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# Imidazole facilitates electron transfer from organic reductants

Brian H. Kipp[1], Chadi Faraj, Guoliang Li, David Njus*

*Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA*

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**Abstract**

In cyclic voltammetry studies at pH 8, imidazole facilitates oxidation of organic compounds that normally lose hydrogen atoms. High concentrations of imidazole shift the oxidizing wave of ascorbic acid, 2,3-dimethoxy-5-methyl-1,4-hydroquinone, and the vitamin E analogue Trolox toward lower potentials. By contrast, imidazole has no effect on the cyclic voltammogram of methyl viologen, which undergoes electron rather than hydrogen-atom transfer. The effect of imidazole is observed at pH 8.0 but only to a lesser extent at pH 5.5 indicating that imidazole must be unprotonated to facilitate oxidation. Digital simulation shows that these results are consistent with a mechanism in which imidazole acts as a proton acceptor permitting concerted proton/electron transfer by the organic reductant.


*Keywords:* Cyclic voltammetry; Ascorbic acid; Hydroquinone; Imidazole

## 1. Introduction

The role of metal ions in catalyzing the oxidation of organic compounds has received considerable attention, but the possibility that other organic compounds may also catalyze these reactions has been less widely considered. In biological systems, where metal ions are almost always sequestered, the role of organic groups in adventitious catalysis of pathological redox reactions may be especially significant.

In studies of cytochrome $b_{561}$, we [1] found evidence that a histidine residue facilitates oxidation of ascorbic acid. The imidazole side chain of the histidine residue appears to act as a proton acceptor, permitting ascorbic acid to lose a hydrogen atom by transferring an electron to the cytochrome's heme and a proton to the imidazole. It is, therefore, of interest to see whether imidazole might promote oxidation of ascorbic acid non-enzymatically. Moreover, because reduced organic compounds typically lose hydrogen atoms upon oxidation, it is of interest to see whether imidazole might also facilitate oxidation of other such compounds. We have explored the effect of imidazole on the redox properties of ascorbic acid, quinone, and the vitamin E analogue Trolox. We report here on the electrochemical behavior of these compounds as analyzed by cyclic voltammetry.

## 2. Materials and methods

### 2.1. Cyclic voltammetry

Cyclic voltammograms were recorded on a BAS 100 W electrochemical analyzer equipped with a glassy carbon working electrode, a Ag/AgCl reference electrode and a platinum wire auxiliary electrode. Sweep rates were 0.1 V/s unless specified otherwise. The glassy carbon electrode was polished with alumina and cleaned by sonication between each recording. Prior to recording, argon was bubbled through the solution to purge dissolved $O_2$. Cyclic voltammograms were also recorded in the buffer solution alone, and this background current was subtracted from all traces shown. Potentials are expressed relative to the Ag/AgCl reference electrode.

### 2.2. Simulation

Digital simulations of cyclic voltammograms were carried out using Digisim v. 3.0 (BioAnalytical Systems). The simulations were done using an expanding space factor of 0.5 and a potential interval of 5 mV. The glassy carbon electrode was assumed to be a planar surface with an area of

* Corresponding author. Tel.: +1-313-577-2520; fax: +1-313-577-6891.
*E-mail address:* dnjus@wayne.edu (D. Njus).
[1] Present address: Biomedical/Health Sciences, Grand Valley State University, Allendale, MI 49401, USA.

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Table 1
Reactions used for digital simulation of ascorbic acid

