Advances in Redox Research 8 (2023) 100068

Contents lists available at ScienceDirect

Advances in Redox Research

journal homepage: www.elsevier.com/locate/arres

Full Length Article

Spectroelectrochemical testing of a proposed mechanism for a redox-based therapeutic intervention: Ascorbate treatment of severe paraquat poisoning

Zhiling Zhao[a,b,1], Eunkyoung Kim[a,b,1], William E. Bentley[a,b,c], Gregory F. Payne[a,b,*]

[a] University of Maryland, Institute for Bioscience and Biotechnology Research, College Park, MD 20742, USA
[b] Robert E. Fischell Institute for Biomedical Devices, University of Maryland, College Park, MD 20742, USA
[c] Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA

A B S T R A C T

The toxicity of paraquat is believed to involve a redox-cycling mechanism that can disrupt cellular redox homeostasis and, also, generate damaging free radicals. It has been suggested that for cases of severe paraquat poisoning the administration of ascorbate (i.e., vitamin C) can confer benefit by quenching the paraquat free radical ($PQ^{+\cdot}$). Here, we used an electrochemical approach that abstracts-away many of the (bio)chemical complexities and isolates the redox-interactions between paraquat and ascorbate. Specifically, we used a series of experiments that coupled electrochemical measurements of electron flow with optical measurements of paraquat's redox-state switching. Our results demonstrate that the reduced absorbate cannot quench the $PQ^{+\cdot}$-radical because they are both reductants. However, oxidation of ascorbate does allow $PQ^{+\cdot}$-radical scavenging. More broadly, we believe this study demonstrates the potential for developing electrochemical approaches to complement existing experimental methods in redox biology.

Introduction

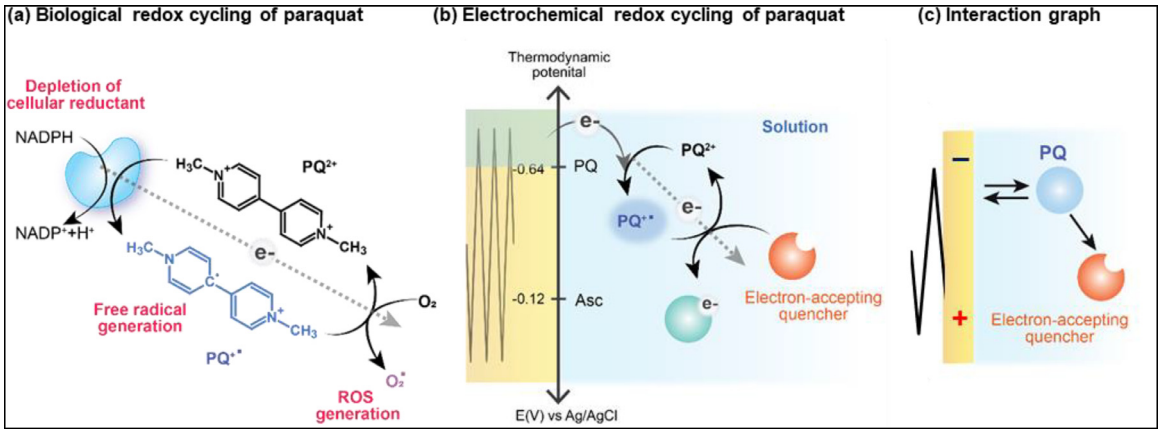
The underlying hypothesis of this work is that our understanding of redox activities in biology could be enhanced by better experimental methods. In general, redox activities involve electron transfer reactions between donors (reductants) and acceptors (oxidants), and often in biology these redox reactions are nested within larger reaction networks. In some cases, the coupling of redox activities is spatially-organized to confer a clear function (e.g., energy transduction through the respiratory electron transfer chain), but in other cases the network organization and function remain unclear (e.g., the set of antioxidants in the serum-interactome that offers protection from reactive oxygen species) [1–10]. Importantly, the "flow" of electrons through such redox networks has both molecular features (e.g., the network's nodes) and electrical features (e.g., the network's electron-transfer links). Most experimental approaches to characterize coupled redox activities employ chemical measurements (e.g., omics) that focus on the molecular features [11–17]. In contrast, we are developing electrochemical methods that focus on the electrical features of coupled redox activities [18–23].

Here, we report *in vitro* measurements of electrical activities to evaluate a proposed hypothesis for a clinical intervention for paraquat poisoning. Paraquat (PQ^{2+}) is an agricultural chemical that is believed to be toxic through the redox mechanisms illustrated in Scheme 1a. PQ^{2+} is believed to accept a single electron from biological reducing equivalents to form a reactive $PQ^{+\cdot}$ free radical that can subsequently donate this electron to O_2 to generate reactive oxygen species (ROS) [24]. Thus, PQ^{2+}'s toxicity could include both a disruption in cellular redox state as well as the generation of damaging ROS [25–28]. It has been hypothesized that in some extreme poisoning cases, the administration of ascorbate (i.e., vitamin C) could confer benefit by scavenging the $PQ^{+\cdot}$ radical, and in fact, some benefits have been reported [25,29–32]. The problem with this hypothesis is that ascorbate is reduced and should be unable to accept electrons to quench the $PQ^{+\cdot}$ radical.

Our *in vitro* approach to study the PQ^{2+}-ascorbate redox interactions is illustrated in Scheme 1b. Here, an electrode is immersed into a solution of the oxidized PQ^{2+} and an oscillating potential is imposed in the voltage range that brackets PQ^{2+}'s redox potential ($E^{\circ} = -0.64$ V vs Ag/AgCl). When the potential is cycled to reducing conditions, an electron can be transferred from the electrode to PQ^{2+} to generate a measurable reducing current while converting the colorless PQ^{2+} into the blue-colored $PQ^{+\cdot}$ radical. When the potential is reversed and cycled to oxidizing conditions, the reaction can be reversed with the generation of an oxidizing current and conversion of the colored $PQ^{+\cdot}$ radical back to the non-radical PQ^{2+}. Scheme 1b illustrates that if an electron-accepting quencher is present, it can accept an electron from $PQ^{+\cdot}$ and this interaction is expected to perturb the observable electrochemical oxidation and reduction currents. Such perturbations are illustrated in the interaction graphs in Scheme 1c. In addition to electrical measurements of activities, simultaneous optical measurements can observe the dynamic redox-state switching of $PQ^{2+}/PQ^{+\cdot}$ and can detect perturbations

* Corresponding author at: University of Maryland, Institute for Bioscience and Biotechnology Research, College Park, MD 20742, USA.
E-mail address: gpayne@umd.edu (G.F. Payne).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.arres.2023.100068
Received 11 February 2023; Accepted 30 March 2023
Available online 31 March 2023



Scheme 1. (a) Biological redox-cycling of paraquat: paraquat (PQ^{2+}) can accept electrons from biological reductants and donate electrons to O_2 to generate reactive oxygen species (ROS). (b) Electrochemical redox-cycling of paraquat: the electrode can donate electrons to reduce PQ^{2+} to the $PQ^{+\cdot}$ radical which can be re-oxidized by donating an electron to an electron-accepting quencher. (c) Interaction graph: paraquat can exchange electrons with the electrode with the direction controlled by the imposed electrode potential (i.e., voltage), and the reduced $PQ^{+\cdot}$ radical can donate an electron to a suitable quencher.

associated with a quenching chemical reaction. Using such spectroelectrochemical measurements we provide evidence that while ascorbate is unable to quench the $PQ^{+\cdot}$ radical, the oxidized dehydroascorbate can quench $PQ^{+\cdot}$.

