) have been used to examine the effects of electrostatic interactions and changing microenvironment on the pH-dependent redox properties of a flavin analog. The  $pK_a$  value of the N1 proton for the reduced flavin analog was determined to be  $\sim 9.7$  in the SAM of disulfide 5, 8.5 in the SAM of disulfide 7, and 6.7 when free in solution. The pyridinium ion of 7 stabilizes the anionic form of reduced flavin analog and provides a dielectric medium more closely resembling that experienced by the flavin analog free in solution. *Antioxid. Redox Signal.* 5, 731–736.

### INTRODUCTION

FLAVOENZYMES are an important class of enzymes present in most plants and animals (16, 23, 37). Flavins [*e.g.*, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)] are coenzymes that catalyze many reactions, such as oxidations, dehydrogenations, hydroxylations of aromatic compounds, and activation of oxygen (7, 11, 15, 17, 37). In addition, current research demonstrates that flavoenzymes can be used to inhibit self-splicing of certain types of genes (19). Of importance in these reactions is the isoalloxazine ring (Fig. 1), the redox active component of the coenzymes. The isoalloxazine moiety of flavins and analogs may undergo a one- or two-electron transfer process (Scheme 1; Et, ethyl; Me, methyl), depending on the apoprotein that it complexes with or the medium in which it resides (12, 16, 18, 35, 37).

In biological systems, noncovalent interactions (including  $\pi$ -stacking, hydrophobic effects, hydrogen bonding, and electrostatic interactions) (2–4, 25, 30, 31, 35) between flavins and apoproteins play an important role in varying the redox potential of the isoalloxazine moiety over a range of as much as 600 mV (37). A fundamental understanding of the factors influencing the redox properties of flavins can be expected to provide insight into biological mechanisms for tuning their redox potentials and may allow control of its catalytic properties in nonbiological systems. In this article, we report the use of self-assembled monolayers (SAMs) to examine the effects of electrostatic interactions and changing microenvironments on the redox potential of the isoalloxazine moiety.

SAMs of terminally functionalized alkyl thiols and disulfides (26, 27, 36, 38) serve as use-

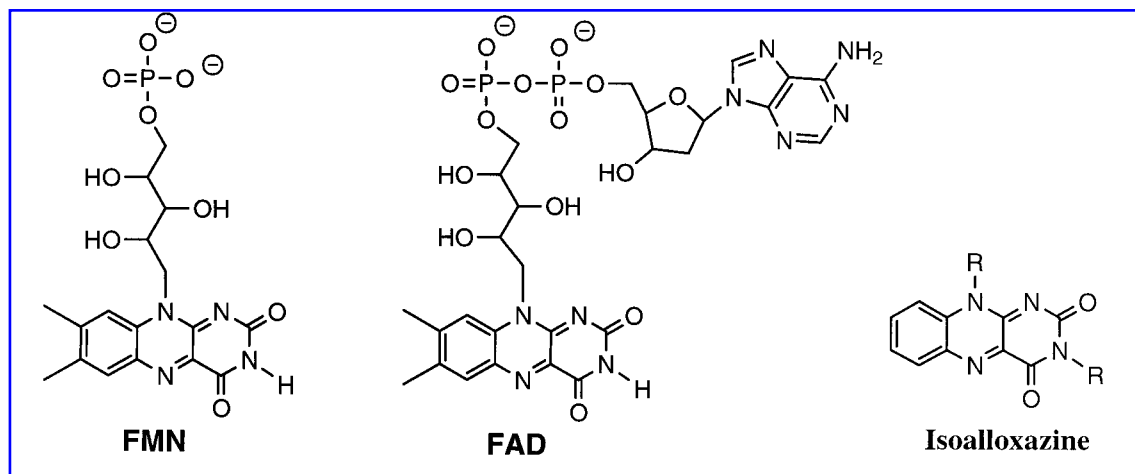


FIG. 1. Structures of flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and the isoalloxazine ring.