| Reaction | Equilibrium | Rate constant | Literature |
|---|---|---|---|
| (1) $AH^{\bullet}+e-=AH-$ | 0.774 V | 1 cm/s | dependent |
| (2) $A-^{\bullet}+e-=A^{=}$ | 0.076 V | 1 cm/s | 0.076 V [12] |
| (3) $AHI^{\bullet}+e-=AHI-$ | 0.500 V | 0.3 cm/s | – |
| (4) $AH^{\bullet}H_2O+e-=AH-H_2O$ | 0.350 V | 0.03 cm/s | – |
| (5) $AH_2+H_2O=AH-+H_3O^+$ | $1.66\times10^{-6}$ | $1\times10^{5}$ $M^{-1}s^{-1}$ | pK 4.04 [6] |
| (6) $AH-+H_2O=A^{=}+H_3O^+$ | $8.3\times10^{-14}$ | 0.001 $M^{-1}s^{-1}$ | pK 11.34 [6] |
| (7) $AH^{\bullet}+H_2O=A-^{\bullet}+H_3O^+$ | 0.0512 | $1\times10^{9}$ $M^{-1}s^{-1}$ | pK – 0.45 [13] |
| (8) $AH^{\bullet}H_2O=A-^{\bullet}+H_3O^+$ | $3.54\times10^{-6}$ M | $1\times10^{6}$ $s^{-1}$ | dependent |
| (9) $AHI-=AH-+I$ | 0.3 M | 5 $s^{-1}$ | – |
| (10) $AHI^{\bullet}=A-^{\bullet}+IH^+$ | 253 M | $1\times10^{8}$ $s^{-1}$ | dependent |
| (11) $AH-H_2O=AH-+H_2O$ | 1000 M | $1\times10^{11}$ $s^{-1}$ | – |
| (12) $A-^{\bullet}+A-^{\bullet}=2A-^{\bullet}$ | 780 $M^{-1}$ | $7.8\times10^{7}$ $M^{-1}s^{-1}$ | $1\times10^{3}$ $M^{-1}$ [2] |
| (13) $2A-^{\bullet}+H_3O^+=AH-+AH_2O$ | $3.6\times10^{14}$ | $3.8\times10^{9}$ $M^{-1}s^{-1}$ | $1.5\times10^{13}$ [2] |
| (14) $2A-^{\bullet}+H_2O=A^{=}+AH_2O$ | 29.88 | 0.5 $M^{-1}s^{-1}$ | dependent |
| (15) $BH^+ + H_2O = B + H_3O^+$ | $1.82\times10^{-10}$ | 10 $M^{-1}s^{-1}$ | pK 8.0 |
| (16) $IH^+ + H_2O = I + H_3O^+$ | $1.44\times10^{-9}$ | 100 $M^{-1}s^{-1}$ | pK 7.1 |

B and I denote buffer and imidazole. Reactions (12)–(14) describe disproportionation of the semidehydroascorbate radical anion; the mechanism and rate constants are from Bielski et al. [2]. Observed values for equilibrium constants are listed in the "Literature" column. Equilibrium constants that are defined when values for other reactions are chosen are identified as "dependent." Reduction potentials in reactions (1)–(4) are relative to the standard hydrogen electrode. A value of 0.5 was used for $\alpha$ in all electron transfer reactions except reaction 4 in which $\alpha = 0.65$.

0.07 $cm^2$. Reactions and parameters used are listed in Tables 1 and 2. All simulations included a buffer with a $pK_a$ equal to the desired pH and a concentration of 0.1 M. In order to model the role of $H_2O$ as a proton acceptor, dissociation constants were divided by 55 M to explicitly include $H_2O$, and $H^+$ was assumed to be entirely hydrated ($H_3O^+$). For comparison with observed cyclic voltammograms (Fig. 8), reduction potentials were reduced by 0.197 V to express them relative to the Ag/AgCl electrode.

### 2.3. Materials

(*R*)-(+)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich. Methyl viologen (1,1′-dimethyl-4,4′-bipyridinium dichloride), 2,3-dimethoxy-5-methyl-1,4-benzoquinone (coenzyme $Q_0$) and imidazole were obtained from Sigma. Ascorbic acid was from Fisher.

## 3. Results

The redox properties of a compound are readily characterized by cyclic voltammetry. For ascorbic acid, the positive potential sweep produces a single wave of oxidizing current (Fig. 1). Because the oxidized product, semidehydroascorbate, disproportionates quickly [2] and essentially irreversibly, the reverse sweep does not elicit a corresponding reducing current.

Cyclic voltammetry was repeated in the presence of imidazole to assess its effect on ascorbate oxidation. Because the interaction between ascorbate and free imidazole is expected to be weak, high concentrations of imidazole were used to maximize the concentration of any ascorbate/imidazole complex. In the presence of 50–200 mM imidazole, the oxidizing current of ascorbic acid shifts progressively toward lower potentials, indicating that ascorbate is oxidized more readily in the presence of imidazole (Fig. 1). A similar shift is observed using histidine in place of imidazole (data not shown). Cyclic voltammograms of the medium lacking ascorbate showed no electrochemical activity whether imidazole was present or not. Also, including ethylenediamine tetraacetic acid (EDTA) in the medium had

Table 2
Reactions used for digital simulation of 2,3-dimethoxy-5-methyl-1,4-benzoquinone