Experimental section

Materials: Paraquat, ascorbate, and dehydroascrobate were purchased from Sigma-Aldrich. All reagents were used as received without further purification. All solutions were prepared using Millipore water (>18 MΩ). The solutions of mediators were prepared in 0.1 M phosphate buffer (pH 7.0) with air being excluded by purging N_2 before the experimental.

Instrumentation: The spectroelectrochemical cell consisted of a honeycomb gold (19 holes of 0.5 mm diameter from Pine Research) working electrode, a Ag/AgCl reference electrode and a counter electrode made of 0.3 mm platinum wire. A potentiostat (CHI 620, CH Instruments) was used for all the electrochemical measurements. UV-Vis spectrophotometer (AvaSpec-ULS2048, Pine Research) was used for the spectral measurements which were carried out simultaneously with the electrochemical measurements.

Results and discussion

Testing the hypothesis that ascorbate can quench the paraquat free radical

To experimentally test if ascorbate (Asc) can quench the $PQ^{+\cdot}$ free radical, we used a honeycomb gold electrode illustrated in Fig. 1a and performed spectroelectrochemical measurements. As illustrated, the reduced $PQ^{+\cdot}$ radical can be formed at the electrode and diffuse into the optical window where it is detected by its optical absorbance at 395 nm (or also at 600 nm). Experimentally, we prepared a buffered solution (0.1 M phosphate; pH 7.0) containing PQ^{2+} (0.2 mM) and measured cyclic voltammograms (CV) by cycling the imposed potential over a narrow range (−0.15 to −0.7 V vs Ag/AgCl; scan rate of 10 mV/s) that brackets the PQ's redox potential (E°= −0.64 V). The three-dimensional plot in Fig. 1b shows the differential absorbance spectra (referenced against the spectrum for the initial 0.2 mM PQ^{2+} solution) for two CV cycles. The cross-sectional panels shown in Fig. 1b illustrate that when the input potential is cycled to reducing values (−0.70 V) large absorbance peaks appear at 395 nm and 600 nm, while these absorbance peaks disappear when the potential is cycled to oxidizing values (−0.15 V). The two-dimensional plot in Fig. 1c summarizes this response (i.e., the appearance and disappearance of absorbance peaks) which is consistent with the electrochemical generation of $PQ^{+\cdot}$ radical and its subsequent electrochemical consumption.

To test the hypothesis that Asc can quench the $PQ^{+\cdot}$ radical, we prepared buffered solutions (0.1 M phosphate; pH 7.0) containing either PQ^{2+} (0.2 mM) or Asc (0.5 mM), or an PQ^{2+}-Asc mixture, and compared the responses from multicycle CV measurements (potential range −0.15 to −0.7 V vs Ag/AgCl; scan rate 10 mV/s). Fig. 2a shows the data from these CV experiments represented as two-dimensional time series plots of the imposed potential input and observed electrical and optical output responses. The first set of output curves in Fig. 2a shows the electrical current. One observation is that the output current response for the PQ^{2+} solution and PQ^{2+}-Asc mixture show comparatively large reducing current peaks (compared to that for the Asc solution). The second observation is that the reduction peaks are similar for the PQ^{2+} solution and the PQ^{2+}-Asc mixture and this observation is not consistent with the hypothesis that Asc can quench the $PQ^{+\cdot}$ radical. Specifically, if the $PQ^{+\cdot}$ radicals were being quenched, then paraquat would be expected to undergo redox-cycling such that the electrons transferred from the electrode to generate the reduced $PQ^{+\cdot}$ radical would be transferred to the quencher with the regenerated PQ^{2+} capable of again accepting electrons from the electrode. Previously, such redox-cycling between paraquat and a quencher has been observed to result amplifications of reducing currents [33], but such amplification are not evident for the PQ^{2+}-Asc mixture.

The second set of output curves in Fig. 2a show the optical absorbance at 395 nm which is detecting the redox-state switching of PQ between its oxidized PQ^{2+} state and its reduced $PQ^{+\cdot}$ radical state. As observed in Fig. 2a for the PQ^{2+} solution, an absorbance peak appears under reductive conditions and disappears under oxidative conditions, while no absorbance change is observed for the Asc solution. The optical absorbance responses for the PQ^{2+} solution and the PQ^{2+}-Asc mixture appear to be nearly identical which again is inconsistent with the hypothesis that Asc can quench the $PQ^{+\cdot}$ radical.

A third output response in Fig. 2a is the time-derivative of the absorbance at 395 nm (the corresponding output absorbance response at 600 nm is summarized in Fig. S1 of Supporting Information). This output response characterizes the dynamics of paraquat's redox-state switching. Interestingly, previous studies have shown that the peaks in the time derivative of the optical signal often align with the peaks in current associated with the reaction responsible for redox-state switching [34]. This time-derivative output response is nearly the same for the PQ^{2+} solution and the PQ^{2+}-Asc mixture again suggesting that Asc does not quench the $PQ^{+\cdot}$ radical.