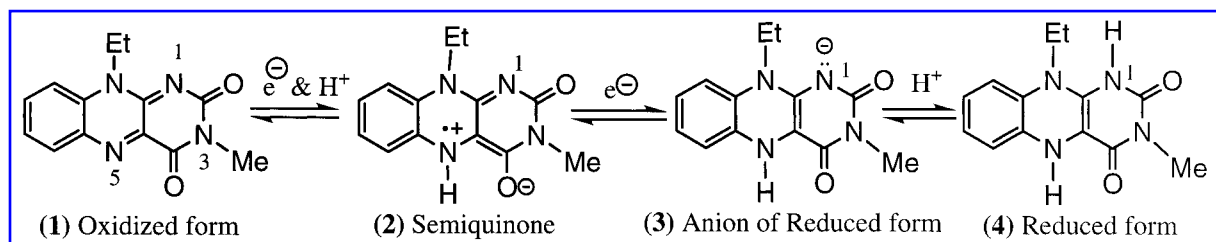
ful models for fundamental studies of the microenvironmental effects on electron-transfer kinetics (5, 6, 10, 13, 14, 21, 22, 29) and other phenomena in interfacial science (24, 28, 32). Our research group is interested in studying the redox properties of flavin analogs in different microenvironments provided by SAMs. Previous studies by our group have found that the pH-dependent redox properties of the isoalloxazine unit in a SAM prepared from disulfide **5** (Fig. 2) are different from those of **1** (Scheme 1) free in solution (34). The  $pK_a$  value of the N1 proton of **4** (Scheme 1) in solution is  $\sim 6.7$ , as determined from the inflection point of the  $E^0$  versus pH plot (9). This value is consistent with those reported from reduced FMN and FAD in aqueous solutions (8, 18). The  $pK_a$  value of the N1 proton of its analog in the SAM of **5** is, however, 3 units higher. This striking difference was attributed to the instability of the anions in the SAM resulting from the electrostatic repulsion in the low dielectric environment of the SAM (1, 20).

In this study, we examine the effects of introducing a cationic species into the SAM to enhance the stability of the anions by electrostatic attraction and  $\pi$ -stacking interactions and by changing the dielectric medium provided by the SAM. We synthesized disulfides **6–8** (Fig. 2) and studied the electrochemical properties of their SAMs by cyclic voltammetry. Their properties are compared with those of a SAM prepared from **5**.

## MATERIALS AND METHODS

Disulfides **5** and **6** were prepared according to a previously published procedure (33). Disulfides **7** and **8** were synthesized by reactions of **6** and dibromide **10**, respectively, with neat pyridine at  $50^\circ\text{C}$  for 24 h as shown in Scheme 2 (Et, ethyl; Ph, phenyl).

SAMs were prepared by placing gold (300 Å thickness)-coated silicon wafers into  $\sim 1$  mM solutions of the disulfides in acetonitrile for



Scheme 1.