| Reaction | Equilibrium | Rate | Literature |
|---|---|---|---|
| (1) $Q+e-=Q-^{\bullet}$ | – 0.035 V | 10 cm/s | – 0.112 V [14] |
| (2) $Q-^{\bullet}+e-=Q^{=}$ | – 0.172 V | 10 cm/s | – 0.172 V [14] |
| (3) $QH^{\bullet}+e-=QH-$ | + 0.185 V | 10 cm/s | + 0.185 V [14] |
| (4) $QH_2I^{+\bullet}+e-=QH_2I$ | + 0.255 V | 10 cm/s | – |
| (5) $QH^{\bullet}H_3O^+e-=QH_2H_2O$ | + 0.330 V | 1 cm/s | – |
| (6) $QH_2+H_2O=QH-+H_3O^+$ | $2.29\times10^{-12}$ | 0.05 $M^{-1}$ $s^{-1}$ | pK 9.9 [15] |
| (7) $QH-+H_2O=Q^{=}+H_3O^+$ | $2.29\times10^{-14}$ | 0.001 $M^{-1}$ $s^{-1}$ | pK 11.9 [15] |
| (8) $QH^{\bullet}+H_2O=Q-^{\bullet}+H_3O^+$ | $2.47\times10^{-8}$ | $1\times10^{4}$ $M^{-1}$ $s^{-1}$ | pK 5.9 [16] |
| (9) $QH_2I=QH_2+I$ | 0.15 M | 5 $s^{-1}$ | – |
| (10) $QH_2I^{+\bullet}=QH^{\bullet}+IH^+$ | 0.00364 M | $1\times10^{7}$ $s^{-1}$ | dependent |
| (11) $QH_2H_2O=QH_2+H_2O$ | 2000 M | $1\times10^{4}$ $s^{-1}$ | – |
| (12) $QH^{\bullet}H_3O^+=QH^{\bullet}+H_3O^+$ | $1.29\times10^{-6}$ M | $1\times10^{8}$ $s^{-1}$ | dependent |
| (13) $QH^{\bullet}+QH^{\bullet}=QH_2+Q$ | $5.64\times10^{7}$ | $8\times10^{7}$ | dependent |
| (14) $BH^+ + H_2O = B + H_3O^+$ | $1.82\times10^{-10}$ | 10 $M^{-1}s^{-1}$ | |
| (15) $IH^+ + H_2O = I + H_3O^+$ | $1.44\times10^{-9}$ | 100 $M^{-1}s^{-1}$ | pK 7.1 |

B and I denote buffer and imidazole. Reaction (13) describes disproportionation of the semiquinone. The rate constant is from Yamazaki and Ohnishi [17]. Observed values for equilibrium constants are listed in the "Literature" column. Equilibrium constants that are defined when values for other reactions are chosen are identified as "dependent." Reduction potentials in reactions (1)–(5) are relative to the standard hydrogen electrode. A value of 0.5 was used for $\alpha$ in all electron transfer reactions except reaction (4) in which $\alpha = 0.65$.

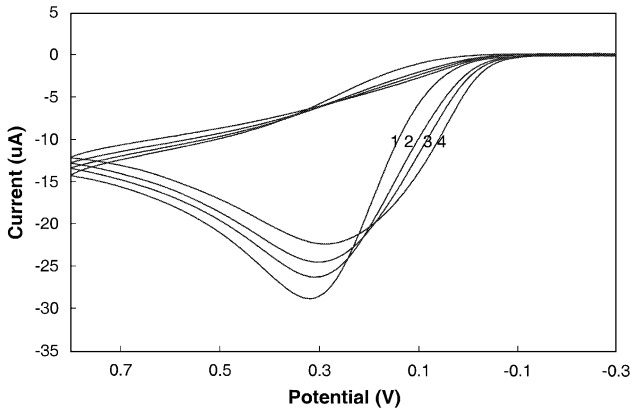


Fig. 1. Cyclic voltammetry of ascorbate. Cyclic voltammograms of ascorbic acid (1 mM) were recorded in 0.1 M KCl, 0.1 M $K_xH_yPO_4$, pH 8.0 in the presence of (1) 0, (2) 50 mM, (3) 100 mM or (4) 200 mM imidazole. At least five separate recordings were averaged to obtain each trace shown.

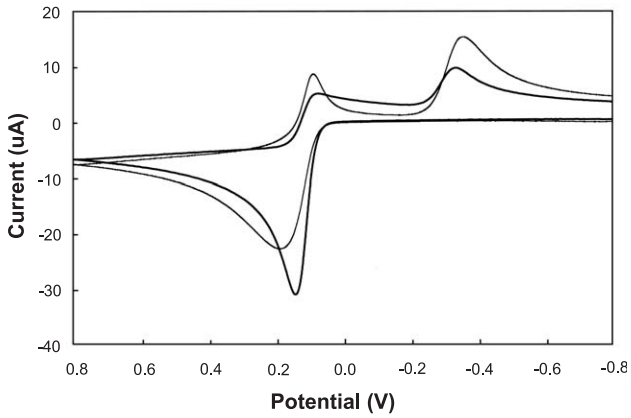


Fig. 3. Cyclic voltammetry of Trolox. Cyclic voltammograms of Trolox (1 mM) were recorded in 0.1 M KCl, 0.1 M $K_xH_yPO_4$, pH 8.0 in the presence of 0 (thin line) or 200 mM imidazole (heavy line). At least five separate recordings were averaged to obtain each trace shown.

no effect excluding the possibility that the shift in the oxidizing wave is caused by trace amounts of metal ions or imidazole-metal complexes.