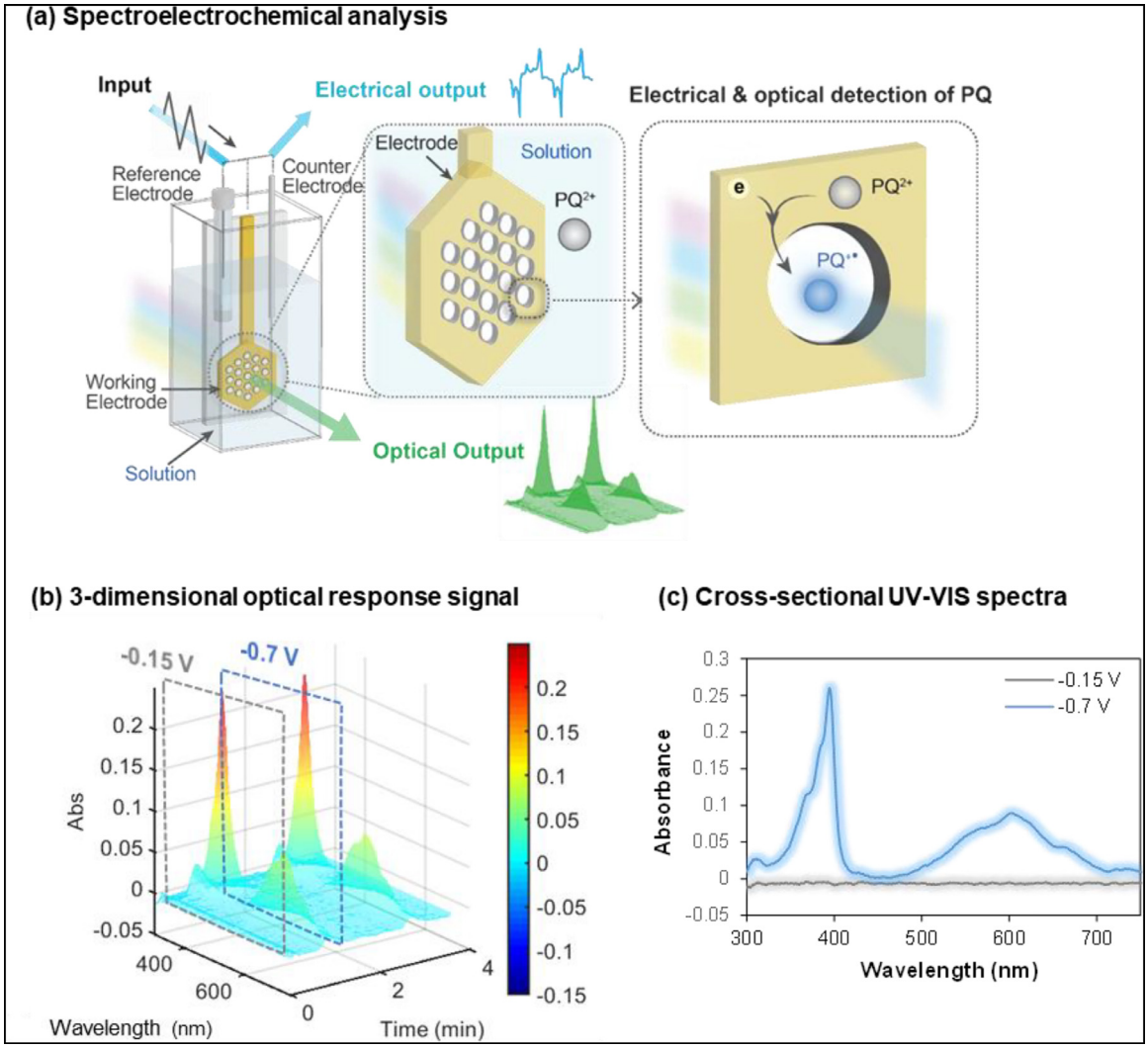


Fig. 1. *Spectroelectrochemical analysis.* (a) Spectroelectrochemistry allows the simultaneous measurement of electrical and optical signals associated with the redox-state switching of paraquat (note: the oxidized PQ^{2+} is colorless while the reduced $PQ^{+\cdot}$ radial is blue-colored). (b) Three-dimensional plot shows the optical absorbance as a function of wavelength and time while a cyclically oscillating input potential was imposed to the underlying electrode: the differential spectra show peaks at 395 nm and 600 nm due primarily to the $PQ^{+\cdot}$ radical. (c) Two-dimensional, cross-sectional plots of optical absorption spectra at two potential slices.

In addition to representing data as input-output time series curves, Fig. 2b shows the results can be represented as phase-plane plots that show the output responses as a function of the input potential. The upper plot in Fig. 2b is the standard cyclic voltammogram (CV) that shows current vs potential. In the region where the imposed potential is most reducing (−0.45 to −0.70 V), the current responses observed for the PQ^{2+} solution and PQ^{2+}-Asc mixture are similar, while the current response in this region for the Asc solution is considerably less. In the region where the imposed potential is most oxidizing (−0.35 to −0.15 V), the observed currents are small and similar for all three solutions. The two optical phase-plane plots (absorbance 395 nm and its time-derivative) show similar responses for the PQ^{2+} solution and PQ^{2+}-Asc mixture, while no optical response is observed for the Asc solution.

As noted, previous studies have shown that when an optical measurement can detect redox-state switching, then the output current responsible for this redox-state switching is correlated to the time derivative of the optical absorbance [34]. This correlation is illustrated by the bottom phase-plane plots in Fig. 2c. Specifically, in the potential region near PQ^{2+}'s redox potential (E° −0.64 V) where PQ^{2+}/ $PQ^{+\cdot}$ redox-state switching is expected, this phase-plane plot shows the current and first-derivative of absorbance at 395 nm are nearly superimposable for the PQ^{2+} solution and PQ^{2+}-Asc mixture (Fig. S1 of the Supporting Information shows the analogous absorbance response at 600 nm).

In summary, the similarity in the output responses between the PQ^{2+} solution and PQ^{2+}-Asc mixture do not support the hypothesis that ascorbate can quench the $PQ^{+\cdot}$ radical.

Testing the hypothesis that dehydroascorbate can quench the paraquat free radical

Ascorbate is a common physiological antioxidant and radical scavenger that can be oxidized by various reactive oxidants (e.g., reactive oxygen species). A single electron oxidation leads to the ascorbate free

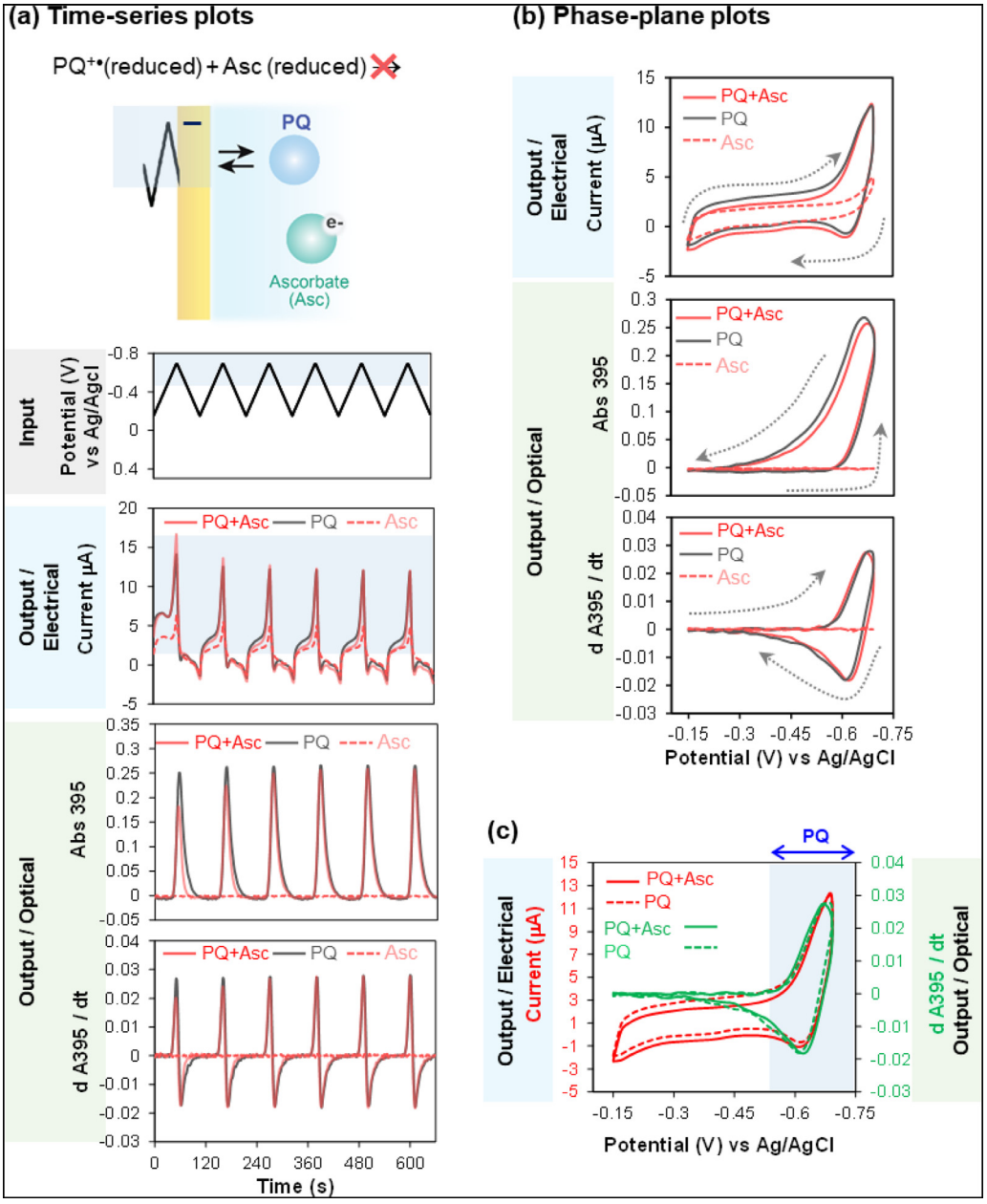
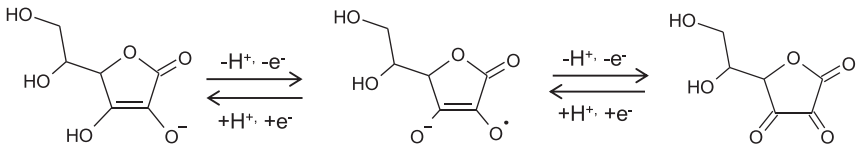


