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A Surface-Modified Gold Minigrid Electrode Which Heterogeneously Reduces Spinach Ferredoxin

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Abstract: The preparation and characteristics of gold minigrid electrode with its surface modified by a polymeric form of 1,1′-dimethyl-4,4′-dipyridyl dichloride are described. The prepared electrode is stable to a wide range of applied potentials and to chemical reaction with molecular oxygen and Fe(III). The electrode has the ability to heterogeneously reduce spinach ferredoxin at a rate which is considerably faster than that of a nonmodified gold minigrid electrode.

The direct electrochemical reduction or oxidation of components of the plant photosynthetic apparatus using a solid electrode is usually an irreversible electrode reaction which prohibits the use of a variety of voltammetric techniques[1,2] for its characterization. The use of mediated, or indirect, electrochemical methods has permitted the application of a growing number of instrumental techniques to the study of this problem.[1-5] This present study was undertaken to determine the electron-transfer features of spinach ferredoxin in an optically transparent thin-layer electrochemical cell (OTTLE cell)[6] based on the successful application of this method to components of the mammalian oxidative phosphorylation apparatus by Heineman et al.[7,8] and in the study of vitamin B_{12}.[9] During the course of this investigation an aspect of 1,1′-dimethyl-4,4′-dipyridyl dichloride (common name methyl viologen, MV) chemistry which has not been previously reported to the authors' knowledge was discovered.

The electrochemistry of MV has been widely studied[3,10-15] and its electrochemical reactions are

$$MV^{2+} + e^- \rightarrow MV^{+\cdot} \quad (1)$$

$$MV^{+\cdot} + e^- \rightarrow MV^0 \quad (2)$$

Prominent among the chemical reactions which complicate the above electrochemical reactions are the dimerization of the cation radical[3,16] and the disproportionation of the cation radical to the dicationic and the neutral species.[17] We wish to report results which indicate that under certain experimental conditions another species, proposed to be polymeric, is formed which is analogous to the polyviologen product of the chemical reactions of a series of viologens described by Simon and Moore.[18]

The significance of these results is that the polymeric form of MV is readily prepared by electrochemical means on a gold minigrid surface. Once formed it is stable to the application of between +0.50 and −0.95 V vs. Ag|AgCl and to chemical degradation with added MV^{2+}, molecular oxygen, and ferric iron. This polymeric film of MV formed on the gold surface is also stable to extended exposure to air. The utility of this surface-modified electrode is that it renders the gold surface electroactive in the reduction and oxidation of spinach ferredoxin. In the absence of the polymeric film and solution, MV^{2+} gold electrodes reduce ferredoxin only at a very slow rate.[19] The results of this investigation show that these electrodes may be prepared simply and that the surface-modified electrode has the capability of heterogeneous, or direct, electron transfer with at least one reasonably large biological molecule from the plant photosynthetic apparatus. The kinetic and analytical utility of this electrode is currently being investigated in our laboratory.

There are important consequences to being able to eliminate the need for mediators in the study of photosynthetic components. Optical studies of the chromophores of the plant photosynthetic apparatus are hampered due to the large absorbances of the mediators (cation radicals of the viologens) in the 390 and 600-nm range.[20] This requires that the mediators be present in low concentration in electrochemical titrations leading to slow rates of reduction. Secondly, the cation radical forms of the viologen mediators produce a large electron paramagnetic resonance (EPR) signal which must be accounted for in studies of the difference spectra often obtained in such studies (i.e., light minus dark EPR spectra). The electrode described in this paper may allow ready potentiometric poising of photosynthetic samples without the need for viologen mediators in solution, thereby permitting much more sensitive optical and EPR measurements.

Experimental Section

The electrochemical instrumentation consisted of a Wenking Model 61RS potentiostat with controlled voltages obtained from a Wavetek Model 112 signal generator and a battery. These voltages were added with a conventional operational amplifier adder circuit before being applied to the potentiostat.