2,3-Dimethoxy-5-methyl-1,4-benzoquinone differs from ascorbic acid in that it exhibits a more typical reversible oxidation/reduction cycle. Cyclic voltammetry elicits a reducing current on the negative sweep and an oxidizing current on the positive sweep (Fig. 2). As with ascorbate, however, the oxidizing peak shifts toward lower potentials in the presence of 50–200 mM imidazole. The reducing peak also shifts in the presence of imidazole, although the observed shifts are much smaller. This indicates that imidazole facilitates reduction as well as oxidation.

The water-soluble vitamin E analogue Trolox also exhibits a well defined peak on oxidation, but the radical can be further oxidized to a number of species [3,4]. Consequently, the reducing sweep exhibits a more complex pattern (Fig. 3). Significantly, however, addition of imidazole shifts the oxidizing current to lower potentials, as is the case with ascorbate and the quinone.

Imidazole does not shift the cyclic voltammograms of all compounds, however. In particular, the cyclic voltammogram of methyl viologen is not affected by 200 mM imidazole (Fig. 4). Methyl viologen differs from the other compounds in that its oxidation/reduction cycle involves only electron transfer. It is not a hydrogen-atom carrier (Fig. 5). At sufficiently high pH, even ascorbate and hydroquinone should deprotonate and act as electron donors. Consistent with this, the oxidizing wave of the quinone is shifted toward lower potentials at pH 9.0. At this pH, the monoanion fraction ($pK_a = 9.85$) is apparently large enough for oxidation of the monoanion to the semiquinone to be the dominant oxidation pathway. At this pH, imidazole causes no further shift in the potential of the oxidation wave as

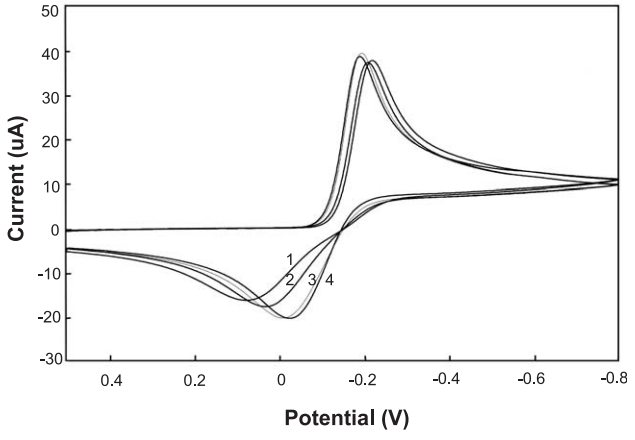


Fig. 2. Cyclic voltammetry of 2,3-dimethoxy-5-methyl-1,4-benzoquinone. Cyclic voltammograms of the quinone (1 mM) were recorded in 0.1 M KCl, 0.1 M $K_xH_yPO_4$, pH 8.0 in the presence of (1) 0, (2) 50 mM, (3) 100 mM, or (4) 200 mM imidazole. At least five separate recordings were averaged to obtain each trace shown.

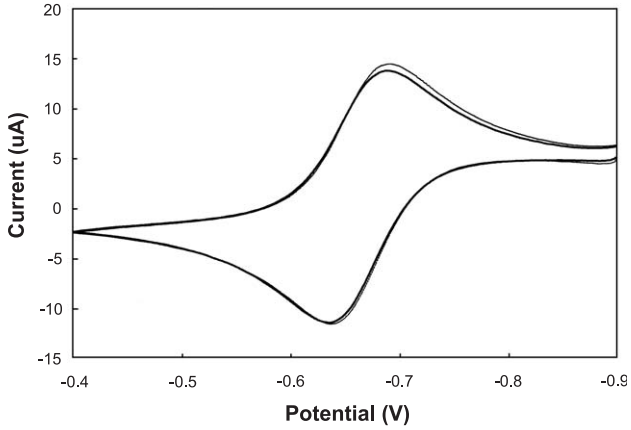


Fig. 4. Cyclic voltammetry of methyl viologen. Cyclic voltammograms of methyl viologen (1 mM) were recorded in 0.1 M KCl, 0.1 M $K_xH_yPO_4$, pH 8.0 in the presence of 0 (thin line) or 200 mM imidazole (heavy line). At least five separate recordings were averaged to obtain each trace shown.