Fig. 2. *Interaction between paraquat and reduced ascorbate.* (a) Interaction graph suggests no interaction between paraquat and the reduced ascorbate (Asc) because either both the $PQ^{+\cdot}$ radical and Asc are reduced, or it is thermodynamically unfavorable for the reduced Asc to transfer an electron to the oxidized PQ^{2+}. Time-series electrical and optical output reponses to a narrow range of cyclically-imposed input potentials (−0.15 V ~ −0.7 V vs Ag/AgCl; solutions contained 0.2 mM paraquat and/or 0.5 mM Asc). (b) Phase-plane plots showing the output responses as a function of the input potential. (c) Phase-plane plot showing that the electrical and optical responses for the redox switching of paraquat are not affected by ascorbate.

radical while the loss of a second electron yields the non-radical dehydroascorbate (DHAsc): dummy

Importantly, various physiological mechanisms are used to re-reduce the ascorbate radical and dehydroascorbate (DHAsc) back to its reduced state [35–38].



(1)

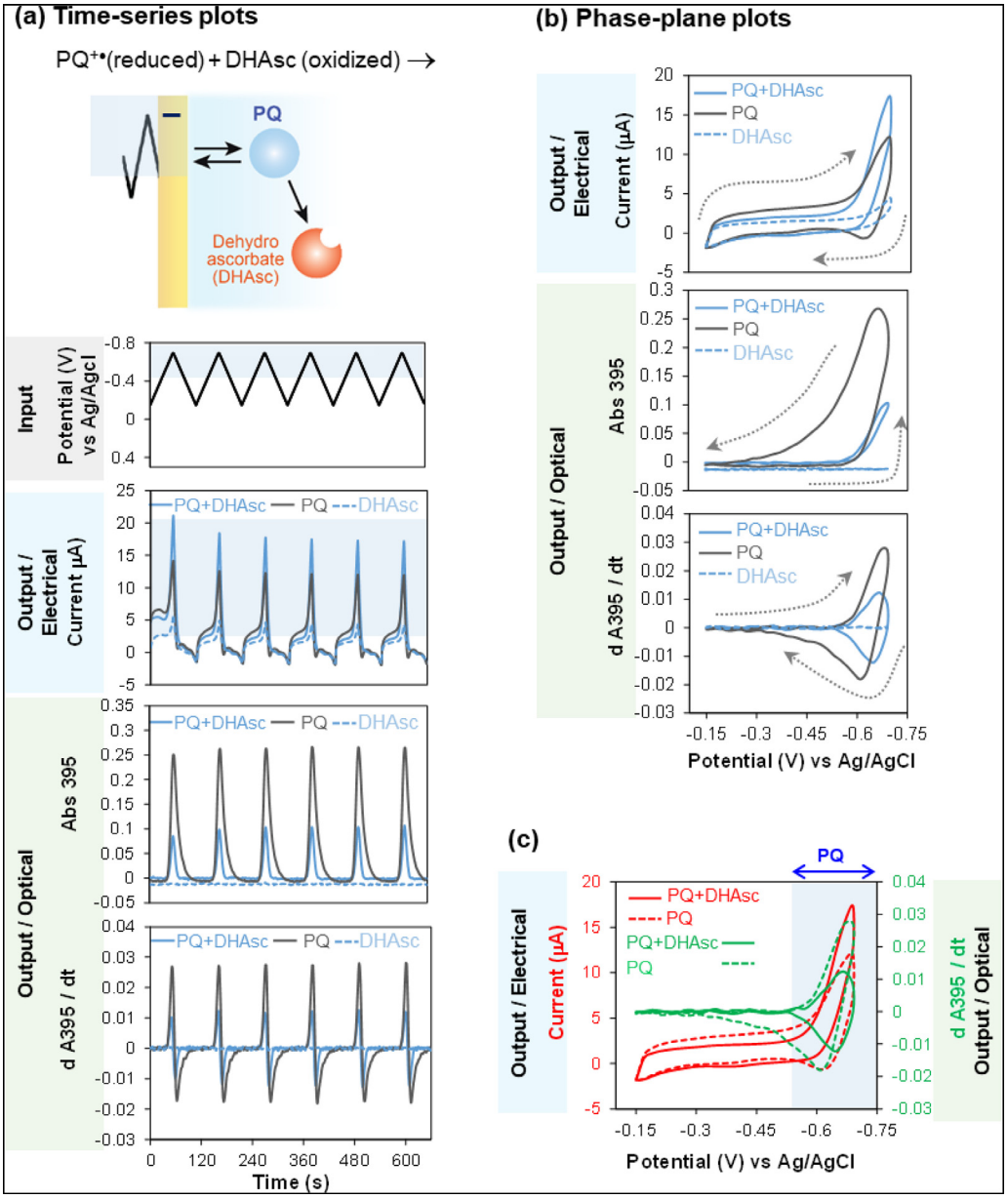


Fig. 3. *Interaction between paraquat and oxidized dehydroascorbate.* (a) Interaction graph suggests the reduced $PQ^{+\cdot}$ radical can be quenched by transferring an electron to the oxidized dehydroascorbate (DHAsc). Time-series electrical and optical output reponses to a narrow range of cyclically-imposed input potentials (−0.15 V ~ −0.7 V vs Ag/AgCl; solutions contained 0.2 mM paraquat and/or 0.5 mM DHAsc). (b) Phase-plane plots showing the output responses as a function of the input potential. (c) Phase-plane plot summarizing the amplified electrical response and attenuated optical response when paraquat was electrochemically reduced and oxidized in the presence of DHAsc.

