Modulating the pH-dependent redox potential of a flavin analog *via* incorporation into a self-assembled monolayer on gold

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We report the first example of modifying the pH-dependent electrochemical properties of a flavin analog on gold by changing the pK_a value of its N^1 proton.

Flavoenzymes are important electron shuttles in a wide range of biological redox reactions.[1] The redox potential of flavin (the redox active moiety of flavoenzymes) varies by at least 600 mV, as governed by binding in different apoproteins. Non-covalent interactions (including π-stacking,[2] hydrophobic effects, hydrogen bonding, and electrostatic interactions)[3] between flavin and apoproteins play important roles; however, it is difficult to examine the effects of individual interactions in complex biological systems. A fundamental understanding of environmental effects on the flavin redox potential can be expected to provide insight into biological mechanisms for tuning the redox potential of flavoenzymes. In addition, control over its redox activity may increase selectivity of electrocatalysis in synthetic devices.[4] Monolayers prepared from alkanethiols or disulfides with electroactive derivatives[5] are commonly used for studying environmental effects on electron-transfer kinetics.[6] The present work presents novel self-assembled monolayer (SAM) formation based on an alkanethiolate derivatized flavin analog[7] and the modulation of its redox properties *via* incorporation into SAMs.

Disulfide **1** was synthesized according to the procedure outlined in Scheme 1.[8] SAMs containing isoalloxazine (the heterocyclic ring system of flavin) were prepared by immersing a gold coated (500 Å) silicon wafer in an acetonitrile solution of **1** and were characterized by cyclic voltammetry (Fig. 1). Flavin and its analogs undergo two reversible one-electron reduction steps with overlapping potentials in aqueous buffer solutions (Scheme 2).[9] The surface coverage was calculated to be *ca.* 5×10^{-10} mol cm^{-2} by integrating the area under the oxidation or reduction peak. This surface coverage coincides with a densely packed film of the flavin based on modeling the molecular area expected for a flavin unit. The pH-dependent formal potential (E^0) of the bound isoalloxazine moiety was determined from the mean value of the cathodic and anodic peak potentials. Upon changing from acidic to basic conditions, the full-widths at half maxima of the peaks increase. This change may be indicative of structural heterogeneity or differences in the rates of electron transfer for the two electron transfer steps (Fig. 1).

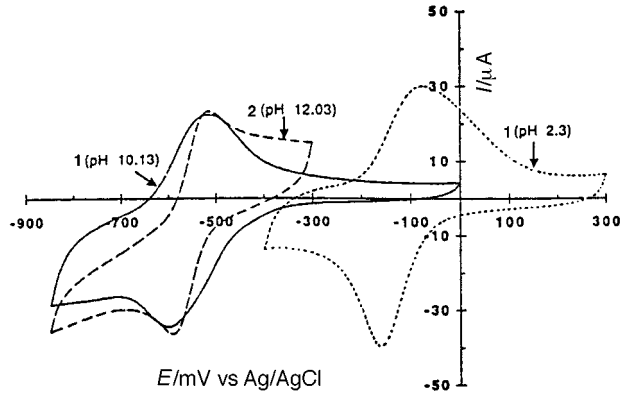
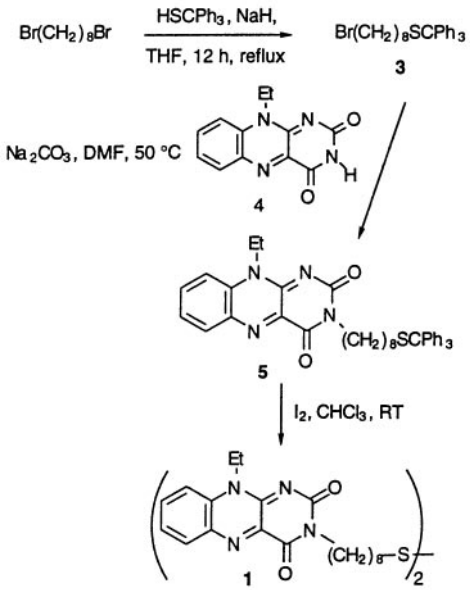
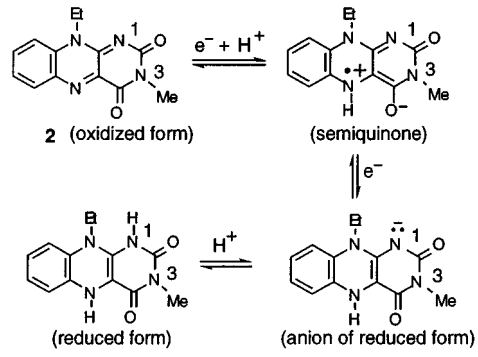


Fig. 1 Cyclic voltammogram (steady state cycle) of (a) (·····) a SAM from **1** in an aqueous buffer solution of pH 2.3, (b) (——) a SAM from **1** in an aqueous buffer solution of pH 10.13, and (c) (- - - - -) **2** (1 mM) in an aqueous buffer solution of pH 12.03. A bare gold working electrode is used in the determination of E^0 of **2**. Scan rates are 0.1 V s^{-1}.