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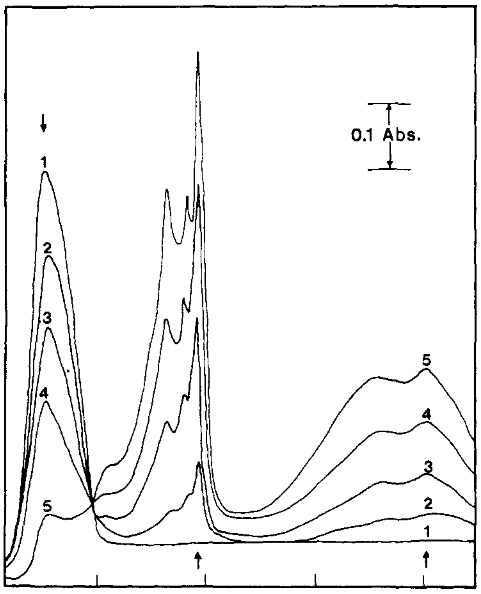

Figure 1. Absorbance spectra of MV vs. applied potential. Solution contained 1.0 mM MV, 0.1 M Tris, and 0.1 M NaCl, pH 7.1. Potentials applied are (1) 0.000, (2) −0.600, (3) −0.625, (4) −0.650, and (5) −0.725 V. Each potential was applied for 10 min.

A conventional integrator circuit employing a Philbrick/Nexus Model 1026 operational amplifier was used to integrate current. The drift of the integrator was less than 0.1% in all experiments. Cyclic voltammetric, coulometric, and polarographic experiments were recorded on a Hewlett-Packard/Moseley 7035 AM X-Y recorder or a Fisher series 100XY recorder. Polarographic experiments were performed using a Heath Model EUA-19-2 polarography module which was chopper stabilized. A Digitec Model 200A voltameter was used to accurately determine the voltage applied to the working electrode.

A Cary Model 17 spectrophotometer was used for all optical measurements. The scanning electron micrographs (SEM) were obtained on an A.M.R. 100A scanning electron microscope with a Kevex energy dispersive x-ray source, Model 5100.

The optically transparent gold minigrid electrodes were obtained from Buckbee-Mears, St. Paul, Minn., and were used in a cell based on the design of Norris, Meckstroth, and Heineman.[8] The cell used in this work was assembled with eight 4/40 screws instead of epoxy to permit removal of the minigrid electrodes for surface analysis. A pair of Al optical grade quartz plates from ESCO Optical Products was mounted in the cell to permit optical measurements in the UV spectral region. A typical cell thickness was 0.025 cm. An Ag|AgCl (1.0 M KCl) reference electrode was used in all cases. The potential of the reference electrode was calibrated vs. a saturated quinhydrone solution at known pH[21] after each analysis and found to be 0.228 ± 0.005 V vs. NHE. All potentials are reported vs. the Ag|AgCl reference electrode unless otherwise indicated. MV was obtained from K and L Laboratories, Plainview, N.Y., and was recrystallized from ethanol with acetone three times. The spinach ferredoxin was a kind gift of Dr. Bacon Ke, C. F. Kettering Research Laboratories, and exhibited a 420-276-nm absorbance ratio of 0.46. All other chemicals were reagent grade and all solutions were prepared in distilled water.

Results and Discussion

The unexpected behavior of MV reported in this paper was first manifest when gold minigrid electrodes were used in a spectroelectrochemical study of MV in preparation for a subsequent study of spinach ferredoxin. Electrodes with which spectroelectrochemical experiments had been previously performed had the characteristic of being capable of generating the blue color of the radical cation of MV when introduced into a solution of MV^{2+}. The cell *was not* connected to any electrode contacts of the potentiostat so that the reduction of MV^{2+} occurred via reducing power stored on the electrode surface. This color was generated even after the electrode had been removed from the cell, washed with distilled water and then acetone, and left exposed to air for periods of over 1 day. No potential was applied to the electrode once it was removed from the cell. Simply mounting the electrode in a cell containing MV^{2+} in 0.1 M Tris buffer, 0.1 M NaCl, pH 7.0, produced the blue color of the radical cation on MV in the solution adjacent to the electrode. This same experiment was repeated but at a solution pH of 6.0 which is sufficiently acidic that sodium dithionite is not capable of reducing MV^{2+}. The Nernst equation defines the negative solution potential limit which can be achieved through chemical reduction, $E = -0.059$ pH at $T = 298$ K, or a limit of −0.354 vs. NHE at pH 6. The formal potential of eq 1 is −0.446 V vs. NHE.[3] It was experimentally verified that MV^{2+} is not reduced at pH 6 by dithionite but the surface-modified gold minigrid electrode did generate the spectrum of the cation radical. Quantitative spectroelectrochemical experiments were then performed to elucidate the nature of the species capable of storing this considerable reducing power even on extended exposure to air.