We next tested the hypothesis that the oxidized DHAsc could quench the $PQ^{+\cdot}$ radical. Specifically, we used the same spectroelectrochemical approach as described in Fig. 2 except we prepared a DHAsc solution (0.5 mM) and an PQ^{2+}-DHAsc mixture (0.2 mM PQ, 0.5 mM DHAsc). The time series input-output curves in Fig. 3a illustrate that the responses of the PQ^{2+}-DHAsc mixture (compared to the PQ^{2+} solution) are markedly different from the responses observed for the PQ^{2+}-Asc mixture. Specifically, the responses for the PQ^{2+}-DHAsc mixture show: amplifications in reductive currents; and attenuations in both optical absorbance at 395 nm and its time derivative. These responses are consistent with the hypothesis that the $PQ^{+\cdot}$ radical is quenched by DHAsc. [Note: despite the thermodynamic driving force, DHAsc does not appear to undergo significant reduction at the electrode.]

The phase-plane plots in Fig. 3b further illustrate these changes. These changes are summarized in the Fig. 3c which shows that in the potential region near PQ^{2+}'s E° value that the addition of DHAsc leads to: an amplification in the reducing current associated with the generation of the $PQ^{+\cdot}$; but an attenuation in the first-derivative of absorbance associated with $PQ^{+\cdot}$ radical.

In summary, the responses observed in Fig. 3 are consistent with the hypothesis that the oxidized DHAsc can accept an electron to quench the $PQ^{+\cdot}$ radical.

Modeling support for the hypothesis that dehydroascorbate can quench the paraquat free radical

The above evidence that DHAsc (but not Asc) can quench the $PQ^{+\cdot}$ radical is based on the experimental observations that DHAsc could amplify the $PQ^{+\cdot}$-generating reduction currents but attenuate the optical signals associated with the accumulation of this radical [33]. To

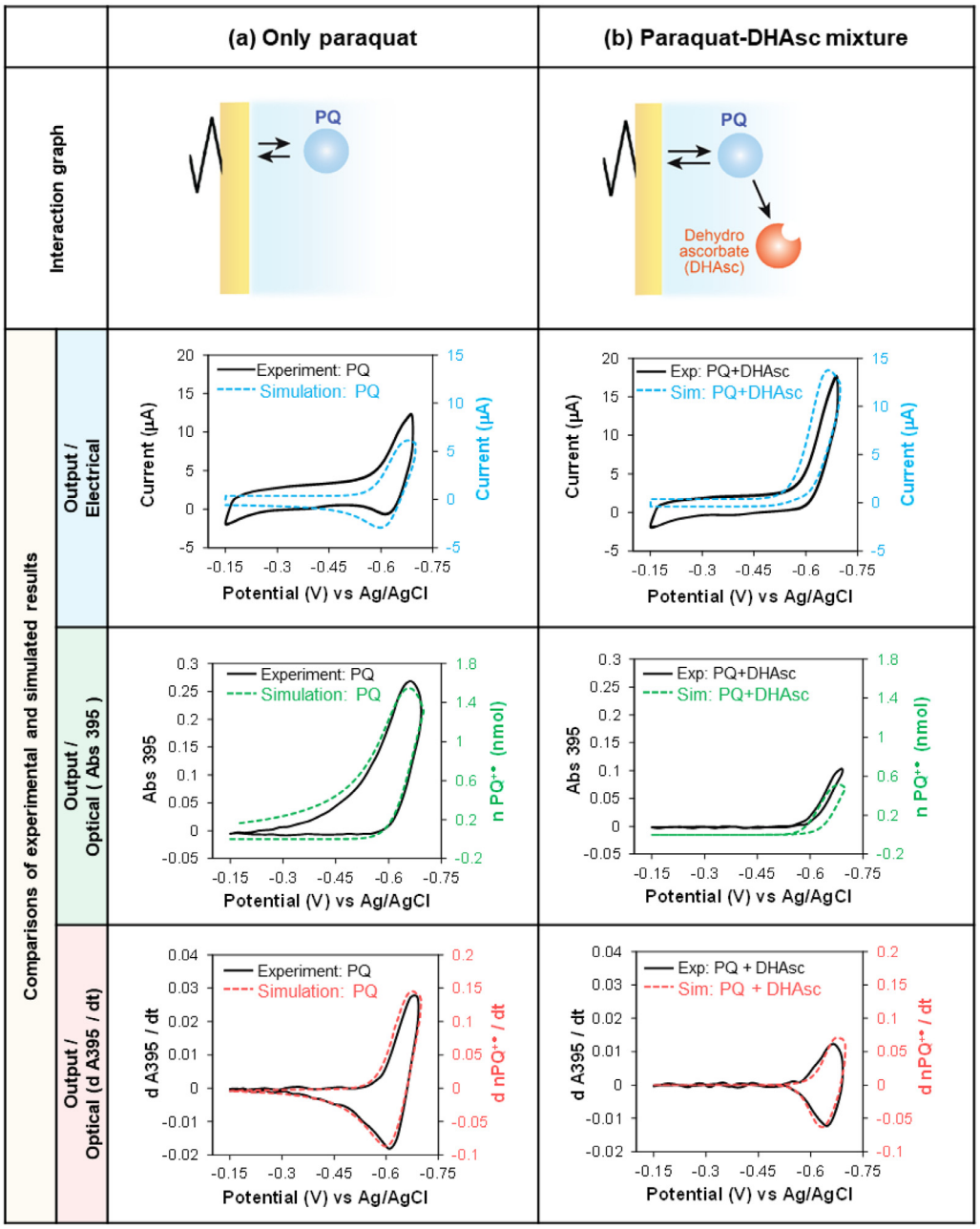


Fig. 4. *Comparison of the simulated and experimental results*. (a) Interaction graph for the potential-dependent exchange of electrons between the electrode and paraquat, and comparison of simulated and experimental phase-plane plots for the electrical and optical output responses. (b) Interaction graph that includes electron transfer from the reduced $PQ^{+\cdot}$ to oxidized DHAsc, and comparison of simulated and experimental results for the electrical and optical responses. The agreement between simulations and experiment supports the hypothesis that the oxidized dehydroascorbate can quench the $PQ^{+\cdot}$ radical.

provide a more detailed evaluation, we compared the response characteristics with those predicted from simulations using a physically-realistic quantitative framework based on a standard electrochemical modeling package (electroanalysis interface of COMSOL; see Supporting Information for details) [20,34]. It is important to emphasize that the purpose of this simulation is to reveal the intrinsic structure of the data and not to create a digital replica. Specifically, this modeling approach accounts for three phenomena: the electrochemical oxidation/reduction of PQ^{2+}; the diffusion of the oxidized PQ^{2+}, the reduced $PQ^{+\cdot}$ radical, and DHAsc; and the electron transfer chemical reaction between $PQ^{+\cdot}$ and DHAsc. Although we performed some initial parameter optimizations (e.g., for diffusion coefficients and reaction rate constants), this model is a minimal model that it neglects aspects that are expected to be of secondary importance (e.g., the electrode shape and size). Further, this model assumes a proportionality between the simulated moles of the $PQ^{+\cdot}$ radical ($nPQ^{+\cdot}$) and the observed optical absorbance, but we did not attempt to discern the proportionality constant.

Fig. 4a shows the interaction graph and compares the simulated and experimental results for a solution containing only PQ^{2+}. The electrical response-characteristics are shown in the upper phase-plane plot and the shape of the i-E curves are similar between simulations and experiment. The optical (or molecular) response-characteristics are shown in the lower two phase-plane plots and the shapes of both the optical (or $nPQ^{+\cdot}$) and time-derivative curves are in good agreement between simulation and experiment.