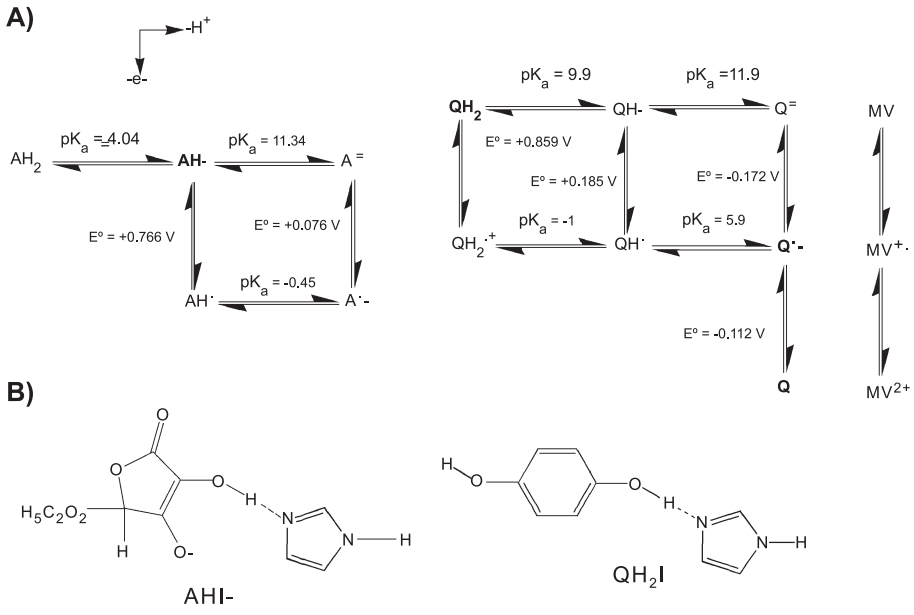


Fig. 5. (A) Electron transfer and protonation reactions of ascorbate, quinone, and methyl viologen. Bold-face type indicates the predominant species at pH 8. Values for reduction potentials and $pK_a$s are from sources cited in Tables 1 and 2. (B) Hypothesized redox complexes formed by hydrogen bonding of imidazole to ascorbate or hydroquinone.

would be expected if the quinone is acting as an electron donor.

To quantitate the shift induced by imidazole, we have used the potential at half-maximum current as an index. This value can be determined more precisely than the potential at maximum current. The shift in the potential increases with imidazole concentration up to the limit of imidazole solubility (Fig. 6). The data can be fit to the following equation to determine the imidazole concentration required to cause 50% of the maximum shift ($EC_{50}$):

$$E(I) - E(0) = \Delta E_{max}[I]/([I] + EC_{50}) \quad (1)$$

$E(I)$ and $E(0)$ are the half-height potentials observed in the presence and absence of imidazole, respectively. $\Delta E_{max}$ is the maximum potential shift (i.e., the shift that would be observed at infinite [$I$]). Data as shown in Fig. 6 were fit to Eq. (1) by a least-squares method to determine best-fit values for $EC_{50}$ and $\Delta E_{max}$. For quinone/imidazole, $EC_{50}$ was about 0.1 M for both the oxidation and reduction peaks. For ascorbate/imidazole, $EC_{50}$ was about 0.15 M. These values were obtained by analyzing several experiments of the type shown in Fig. 6. Results were similar whether the potential was measured as half-height potential, peak potential, or as $E_{1/2}$ from a semi-integral plot [5]. $EC_{50}$ is a measure of the effective imidazole concentration, but care should be exercised in equating it with $K_D$, the dissociation constant for the hypothesized imidazole/reductant complex. Cyclic voltammetry is a kinetic experiment and may not accurately reflect equilibrium properties such as $K_D$. Digital simulation suggests, however, that $K_D$ values are close to the $EC_{50}$ values measured here.

If imidazole facilitates oxidation of H-atom carriers by acting as a proton acceptor, then it should be less effective below its $pK_a$ of 7.1. To test this, the pH dependence of the imidazole effect was examined. At pH 5.5, 200 mM imidazole has very little effect on the cyclic voltammogram

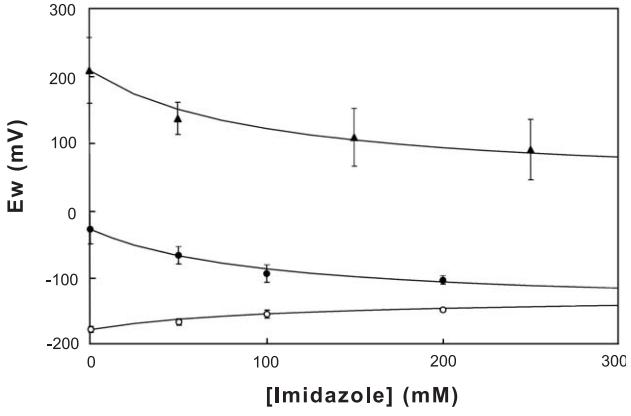


Fig. 6. Dependence of ascorbic acid and benzoquinone potential shifts on imidazole concentration. Half-height potentials from individual traces as shown in Figs. 1 and 2 were measured and averaged for each imidazole concentration. (▲) Ascorbate; (●) quinone oxidizing sweep; (○) quinone reducing sweep. Each point is the average (± SD) of six or more separate measurements. Lines were calculated from Eq. (1) using a value of 0.1 M for $EC_{50}$ and $\Delta E_{max}$ values of 175 mV (ascorbate), 120 mV (quinone oxidizing sweep), and 45 mV (quinone reducing sweep).