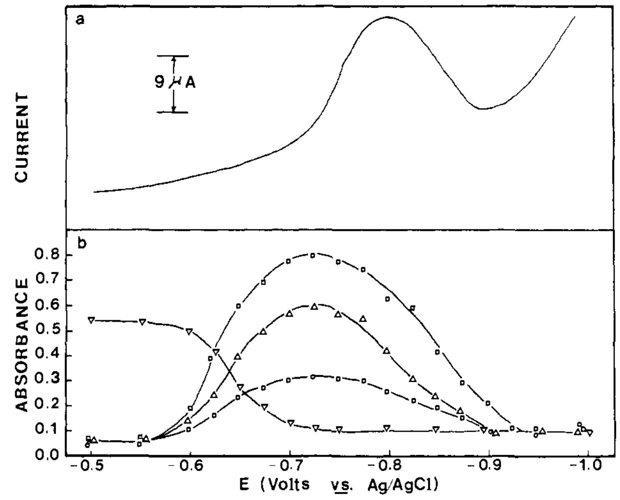

Figure 2. (a) Voltammogram of 1.0 mM MV^{2+} at a clean gold minigrid electrode. Solution conditions same as in Figure 1, electrode area 0.75 cm^2, scan rate 0.020 V/s. (b) Change in absorbance vs. applied potential. Solution conditions same as in Figure 1: (▽) −255 nm, (□) −395 nm, (△) −370 nm, and (○) −605 nm. Each spectrum was obtained after a new potential had been applied for 10 min.

Typical absorbance spectra obtained in spectropotentiostatic experiments are shown in Figure 1. The 255-nm absorbance maximum of MV^{2+} disappears as the applied potential becomes more negative while the 395- and 605-nm absorbance maxima of the cation radical of MV increases. Calculation of the concentration of MV in the cell from the change in absorbance at 605 nm from published values[3] of the molar absorptivity yields $[MV^{2+}] = 0.997$ mM. Figure 2a shows a voltammogram of MV^{2+} at the gold minigrid electrode and Figure 2b shows the corresponding changes in absorbance for the absorbance maximum of MV^{2+} at 255 nm and the absorbance maxima for the cation radical of MV at 395, 370, and 605 nm. This figure clearly indicates that the cation radical as well as the dication are no longer present in the solution when potentials positive of the second reduction process, eq 2, are being maintained. The current peak at −0.8 V is due to the reduction of the dication to the cation radical, eq 1, and the increase in current starting at −0.9 V is due to the reduction of the cation radical to the neutral, eq 2, and background current. To verify that the second reduction process was not occurring, thin-layer coulometric experiments were performed to determine the number of equivalents injected into the sample at these applied potentials. These experiments were performed

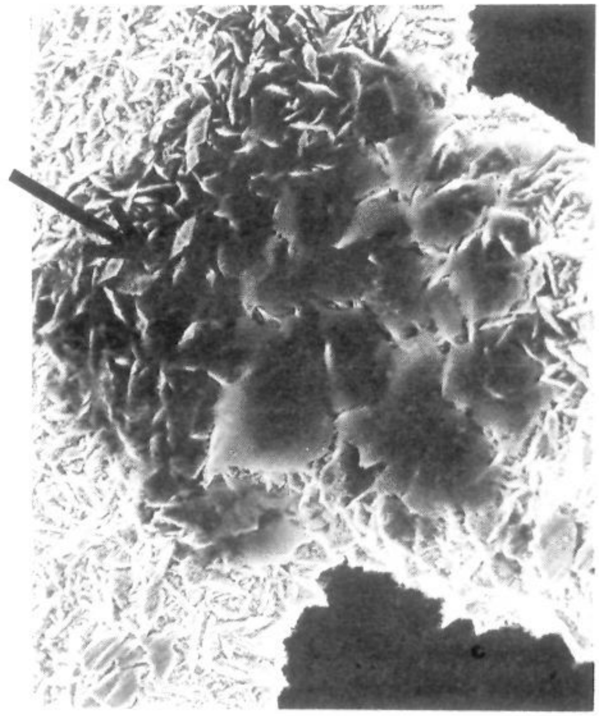

Figure 3. Scanning electron micrograph of a surface-modified gold minigrid electrode: magnification 2340× at 20 kV; arrow indicates deposit.