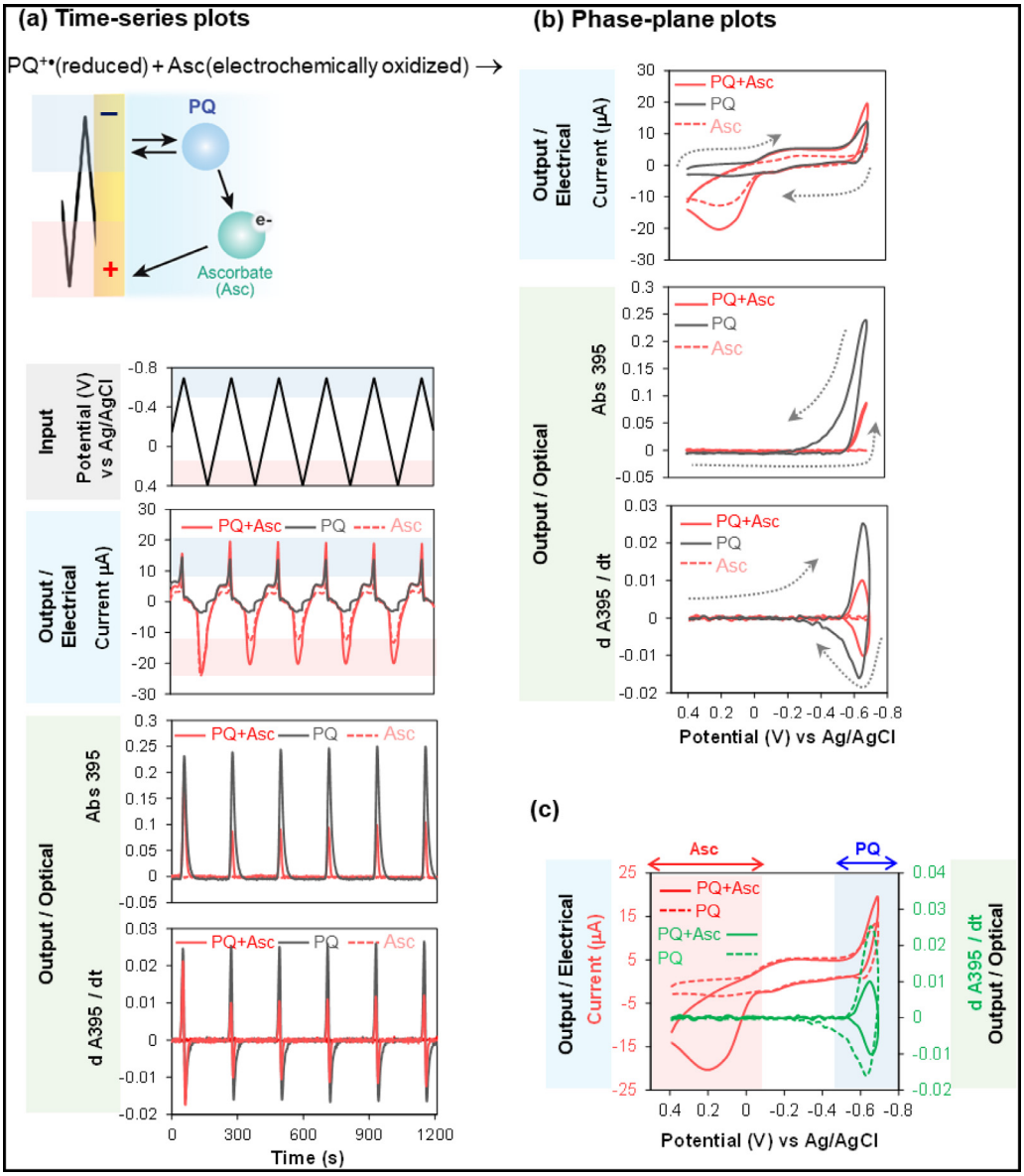


Fig. 5. *Interaction between paraquat radical and electrochemically oxidized ascorbate.* (a) Interaction graph suggests the reduced $PQ^{+\cdot}$ radical can be quenched by transferring an electron to ascorbate if the ascorbate is electrochemically-oxidized. Time-series electrical and optical output responses to a broad range of cyclically-imposed input potentials (+0.4 V ~ −0.7 V vs Ag/AgCl; solutions contained 0.2 mM paraquat and/or 0.5 mM Asc). (b) Phase-plane plots showing the output responses as a function of the input potential. (c) Phase-plane plot summarizing the amplified electrical response and attenuated optical response of paraquat when it was tested in the presence of Asc and with potential inputs sufficient to oxidize Asc.

Fig. 4b shows the interaction graph and compares the simulated and experimental results for a PQ^{2+}-DHAsc mixture. Again, the shapes for the electrical and optical phase-plane plots are similar between the simulations and the experimental observations. This agreement indicates that the simulations provide a theoretically-based framework that reveals the intrinsic structure of the response-characteristics as evaluated from the phase-plane plots.

Importantly, the comparison of Fig. 4a and 4b illustrates that the simulations predict several important features that were observed in the experimental phase-plane plots. Most obvious is that the simulations predict that DHAsc should amplify the electrical features associated with PQ^{2+}-reduction but attenuate the optical features associated with $PQ^{+\cdot}$-accumulation (e.g., the absorbance and its time derivative). Less obvious is that simulations predict the DHAsc-induced perturbations to the shapes of the phase-plane curves. For instance, comparison of the i-E curves illustrates that while DHAsc amplifies the PQ^{2+}-reduction current, it attenuates the $PQ^{+\cdot}$-oxidation current (i.e., DHAsc leads to a rectification of the electrochemical currents). This rectification results in a significant change in the shapes of the i-E curves between Fig. 4a and 4b. In contrast, DHAsc leads to an attenuation in the optical outputs but less dramatic changes in the shapes of these optical phase-plane plots.

The agreement between simulations and experiment provides further confidence in the conclusion that the oxidized dehydroascorbate (but not the reduced ascorbate) can quench the $PQ^{+\cdot}$ radical.

Oxidation of ascorbate allows for quenching of the paraquat free radical

In a final experiment, we tested whether oxidation of ascorbate would allow for quenching of the $PQ^{+\cdot}$ radical. In this experiment, we used the same set of solutions as in Fig. 2 but performed CV measurements over a broader potential range (+0.4 to −0.7 V vs Ag/AgCl; 10 mV/s) to include values that are more oxidative than the redox po-

tential for ascorbate (E° −0.12 V vs Ag/AgCl). The network interaction graph in Fig. 5a proposes that $PQ^{2+}/PQ^{+\cdot}$ can be reversibly oxidized/reduced at the electrode; electron transfer between paraquat and ascorbate is unidirectional (the $PQ^{+\cdot}$ radical can donate an electron to the oxidized form of Asc); and ascorbate can be oxidized at the electrode (but the oxidized ascorbate cannot be reduced at the electrode). [Note: (i) the molecular structure of the electrochemically-generated oxidized ascorbate species is unknown; and (ii) the proposed interaction graph is based on experimental observations discussed below.]