To examine the importance of microenvironment on the one-electron processes of Scheme 2, we compared the pH dependent redox potential of flavin analog **2** in solution (shown in Scheme 2) with that of immobilized isoalloxazine. As shown in Fig. 2, the formal potential of **2** dissolved in solution is linearly related to pH, with a slope of −0.057 V (pH unit)$^{-1}$ up to pH 6.7. After this inflection point the redox processes of **2** remain reversible (Fig. 1); however, the slope changes to −0.027 V (pH unit)$^{-1}$ at higher pH values;[10] The pH at the inflection point is the pK_a of the N^1-proton in the reduced form.[9–11] A pK_a value of 6.7 for the N^1-proton in the reduced form of **2** and the decrease in slope are consistent with those reported for mononucleotide (FMN) and flavin adenine dinucleotides (FAD).[9,12] In contrast, the slope of the E^0–pH plot for the monolayer of **1** is uniform until pH ≈ 10



Scheme 1



Scheme 2

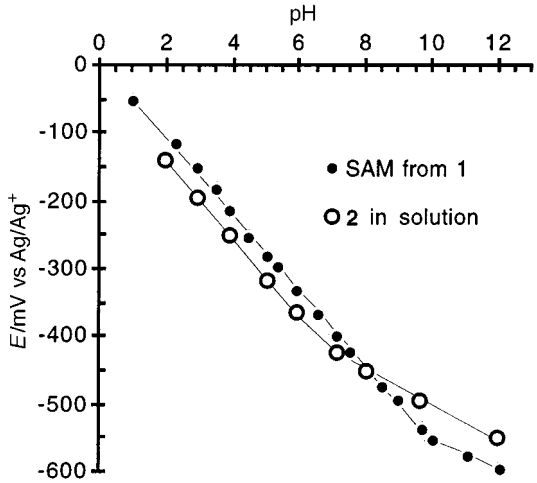
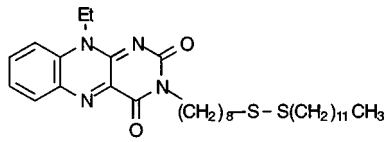


Fig. 2 The pH dependence of redox potential (E^0) of isoalloxazine of **1** in SAM compared with **2** (1 mM) dissolved in aqueous buffer solution.

(Fig. 2). The intersection point of the linear portions yields a pK_a value of 9.7. This dramatic increase in the pK_a value indicates that the microenviroment of the N^1-proton in SAM is less polar than the aqueous solution. These results are in line with the observations of Whitesides and coworkers who demonstrated by contact angle measurements increased pK_a values of acidic groups of ω-mercaptoalkanecarboxylic and phosphonic acids upon incorporation into monolayers.[13] They proposed that a low interfacial dielectric constant and/or electrostatic interactions cause the unfavorable formation of negatively charged species in a closely packed monolayer compared with that in water.[13]

We prepared mixed monolayers from unsymmetrical disulfide **6** and dodecyl disulfide or octyl disulfide thereby reducing the surface coverage of the isoalloxazine moiety to



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about 10% of that in the monolayer from **1**.[14] This reduction of charge/charge repulsion at the interface did not lower the pK_a value of the N^1-proton. In addition, the change in alkyl chain length of the mixed SAM in which N^1-proton is embedded did not influence its pK_a value. These results suggest that the low dielectric constant of the underlying alkyl chains may be the predominant factor influencing the dissociation constant.

In summary, our work demonstrates that the pH-dependent electrochemical properties of the isoalloxazine moiety can be modified *via* incorporation into SAMs, as well as the known mechanisms such as selective H-bonding and π-stacking.

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14 In addition to the decrease in peak areas, the full-widths at half maxima and the separation between the anodic and cathodic peaks (*ca.* 70 mV) in the mixed SAMs are smaller than those of the SAM from **1**.

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