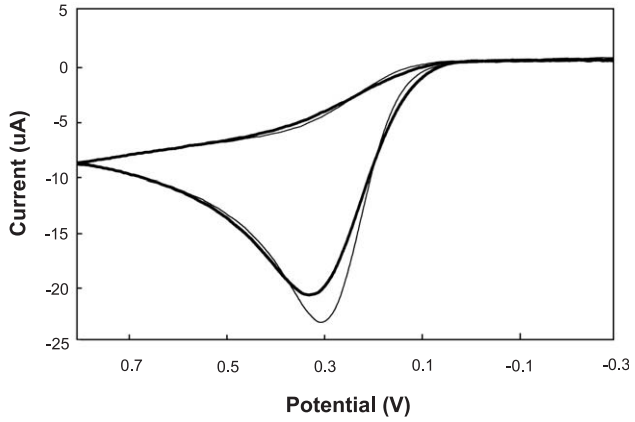


Fig. 7. Cyclic voltammetry of ascorbic acid at pH 5.5 in the presence and absence of imidazole. Cyclic voltammograms of ascorbic acid (1 mM) were recorded in 0.1 M KCl, 0.1 M $K_xH_yPO_4$, pH 5.5 in the absence of imidazole (thin line) or in the presence of 200 mM imidazole (heavy line). Duplicate recordings were averaged to obtain the traces shown.

of ascorbic acid (Fig. 7). A similar lack of effect is observed for the quinone at pH 5.5 (data not shown).

The results, therefore, are consistent with a mechanism in which unprotonated imidazole accepts a proton from the reductant, facilitating electron transfer to the electrode. Digital simulation provides an important validation of the mechanistic basis for these results. Before addressing the effect of imidazole, however, it is necessary to account for the cyclic voltammograms of the organic reductants in water. Consider the case of ascorbic acid first. Because ascorbate has $pK_a$s of 4.04 and 11.34 [6], the monoanion is the predominant species in the pH range examined here. Deakin et al. [7] showed that the potential of the oxidation peak of ascorbic acid does not depend on pH over the range 4.75–7.75, indicating that the ascorbate monoanion is the species oxidized. Although the ascorbate dianion oxidizes readily, it does not make an appreciable contribution to the oxidation current at the sweep rate used here, because the monoanion does not deprotonate quickly enough.

Oxidation of the ascorbate monoanion to the semidehydroascorbate free radical occurs at a relatively high potential ($E^\circ = +0.766$ V [8,9]), however, and this is not consistent with the potential of the observed peak. The low apparent reduction potential of the ascorbate monoanion appears to be attributable to water, because replacing water with methanol shifts the oxidizing peak to higher potentials (data not shown). A reasonable explanation is that $H_2O$ can serve as a proton acceptor like imidazole, facilitating the oxidation of the ascorbate monoanion directly to the semidehydroascorbate radical anion (Fig. 5). In fact, even a very weak association ($K_D = 10^3$ M) provides a path for ascorbate oxidation that is sufficiently rapid to account for the observed cyclic voltammogram (Fig. 8A). The $H_2O$/ascorbate association must be weak, so the concentration of the complex will be negligible and will not affect measured equilibrium constants such as standard reduction potentials or $pK_a$ values. This also means that $H_2O$ will not compete effectively with imidazole or other compounds binding to ascorbate.

Having modeled the cyclic voltammogram of ascorbic acid in water, the imidazole effect can be added by introducing a complex formed between the ascorbate monoanion and the unprotonated imidazole (Fig. 8B). In this model,