The upper time series input-output curves in Fig. 5a show: significant oxidation currents associated with Asc-oxidation; significant reduction currents associated with PQ^{2+}-reduction; and both the oxidation and reduction currents are amplified for the PQ^{2+}-Asc mixture. The molecular-level interpretation of this response is that when the potential is cycled into the oxidative region Asc is oxidized at the electrode and this oxidized Asc remains in the electrode vicinity as the potential is cycled into the reducing region (the oxidized ascorbate cannot be re-reduced electrochemically). When the potential is cycled to allow PQ^{2+}-reduction, the $PQ^{+\cdot}$ radical that is formed can be quenched by the oxidized Asc and this leads to a redox-cycling of paraquat that results in an amplified reduction current. Interestingly, the amplified oxidation current suggests that the $PQ^{+\cdot}$-Asc interaction re-reduces the oxidized Asc allowing it to be re-oxidized at the electrode in the subsequent oxidation cycle. (Note: Fig. S2 shows control CV results with paraquat and DHAsc using the same broad input potential range).

The lower two time series input-out plots in Fig. 5a show attenuation of the optical signals associated with the $PQ^{+\cdot}$ radical (both the absorbance at 395 nm and its time-derivative). This optical attenuation is consistent with a quenching of the $PQ^{+\cdot}$ radical by the oxidized Asc.

The upper phase-plane plot in Fig. 5b further shows: peak currents for Asc's electrochemical oxidation (but no corresponding peak for the electrochemical re-reduction of the oxidized Asc); peak currents for PQ^{2+}-reduction; and amplified currents for the PQ^{2+}-Asc mixture. The bottom two phase-plane plots show that in the presence of electrochemically-oxidized Asc, the optical signals associated with the $PQ^{+\cdot}$ radical are attenuated. These summarized changes are shown in Fig. 5c, which indicate that electrochemically oxidized Asc can quench $PQ^{+\cdot}$.

Conclusions

Here, we used an electrochemical approach to test a specific redox-based hypothesis suggested for the treatment of severe paraquat poisoning. Specifically, we examined whether the $PQ^{+\cdot}$ radical could be quenched by the reduced and/or oxidized ascorbate. One function of the electrode is to serve as the source of electrons for the formation of the $PQ^{+\cdot}$ radical and a sink of electrons for its subsequent oxidation. By comparing Scheme 1a and Scheme 1b, the electrode simplifies the experimental system by abstracting-away: the "upstream" $PQ^{+\cdot}$-generating reactions (e.g., NADPH-mediated reduction reactions); and eliminates the downstream generation of reactive species (Note: O_2 was excluded from our experimental system). The second function of the electrode is to quantitatively count the "flow" of electrons into/from the experiment. We complemented these electrical measurements of redox activities with optical measurements of the redox-state switching of the $PQ^{2+}/PQ^{+\cdot}$ redox couple. The consistent conclusion from a series of experiments, is that the $PQ^{+\cdot}$ radical can be quenched by the oxidized (but not the reduced) ascorbate. If, as suggested from retrospective studies [25,29–32], ascorbate does confer benefit, then this could occur either if some of the administered ascorbate is oxidized or if the reduced ascorbate quenches downstream reactive species.

While this work tests a specific hypothesis, we believe it illustrates the broader concept that electrochemistry offers several benefits for studies in redox biology. Compared to adding chemical reagents to induce oxidation and reduction reactions, the electrical inputs can be imposed precisely, repeatedly and in different sequences to enable rigorous hypothesis-testing. Further, compared to many alternative molecular measurement methods, electrode-based activity measurements are simple, rapid and sensitive, and they can be coupled to simultaneous optical measurements to provide complementary molecular-level information (i.e., of the redox-state switching of paraquat). Finally, electrochemical systems are inexpensive, miniaturizable and provide data in an electronic format convenient for real time automated analysis. Thus, we envision that electrochemistry could provide a valuable complement to existing methods in redox biology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Science Foundation (CBET #1932963 and MCB #2227598), the Defense Threat Reduction Agency (HDTRA1-19-0021), and the Betty and Gordon Moore Foundation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.arres.2023.100068.

References

[1] R. Vermeulen, E.L. Schymanski, A.L. Barabási, G.W. Miller, The exposome and health: where chemistry meets biology, Science 367 (6476) (2020) 392–396 (80-.), doi:10.1126/science.aay3164.
[2] M.M. Cortese-Krott, A. Koning, G.G.C. Kuhnle, P. Nagy, C.L. Bianco, A. Pasch, D.A. Wink, J.M. Fukuto, A.A. Jackson, H. Van Goor, K.R. Olson, M. Feelisch, The reactive species interactome: evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine, Antioxid. Redox Signal. (2017) 684–712 Mary Ann Liebert, Inc, doi:10.1089/ars.2017.7083.
[3] A. Buico, C. Cassino, M. Ravera, P.G. Betta, D Osella, Oxidative stress and total antioxidant capacity in human plasma, Redox Rep. 14 (3) (2009) 125–131, doi:10.1179/135100009X392557.
[4] J. Santolini, S.A. Wootton, A.A. Jackson, M. Feelisch, The redox architecture of physiological function, Curr. Opin. Physiol. (2019) 34–47 Elsevier Ltd June, doi:10.1016/j.cophys.2019.04.009.
[5] G. Colombo, M. Clerici, D. Giustarini, R. Rossi, A. Milzani, Dalle-Donne, I. Redox albuminomics: oxidized albumin in human diseases, Antioxid. Redox Signal. 17 (11) (2012) 1515–1527, doi:10.1089/ars.2012.4702.
[6] G. Sabbioni, J. Turesky, R. biomonitoring human albumin adducts: the past, the present, and the future, Chem. Res. Toxicol. 30 (1) (2016) 332–366, doi:10.1021/acs.chemrestox.6b00366.
[7] A. Bachi, I. Dalle-Donne, A. Scaloni, Redox proteomics: chemical principles, methodological approaches and biological/biomedical promises, Chem. Rev. (2013) 596–698, doi:10.1021/cr300073p.
[8] A. Mannaa, F.G. Hanisch, Redox proteomes in human physiology and disease mechanisms, J. Proteome Res. (2019), doi:10.1021/acs.jproteome.9b00586.
[9] N.S. Weliwatte, O. Simoska, D. Powell, M. Koh, M. Grattieri, L. Whittaker-Brooks, C. Korzeniewski, S.D Minteer, Deconvoluting charge transfer mechanisms in conducting redox polymer-based photobioelectrocatalytic systems, J. Electrochem. Soc. 169 (8) (2022) 85501.
[10] F. Dong, O. Simoska, E. Gaffney, S.D Minteer, Applying synthetic biology strategies to bioelectrochemical systems, Electrochem. Sci. Adv. 2 (6) (2022) e2100197.
[11] C. Lennicke, J. Rahn, N. Heimer, R. Lichtenfels, L.A. Wessjohann, B. Seliger, Redox proteomics: methods for the identification and enrichment of redox-modified proteins and their applications, Proteomics 16 (2) (2016) 197–213, doi:10.1002/pmic.201500268.
[12] J.A. Reisz, K.M. Chessler, M. Dzieciatkowska, A. D'Alessandro, K.C. Hansen, Blood and plasma proteomics: targeted quantitation and posttranslational redox modifications, Methods Mol. Biol. 1619 (2017) 353–371, doi:10.1007/978-1-4939-7057-5_24.
[13] Y.M.M. Go, J.D. Chandler, D.P. Jones, The cysteine proteome, Free Radic. Biol. Med. 84 (2015) 227–245, doi:10.1016/j.freeradbiomed.2015.03.022.

