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Voltammetric Simulations of Two-Electron/Two-Proton Coupled Mechanisms: An Indirect Method for Obtaining Reduction Potentials and Acid Dissociation Constants?

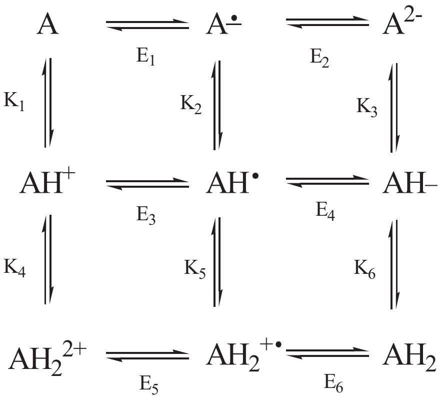
Cyclic voltammograms were simulated using DigiSim® software for reaction mechanisms involving two electron transfer steps coupled to two protons. Since reduction potentials and acid dissociation constants for this reaction mechanism cannot be measured independently, DigiSim may provide an indirect method for simultaneously determining these values. The interrelationship between reduction potentials and acid dissociation constants for this mechanism is presented.

A number of biological cofactors transfer two electrons coupled to two protons according to the following reaction:

$$A + 2H^{+} + 2e^{-} \Leftrightarrow AH_2 \qquad (1)$$

These cofactors include flavins (FAD and FMN) and quinones (coenzyme Q), and they have been electrochemically characterized extensively. The three oxidation states—oxidized (A), semiquinone ($A^{\bullet}$), and hydroquinone (A^{2-})—can also exist in three possible ionization states, for a total of nine separate species. The hypothetical mechanism for electron/proton transfer of this center is illustrated in ***F1***. Each electron transfer reaction step is characterized by a reduction potential (E), and each protonation step is characterized by an acid dissociation constant (K).

F1. Hypothetical reaction mechanism for a two-electron/two-proton coupled reaction.



For two electrons to be transferred in a single step (n = 2), the reduction potential for the first electron transfer step must be *more negative* than the reduction potential for the second electron transfer step (e.g. $E_1 < E_2$). The addition of the second electron is more thermodynamically favorable than the addition of the first electron, and thus the transfer of two electrons occurs in a single step. In cyclic voltammetry, the difference in peak potentials for the cathodic and anodic waves (ΔE_{peak}) is predicted to be 30 mV for a reversible reaction. When proton transfer steps are thermodynamically coupled to electron transfer steps, a pH-dependent reduction potential is observed. The magnitude of the pH-dependence of the reduction potential depends on the differences between the acid dissociation constants of the different oxidation states. The pH-dependent reduction potential of free flavin—$\Delta E^{\circ\prime}/\Delta pH$ = -30 mV/pH unit (1)—is characteristic of a proton-coupled n = 2 reaction mechanism.

Experimentally measuring individual reduction potentials and acid dissociation constants for each step in ***F1*** is problematic. It is not possible to establish a solution potential under standard conditions such that only two oxidation states in a single ionization state exist. Therefore, the reduction potentials in ***F1*** represent hypothetical values. Since reduction potentials cannot be measured directly, computational methods may provide an indirect method for establishing these values. DigiSim is a powerful cyclic voltammetry simulator that allows one to explore virtually any reaction mechanism and then compare the simulations with experimental data (2). This program was recently used to simulate multiple electron transfer reactions coupled to proton transfer (3). Rules were established to describe the interrelationship between all the equilibrium constants for electron and proton transfer in order to reproduce experimental results.

The rules for reproducing a pH-dependent reduction potential that appears to be coupled to two electrons (i.e. $\Delta E^{\circ\prime}/\Delta pH$ = -30 mV/pH unit) are described as follows:

1) For two electrons to be transferred in a single step, the reduction potential for the first electron transfer step must be *more negative* than the reduction potential for the second electron transfer step (e.g. $E_1 < E_2$).
2) In order to observe a pH-dependent reduction potential, the reduction potentials for different ionization states need to be different (e.g. $E_1 \neq E_3$). The difference in reduction potential must span the range of the experimentally observed pH-dependent reduction potentials.
3) In order to observe a pH-dependent reduction potential, the pK_a's for different oxidation states need to be different (e.g. $K_1 \neq K_3$). Note that if $E_1 \neq E_3$, then $K_1 \neq K_2$.
4) The pK of a reduced species needs to be greater than that of an oxidized species in order to observe $\Delta E/\Delta pH < 0$. In the case of a multi-step

mechanism (e.g. n = 2), the pK of at least one reduced species needs to be greater than that of an oxidized species to observe $\Delta E/\Delta pH < 0$.