by applying a potential of −0.950 V for 10 min, stepping the applied potential back to 0.000 V, and integrating the charge. This procedure was followed instead of using a step from 0.000 to −0.950 V because of the considerable background current at −0.950 V due to the hydrogen over voltage limit for gold at this pH. This experiment was performed seven times for a MV^{2+} concentration of 1.00 mM and one time over a concentration range including 0.10, 0.25, 0.50, and 2.00 mM to test the possible contribution of nonfaradaic current to the integrated current. The n value for these 11 analyses was 1.00 with an average deviation of 0.05. This same kind of experiment was performed at an Hg|Au amalgam electrode[22] to verify the n value of 1.00 for reduction to the first step and the n value of 2 for the reduction to the second step (i.e., reduction from the dication to the neutral form). These experiments gave n values of 1.0 and 2.0 ± 10% for the two reduction steps, respectively. The n value of 2.0 for the step to −1.18 V could only be obtained for experiments performed prior to coulometric experiments for the first reduction wave. If the potential was stepped to −0.950 V the n value was 1.0. However, once a potential of −0.950 V had been applied in a coulometric experiment, it was not possible to obtain an n value of 2.0 when the experiment of stepping to −1.18 V was repeated. Application of a potential in the range where the cation radical of MV was heterogeneously formed, but not negative enough to produce the neutral MV, results in the formation of the polymer film as shown in Figure 2b. This occurred over a more negative potential range at Hg|Au (−0.850 to −0.980 V compared to −0.750 to −0.950 V at Au). The potential range at Au is indicated in Figure 2b.

These experiments confirm that one reducing equivalent has been injected and resulted in the complete loss of the absorbance due to the cation radical *and* the dication of MV. Further, the spectra of the cation radical and the dication could be reversibly generated by stepping back and forth between −0.300 and −0.800 V. This was not possible once a potential of −0.950 V had been applied.

Several experiments were performed to substantiate that a MV polymeric film is formed on the electrode surface and to eliminate other possible species such as salts of the cation radical of MV. As previously indicated, once the potential of −0.950 V had been applied for 10 min, the 255-nm absorbance peak due to the dication could not be generated electrochemically even when the potential was stepped to +0.500 V and held there for 1 h. Air-saturated buffer, 0.1 M Tris, 0.1 M NaCl, pH 7.1, was introduced into the cell and again the oxygen in the buffer and the application of potential extremes did not result in the generation of the 255-nm peak. A solution of 1.0 mM MV^{2+} in the same buffer medium was introduced and as long as the applied potential was not more negative than −0.800 V, the 255-nm peak was reversible and never became larger than expected for 1.0 mM MV. A solution of 1.0 mM Fe(III) was introduced into the cell and again the 255-nm peak could not be regenerated on the application of extremes in potential, +0.500 to −0.950 V. To further check the effect of Fe(III), buffer and then MV^{2+} were introduced and the cyclic voltammogram obtained was identical with those obtained prior to the introduction of Fe(III).

Spectropotentiostatic experiments using the Hg|Au minigrid electrode were also performed. Similar results were obtained but the potential at which the absorbance due to the cation radical of MV was lost shifted 50 mV more cathodic.

The reaction which leads to the loss in absorbance due to the cation radical of MV prior to the second reduction step is most probably a chemical reaction occurring in solution. The potential range over which this chemical reaction occurs is far enough removed from the formal potential ($E^{0\prime}$) of the first reduction process that there is little of the dication present and there is not enough thermodynamic control of the dication to cation radical concentration ratio. To investigate the product of this chemical reaction, electrodes prepared as described earlier were subjected to analysis by scanning electron microscopy (SEM). Figure 3 shows the results obtained from these experiments. Amorphous deposits were found on the surface of the gold minigrid electrode. SEM's of pristine gold minigrid electrodes showed no evidence of these deposits when their entire surfaces were examined. Qualitatively, the surface-modified electrode is clearly duller in appearance than the clean gold surface. These results indicate that a film is formed on the surface of the electrode and, as can be seen from Figure 3, deposition of considerably more than a few layers of product occurs at certain sites. This was tested by obtaining the SEM's for two different concentrations of MV^{2+} (0.5 and 5.0 mM) used for preparing the electrode. Qualitatively, there were more sites at which this deposition was evident in the case of 5.0 mM. Surface analysis of these electrodes using ESCA and Auger spectroscopy is planned.[23]