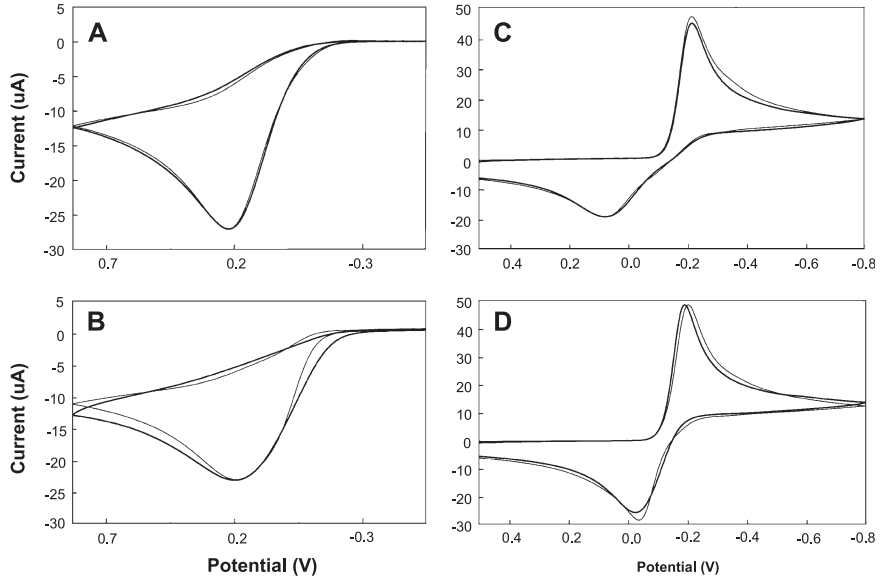


Fig. 8. Observed and simulated cyclic voltammograms of ascorbic acid and quinone. Panels A and B show 1 mM ascorbic acid at pH 8.0. Panels C and D show 1 mM quinone at pH 8.0. Panels B and D show results including 0.2 M imidazole. Digital simulations were performed using reactions and parameters as listed in Tables 1 and 2. Observed traces are indicated by heavy lines and simulated traces by thin lines.

imidazole, like $H_2O$, acts as a proton acceptor, facilitating oxidation of the ascorbate monoanion directly to the radical anion. Because this mechanism requires imidazole to function as a proton acceptor, the effect is greatly diminished when the pH is below the $pK_a$ of imidazole.

A similar analysis permits digital simulation of the electrochemical behavior of 2,3-dimethoxy-5-methyl-1,4-benzoquinone. As with ascorbic acid, the oxidation peak of the quinone occurs at too low a potential to be compatible with oxidation of the hydroquinone to the semiquinone cation. Again, the oxidizing current can be rationalized by formation of a weak complex with $H_2O$ permitting oxidation of the hydroquinone diagonally to the semiquinone (Fig. 8C). The water/hydroquinone complex is sufficiently weak that it does not affect measured reduction potentials or $pK_a$ values, nor does it interfere with binding to imidazole. Complexing with the unprotonated imidazole then accounts for the shift caused by imidazole (Fig. 8D).

In these simulations, the observed data could be fit even more precisely by adding other interactions, such as binding of $H_2O$ and imidazole to other OH groups on ascorbate and hydroquinone. The objective here is to test the redox complex mechanism, however, and this test is more robust if we minimize the number of parameters. For that reason, consideration was limited to those interactions necessary for the redox complex mechanism.

## 4. Discussion

Organic compounds, such as quinone and ascorbate, typically oxidize by losing H atoms rather than electrons. We have proposed here that imidazole facilitates electron transfer from these compounds by acting as a proton acceptor. Formation of a complex between the organic reductant and imidazole allows the hydrogen-atom donor to transfer electrons to the electrode while the residual proton is transferred within the complex. This hypothesis is supported by the observation that imidazole shifts the oxidation current of hydrogen atom donors (ascorbate, quinone and Trolox) to lower potentials. By contrast, oxidation of the electron donor methyl viologen is not affected. In addition, the pH dependence of the effect indicates that imidazole must be in the unprotonated state. Finally, digital simulation confirms that the hypothesized mechanism can account for the observed cyclic voltammograms.

Other possibilities for the effect of imidazole need to be excluded. An affect of adsorbed imidazole on surface potential of the electrode is unlikely, because it is the neutral unprotonated form of imidazole that is effective. Moreover, the high ionic strength of the medium should minimize any effect of surface charge. The mechanism by which organic compounds react with glassy carbon electrodes is presently obscure and may involve adsorption of the compound to the electrode [10,11]. If imidazole adsorbs to the glassy carbon surface, the adsorption would have to be weak. This is indicated by the high imidazole concentration required and by the fact that the imidazole effect is readily reversible. Following an experiment in which the imidazole effect is observed, the imidazole-free cyclic voltammogram can be recovered simply by rinsing the electrode with distilled water and placing it into an imidazole-free solution.

Finally, we have observed that imidazole also catalyzes the oxidation of ascorbate, the hydroquinone and Trolox in solution with $O_2$ as the electron acceptor (Kipp et al., unpublished results). This process can be monitored by following $O_2$ reduction using an oxygen electrode. Given the rate of the reactions and the solubility of the reactants, it is most likely that the reactants are free in solution. In any case, this $O_2$ reduction occurs in the absence of surface effects of the glassy carbon electrode.