[14] J.M. Hansen, D.P. Jones, C. Harris, The redox theory of development, Antioxid. Redox Signal. 32 (10) (2020) 715–740, doi:10.1089/ars.2019.7976.
[15] K.R. Wehmeyer, R.J. White, P.T. Kissinger, W.R. Heineman, Electrochemical affinity assays/sensors: brief history and current status, Annu. Rev. Anal. Chem. 14 (2021) 109–131.
[16] E.A. Hussein, B. Rice, R.J. White, Recent advances in ion-channel probes for nanopore sensing: insights into the probe architectures, Anal. Chim. Acta 1224 (2022) 340162.
[17] S.W. Abeykoon, R.J. White, Continuous square wave voltammetry for high information content interrogation of conformation switching sensors, ACS Meas. Sci. Au. (3) (2022) 1–9.
[18] Y. Liu, C.Y. Tsao, E. Kim, T. Tschirhart, J.L. Terrell, W.E. Bentley, G.F. Payne, Using a redox modality to connect synthetic biology to electronics: hydrogel-based chemo-electro signal transduction for molecular communication, Adv. Healthc. Mater. 6 (1) (2017) 1600908, doi:10.1002/adhm.201600908.
[19] E. Kim, T.E. Winkler, C. Kitchen, M. Kang, G. Banis, W.E. Bentley, D.L. Kelly, R. Ghodssi, G.F Payne, Redox probing for chemical information of oxidative stress, Anal. Chem. 89 (3) (2017) 1583–1592, doi:10.1021/acs.analchem.6b03620.
[20] Z. Zhao, S. Wu, E. Kim, C. Chen, J.R. Rzasa, X. Shi, W.E. Bentley, G.F Payne, System-level network analysis of a catechol component for redox bioelectronics, ACS Appl. Electron. Mater. 4 (5) (2022) 2490–2501.
[21] M. Kang, E. Kim, S. Chen, W.E. Bentley, D.L. Kelly, G.F. Payne, Signal processing approach to probe chemical space for discriminating redox signatures, Biosens. Bioelectron. 112 (2018) 127–135, doi:10.1016/j.bios.2018.04.032.
[22] E. Kim, Z. Keskey, M. Kang, C. Kitchen, W.E. Bentley, S. Chen, D.L. Kelly, G.F. Payne, Validation of oxidative stress assay for schizophrenia, Schizophr. Res. 212 (2019) 126–133, doi:10.1016/j.schres.2019.07.057.
[23] E. Kim, Z. Zhao, J.R. Rzasa, M. Glassman, W.E. Bentley, S. Chen, D.L. Kelly, G.F. Payne, Association of acute psychosocial stress with oxidative stress: evidence from serum analysis, Redox Biol. 47 (2021) 102138.
[24] Z.E. Suntres, Exploring the potential benefit of natural product extracts in paraquat toxicity, Fitoterapia 131 (2018) 160–167.
[25] T. Blanco-Ayala, A.C. Andérica-Romero, J. Pedraza-Chaverri, New insights into antioxidant strategies against paraquat toxicity, Free Radic. Res. 48 (6) (2014) 623–640, doi:10.3109/10715762.2014.899694.
[26] D.A. Drechsel, M. Patel, Chapter 21 paraquat-induced production of reactive oxygen species in brain mitochondria, Methods Enzymol. 456 (A) (2009) 381–393, doi:10.1016/S0076-6879(08)04421-2.
[27] R.J. Dinis-Oliveira, J.A. Duarte, A. Sánchez-Navarro, F. Remião, M.L. Bastos, F. Carvalho, Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment, Crit. Rev. Toxicol. (2008) 13–71, doi:10.1080/10408440701669959.
[28] L.L. Smith, Mechanism of paraquat toxicity in lung and its relevance to treatment, Hum. Exp. Toxicol. 6 (1) (1987) 31–36, doi:10.1177/096032718700600105.
[29] J.M. Moon, B.J Chun, The efficacy of high doses of vitamin C in patients with paraquat poisoning, Hum. Exp. Toxicol. 30 (8) (2011) 844–850, doi:10.1177/0960327110385633.
[30] S.Y. Hong, K.Y. Hwang, E.Y. Lee, S.W. Eun, S.R. Cho, C.S. Han, Y.H. Park, S.K Chang, Effect of vitamin C on plasma total antioxidant status in patients with paraquat intoxication, Toxicol. Lett. 126 (1) (2002) 51–59, doi:10.1016/S0378-4274(01)00431-3.
[31] M. Rodrigues da Silva, A. Schapochnik, M. Peres Leal, J. Esteves, C. Bichels Hebeda, S. Sandri, C. Pavani, A. P. Carolina Ratto Tempestini Horliana, S.H. Farsky, A. Linodos-Santos-FrancoID, Beneficial effects of ascorbic acid to treat lung fibrosis induced by paraquat, PLOS ONE 13 (2018) e0205535, doi:10.1371/journal.pone.0205535.
[32] Z.E. Suntres, Role of antioxidants in paraquat toxicity, Toxicology 180 (1) (2002) 65–77, doi:10.1016/S0300-483X(02)00382-7.
[33] E. Kim, M. Kang, T. Tschirhart, M. Malo, E. Dadachova, G. Cao, J.J. Yin, W.E. Bentley, Z. Wang, G.F. Payne, Spectroelectrochemical reverse engineering demonstratesthat melanin's redox and radical scavenging activities are linked, Biomacromolecules 18 (12) (2017) 4084–4098, doi:10.1021/acs.biomac.7b01166.
[34] Z. Zhao, E. Kim, D. Motabar, W.E. Bentley, G.F. Payne, Spectroelectrochemical network measurements for redox bioelectronics, Chem. Mater. (2023), doi:10.1021/acs.chemmater.2c02707.
[35] M. Eigenschink, D. Savran, C.P. Zitterer, S. Granitzer, M. Fritz, D.M. Baron, E.W. Müllner, U. Salzer, Redox properties of human erythrocytes are adapted for vitamin C recycling, Front. Physiol. 12 (2021) 2222, doi:10.3389/FPHYS.2021.767439/BIBTEX.
[36] J. Shen, P.T. Griffiths, S.J. Campbell, B. Utinger, M. Kalberer, S.E. Paulson, Ascorbate oxidation by iron, copper and reactive oxygen species: review, model development, and derivation of key rate constants, Sci. Rep. 11 (1) (2021) 7417, doi:10.1038/s41598-021-86477-8.
[37] Y.R. Li, H. Zhu, Vitamin C for sepsis intervention: from redox biochemistry to clinical medicine, Mol. Cell. Biochem. (2021) 4449–4460, doi:10.1007/s11010-021-04240-z.
[38] Y.J. Tu, D. Njus, H.B. Schlegel, A theoretical study of ascorbic acid oxidation and HOO˙/O2˙– radical scavenging, Org. Biomol. Chem. 15 (20) (2017) 4417–4431, doi:10.1039/C7OB00791D.