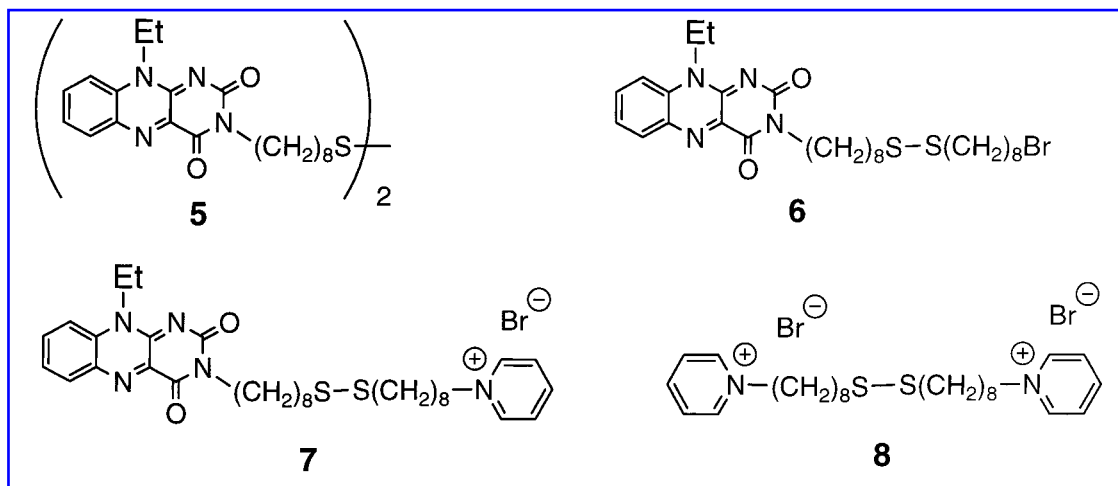


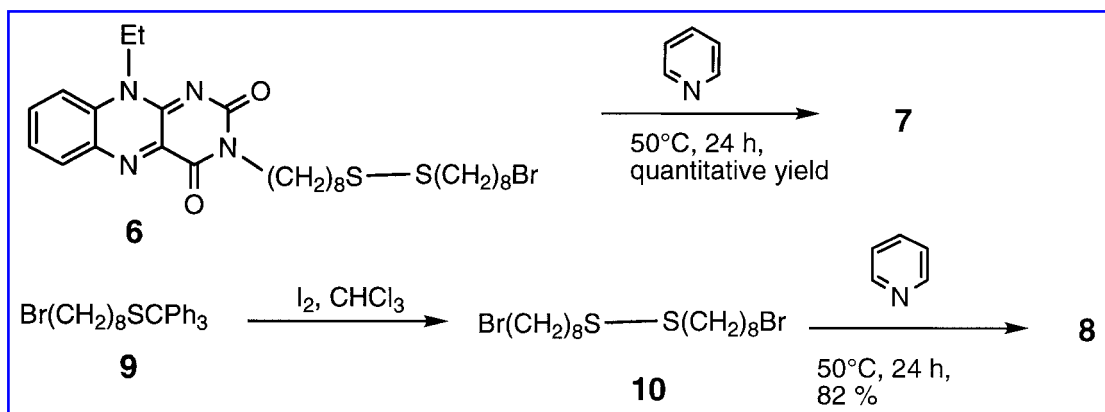
FIG. 2. Structures of disulfides 5–8. Et, ethyl.

24–48 h. The redox potentials were determined over a range of pH values using cyclic voltammetry. A standard three-electrode cell was used. The piece of gold with the SAM was used as the working electrode, a Bioanalytical Systems Ag/AgCl electrode was used as the reference electrode, and a platinum wire was used as the counter electrode. The buffer solutions were purged with argon and maintained under an argon atmosphere during the electrochemical experiments. The surface coverage of the isoalloxazine moiety in different SAMs was calculated from the integrated area of the reduction or oxidation peak and is reported without correction for roughness factor. The formal potentials ( $E^0$ ) of the isoalloxazine moiety in the SAMs at different pH values were determined from the average of the reduction

and oxidation peak potentials in the cyclic voltammograms at steady state.

## RESULTS AND DISCUSSION

The surface densities of the isoalloxazine moiety in the SAMs from **5**, **6**, and **7** were calculated to be approximately  $3.6 \times 10^{-10}$ ,  $2.1 \times 10^{-10}$ , and  $1.5 \times 10^{-10}$  mol/cm<sup>2</sup>, respectively. The area per isoalloxazine unit (determined from surface coverage) in the SAMs from **5** was about two times larger than the calculated area of 21.4 Å<sup>2</sup> per molecule in a close-packed SAM of alkanethiols on gold (36). It is unlikely that the methylene chains in the SAMs from **5** are close-packed or in a high degree of order due to the short methylene chains and the large



Scheme 2.