These results support the contention that imidazole facilitates the transfer of electrons to and from organic redox compounds, consistent with the mechanism we proposed for cytochrome $b_{561}$. The crux of the mechanism is that imidazole acts as a repository for the proton that must be transferred to or from the substrate in conjunction with the electron. The models do not specify a mechanism for imidazole-mediated proton transfer, but we hypothesize that imidazole associates with the reduced compound by hydrogen bonding through the hydrogen atom that is lost in the electron transfer reaction (Fig. 5). This is a natural mechanism for transferring the residual proton to the imidazole, and, in the case of ascorbic acid, it is supported by molecular modeling studies [1].

The fact that imidazole catalyzes electron transfer from ascorbic acid, the quinone, and Trolox suggests that the mechanism may be more generally applicable. For biological systems, this implies that histidine and other proton-transferring groups may catalyze a slow rate of spontaneous oxidations leading to the continuous formation of low levels of free radicals. Although the effects reported here require imidazole concentrations far higher than likely to exist in vivo, physiological relevance is likely in two significant cases. First, imidazole-containing sites that bind organic reductants with greater affinity should catalyze reactivity at correspondingly lower concentrations. This will be the case for enzymes that bind redox substrates with high affinity, a condition already analyzed for cytochrome $b_{561}$ [1]. Second, the effects observed here are recorded on a time scale of seconds. Imidazole catalyzes $O_2$ reduction by these same compounds at the same concentration on a time scale of minutes. At lower concentrations, free radical generation will be slower, but pathological effects caused by damaging effects of free radicals are likely to be cumulative, so slow catalysis over a period of years or even decades may be significant.

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## References


[1] D. Njus, M. Wigle, P.M. Kelley, B.H. Kipp, H.B. Schlegel, Mechanism of ascorbic acid oxidation by cytochrome $b_{561}$, Biochemistry 40 (2001) 11905–11911.

[2] B.H.J. Bielski, A.O. Allen, H.A. Schwarz, Mechanism of the disproportionation of ascorbate radicals, J. Am. Chem. Soc. 103 (1981) 3516–3518.

[3] M.J. Thomas, B.H.J. Bielski, Oxidation and reaction of trolox c, a tocopherol analog, in aqueous solution. A pulse-radiolysis study, J. Am. Chem. Soc. 111 (1989) 3315–3319.

[4] V.W. Bowry, K.U. Ingold, Extraordinary kinetic behavior of the α-tocopheroxyl (vitamin E) radical, J. Org. Chem. 60 (1995) 5456–5467.

[5] A.J. Bard, L.R. Faulkner, Electrochemical Methods, 2nd ed., Wiley, New York, 2001.

[6] M.M. Taqui Khan, A.E. Martell, Kinetics of metal ion and metal chelate catalyzed oxidation of ascorbic acid. IV. Uranyl ion catalyzed oxidation, J. Am. Chem. Soc. 91 (1969) 4668–4672.

[7] M.R. Deakin, P.M. Kovach, K.J Stutts, R.M. Wightman, Heterogeneous mechanisms of the oxidation of catechols and ascorbic acid at carbon electrodes, Anal. Chem. 58 (1986) 1474–1480.

[8] N.H. Williams, J.K. Yandell, Aust. J. Chem. 35 (1982) 1133–1144.

[9] C. Creutz, The complexities of ascorbate as a reducing agent, Inorg. Chem. 20 (1981) 4449–4452.

[10] P. Chen, R.L. McCreery, Control of electron transfer kinetics at glassy carbon electrodes by specific surface modification, Anal. Chem. 68 (1996) 3958–3965.

[11] S.H. DuVall, R.L. McCreery, Control of catechol and hydroquinone electron-transfer kinetics on native and modified glassy carbon electrodes, Anal. Chem. 71 (1999) 4594–4602.

[12] T. Iyanagi, I. Yamazaki, K.F. Anan, One-electron oxidation-reduction properties of ascorbic acid, Biochim. Biophys. Acta 806 (1985) 255–261.

[13] G.P. Laroff, R.W. Fessenden, R.H. Schuler, Electron spin resonance spectra of radical intermediates in the oxidation of ascorbic acid and related substances, J. Am. Chem. Soc. 94 (1972) 9062–9073.

[14] V.A. Roginsky, C. Michel, W. Bors, Reactivity of semiquinones with ascorbate and the ascorbate radical as studied by pulse radiolysis, Arch. Biochem. Biophys. 384 (2000) 74–80.

[15] C.A. Bishop, L.K.J. Tong, Equilibria of substituted quinones at high pH, J. Am. Chem. Soc. 87 (1965) 501–505.

[16] K.B. Patel, R.L. Willson, J. Chem. Soc., Faraday Trans. I 69 (1973) 814–825.

[17] I. Yamazaki, T. Ohnishi, Biochim. Biophys. Acta 112 (1966) 469–481.