Due to the interrelationship between the reduction potentials and acid dissociation constants for this mechanism, it follows that the acid dissociation constants also cannot be measured directly. The acid dissociation constant represents a ratio of two ionization states of a single oxidation state. However, the equilibrium constants for electron and proton transfer are such that conditions cannot be established whereby only two ionization states of a single oxidation state are in equilibrium. At any established solution potential and pH, the species at equilibrium in solution are an oxidized species and a protonated reduced species. For example, at high pH the species at equilibrium are A and AH^-, and at low pH, AH^+ and AH_2. Therefore, the acid dissociation constant also represents a hypothetical value.

Since reduction potentials and acid dissociation constants for this reaction mechanism cannot be determined directly, DigiSim was used to reproduce experimental observations for selected equilibrium constants. This cyclic voltammetry simulator may provide an indirect method for obtaining reasonable values for these important thermodynamic values. In addition, a comparison is made here between acid dissociation constants that were indirectly determined via calculations using DigiSim and values that have been previously reported based on estimations from spectroscopic data (4). A discussion is presented based on the validity of the calculated versus the measured values.

Experimental

Cyclic voltammogram simulations were carried out using DigiSim. The mechanism for the two-electron/two-proton reaction of a flavin center was simulated in multiple single steps as illustrated in Scheme 1, except the AH_2^{2+} and A^{2-} species were omitted. These two species can be included in the simulations, but in this application they were omitted. Reduction potentials and acid dissociation constants for AH_2^{2+} and A^{2-} can be chosen so they do not contribute to the overall observed current due to the other equilibrium constants, reduction potentials and acid dissociation constants for the other species used in the simulations. A buffer component was also incorporated in the reaction mechanism to maintain a constant pH. The buffer was given a pK_a value for the simulated pH, and the buffer concentration for the acid and conjugate base were set at a thousand times larger than the concentration of the FAD center. All heterogenous rate constants for electron transfer were 10^4 cm/s, and homogenous rate constants for proton transfer were 10^{10} L/mole•s. The cyclic voltammograms were calculated for a 2 mV/s scan rate. Under the selected conditions, neither electron transfer nor proton transfer steps were rate limiting at the simulated scan rates.

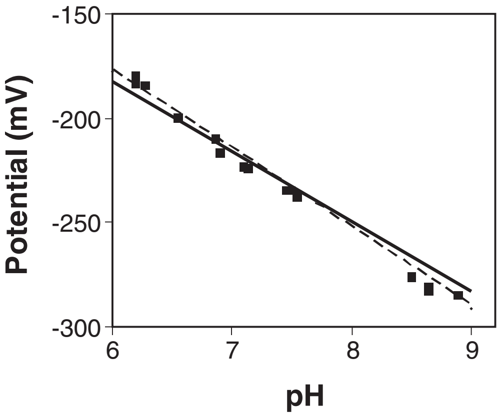
Discussion

The experimentally observed pH-dependent reduction potential of free flavin has been simulated based on a two-electron transfer process coupled to two proton transfer steps (voltammograms not shown) (3). As expected for a two-electron/two-proton coupled reaction mechanism, the pH-dependence of the reduction potential was approximately -30 mV per pH unit (see dashed line in ***F2***), and the peak separation (ΔE_{peak}) was approximately 30 mV (see dashed line in ***F3***). The acid dissociation constants and reduction potentials of individual steps for the mechanism illustrated in ***F1*** that were used in the calculations are listed in ***T1*** and ***T2***, respectively.

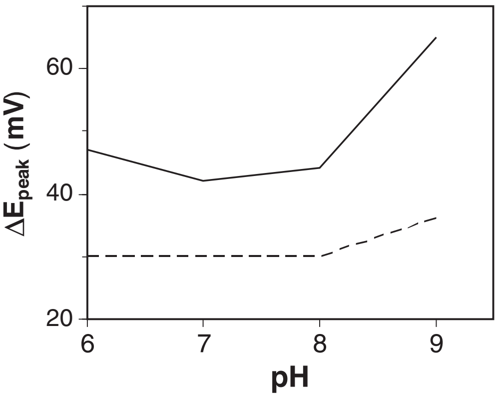
As previously noted (3), an accurate reproduction of the experimental observations for FAD require acid dissociation constants values such that $pK_2 > pK_6 > pK_1 > pK_5$. Other values for the redox potentials and acid dissociation constants can be used to reproduce the pH-dependent reduction potential of free FAD. Selecting larger potential differences between E_1 and E_4 requires larger differences between acid dissociation constants. For example, a 200 millivolt difference in reduction potential between two ionization states requires a $\Delta pK_a > 3$ and all four pK_a's to differ by at least six.