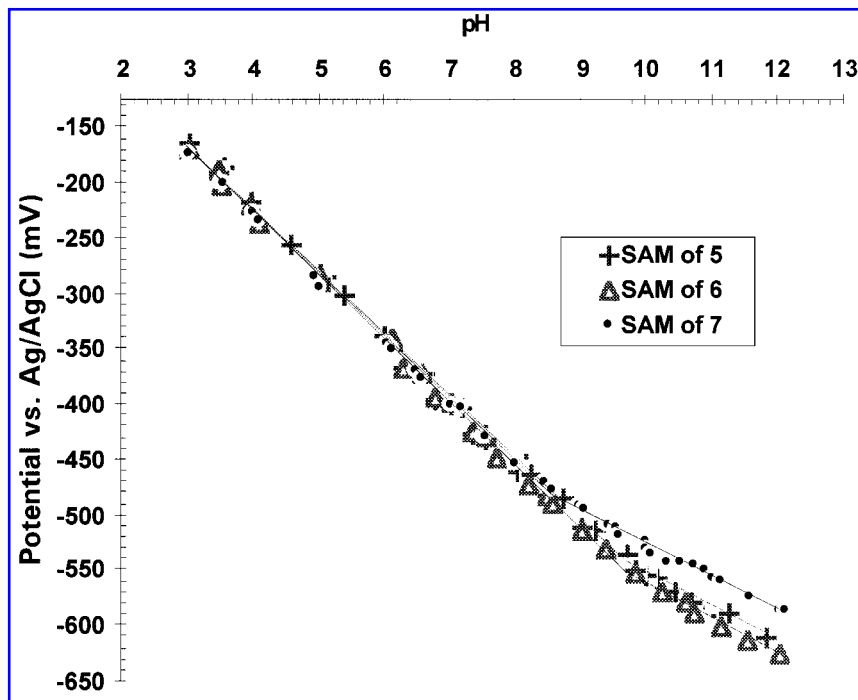


FIG. 3. Plot of the pH dependence of the redox potential ( $E^0$ ) of isoalloxazine in different SAMs.

isoalloxazine head group. The surface densities of the isoalloxazine moiety in the SAMs of unsymmetrical disulfides 6 and 7 were not half of that of the SAMs of 5. Apparently, the size and charge of the other head group influenced the surface densities of these unsymmetrical disulfides and hence the isoalloxazine unit in these SAMs. The surface coverage of the isoalloxazine unit in a SAM of 7 was less than that of a SAM of 6 and is presumably caused by the electrostatic repulsions and larger size of the pyridinium ring in 7 compared with the bromine atom in 6.

As shown in the plot of formal potentials ( $E^0$ ) versus pH (Fig. 3), the pH-dependent redox potentials of the flavin analog in the SAM prepared from 7 differ significantly from those of the SAM of 5. The inflection point of the plot yields a  $pK_a$  value of  $\sim 8.5$  for the N1 proton of the reduced isoalloxazine in the SAM of 7. This is approximately a 10-fold increase in the acid dissociation constant ( $K_a$ ) compared with that in the SAM of 5. Cyclic voltammetry studies of the SAMs prepared from disulfide 8 showed no redox activity as expected. This suggested that the difference in the pH-dependent redox potential observed for the SAM of 7 compared

with the SAM of 5 is not caused by any redox activity of the pyridinium moiety.

The substantial increase in the stability of the anion of reduced isoalloxazine in the SAM of 7 compared with the SAM of 5 could be attributed to several reasons. First, there is less repulsion among the anions because the surface density of the isoalloxazine rings in the SAMs of 7 is only  $\sim 40\%$  of that in the SAMs of 5. Second, the presence of positively charged pyridinium ions in the SAM stabilizes the anion of the reduced isoalloxazine by electrostatic attraction and  $\pi$ -stacking interactions. Third, the positively charged pyridinium ions in the SAM change the dielectric medium provided by the SAM, thus providing a microenvironment more closely resembling flavin free in solution.

To examine the effect of reduced surface coverage of the flavin analog in a SAM, we studied the pH-dependent redox potentials of a SAM formed from disulfide 6. Surprisingly, there is no significant change in the  $pK_a$  value of the N1 proton of reduced isoalloxazine in the SAM of 6 compared with that of a SAM of 5. A similar result was observed when a dodecyl chain instead of a bromooctyl chain was present in the unsymmetrical disulfide (34). One

would expect reduced repulsion and increased stability at the anions as the density of the isoalloxazine moiety decreased. The observed results may be due to the low dielectric environment provided by the dodecyl or bromooctyl chains being unfavorable for anion formation. Nevertheless, the results suggested that the effect of decreased surface density of the isoalloxazine moiety was not significant compared with the stabilizing effects provided by the pyridinium ions through electrostatic attractions,  $\pi$ -stacking interactions, and changing of the dielectric medium in the monolayer of **7**. To differentiate further the effects of electrostatic attractions,  $\pi$ -stacking interactions, hydrophobicity, and dielectric environments, studies on isoalloxazine-tethered unsymmetrical disulfides with other functional groups (such as hydroxyl or quaternary ammonium groups) are under way.

In summary, we have demonstrated the ability to modulate the redox potential of a flavin analog in a monolayer by adjusting the properties of the other monolayer components.

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## ABBREVIATIONS

FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; SAM, self-assembled monolayer.

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