To illustrate the importance of the interrelationship between acid

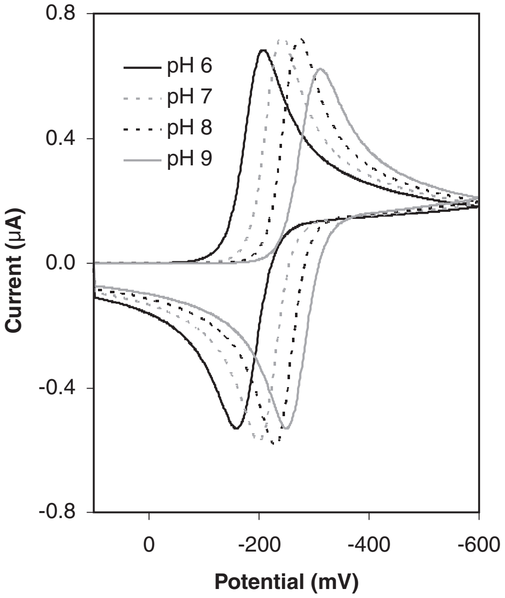
F2. Reduction potential versus pH using both experimentally estimated (solid line [4]) and calculated (dashed line [3]) values for the acid dissociation constants. Symbols represent experimental values for free FAD extracted from (1).



F3. Peak separation versus pH using both experimentally estimated (solid line [4]) and calculated (dashed line [3]) values for the acid dissociation constants.



F4. Simulated cyclic voltammograms as a function of pH for an n = 2 redox process coupled to two protons using experimentally estimated values for the acid dissociation constants for FAD (4).



dissociation constants, simulations of cyclic voltammograms were carried out using estimated acid dissociation constants of free FAD that have been reported in the literature (4). In this previous study, the acid dissociation constants do not follow the relationship described above (i.e. $pK_2 > pK_6 > pK_1 > pK_5$). Rather, the acid dissociation constants for the monoprotic semiquinone was estimated to be larger than that of oxidized FAD ($pK_5 > pK_1$) (***T1***). The simulated voltammograms based on these values as listed in ***T1*** are shown in ***F4***.

The pH-dependent reduction potential of FAD was reproduced (see solid line in ***F2***) using the previously reported acid dissociation constants as estimated by spectroscopic methods. However, the pH-dependent reduction potential of FAD was reproduced more accurately (see dashed line in ***F2***) using previously reported acid dissociation constants as determined by DigiSim. Moreover, a large peak separation (ΔE_{peak} > 30 mV) was observed for simulated voltammograms using acid dissociation constants estimated from spectroscopic data (solid line in ***F3***). The peak separation illustrated by the dashed line in ***F3*** (ΔE_{peak} = 30 mV) was determined from simulations using the acid dissociation constants that were previously reported using DigiSim (3), and this peak separation is characteristic of an n = 2 reaction mechanism.

Summary

The pH-dependent reduction potential of flavin was simulated using DigiSim based on the electron transfer mechanism illustrated in ***F1***. The values for reduction potentials and acid dissociation constants used in the simulations were based on those previously reported (3,4). Since the experimental data is best reproduced using equilibrium constants for electron and proton transfer obtained from simulations using DigiSim, this methodology may provide an indirect method for obtaining reasonable values for reduction potentials and acid dissociation constants of proton-coupled n = 2 reaction mechanisms.

References

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T1. Acid dissociation constants for FAD.

Method	pK_1	pK_2	pK_5	pK_6
Indirect calculation via DigiSim (3)	7.4	8.6	6.7	7.8
Spectroscopic estimation (4)	0	8	2	6

T2. Reduction potentials for FAD (mV).

Method	E_1	E_3	E_4	E_6
Indirect calculation via DigiSim (3)	-620	-550	80	150
Calculated via DigiSim (using pK_a's from (4))	-316	157	-186	280