It is proposed that the product of the chemical reaction which coats the electrode is a polymer of MV. The recent work of Simon and Moore[18] describes the chemical preparation of a series of polyviologens for use as photochromic redox polymers with film-forming properties. Several characteristics of the polymers of viologens described in their work are found in our electrochemical studies. When high concentrations of MV^{2+} (10.0 mM) are used and the electrodes are removed from the cell immediately after application of −0.950 V for 10 min, a blue color is visible on the gold minigrid electrode. The blue color is still visible even after washing with water, acetone, and exposure to air for over 1 day. Further, bulk electrolyses of MV^{2+} at a mercury pool and also at a platinum foil electrode go from colorless to deep blue and finally to an almost colorless solution. The course of the bulk electrolysis was followed by periodically recording a polarogram. At the end of the electrolysis there was no measurable current due to reductions of either reaction given in eq 1 and 2. When this solution was reduced at −1.10 V an absorbance maximum in the 250-nm region and an amber color result. Treatment of this

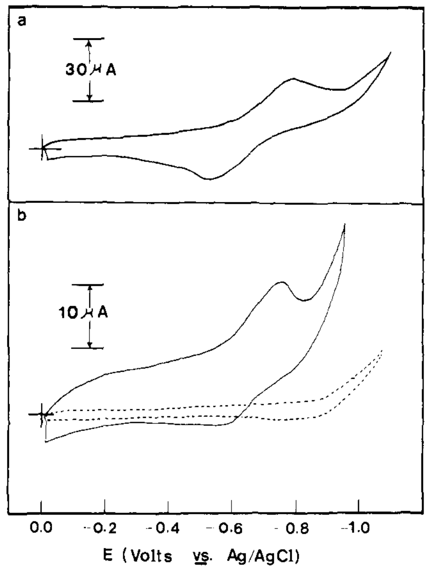

Figure 4. (a) Cyclic voltammogram of MV. Solution conditions same as in Figure 1, recorded prior to electrolysis. (b) Cyclic voltammogram using a surface-modified gold minigrid electrode: (——) 0.30 mM spinach ferredoxin; (- - -) same electrode after rinsing cell and introducing buffer alone.

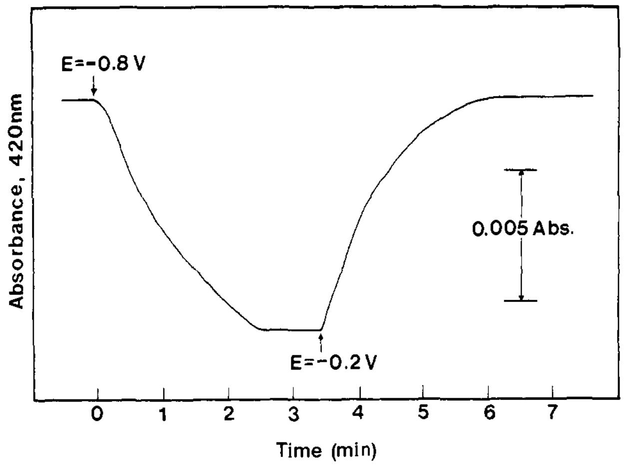

Figure 5. Change in absorbance at the 420-nm absorbance maximum of spinach ferredoxin on heterogeneous reduction and oxidation at a surface-modified gold minigrid electrode. Solution conditions: 0.30 mM spinach ferredoxin; 0.1 M Tris; 0.1 M NaCl, pH 7.1.

solution with acetone results in the formation of a film-type precipitate. These two observations are characteristic of the series of chemically prepared polyviologens reported by Simon and Moore.[18] The structure of the proposed polymer of MV is not known but end to end linkages would likely occur via the nitrogens or a sandwich-type polymer formed via π-cloud overlap may be occurring.

Our experiments have been directed at trying to eliminate the possibility of salts of the radical cation of MV, or other species, being responsible for the observed phenomena. Brown solid has been prepared in the bulk mercury electrolyses which on exposure to oxygen in solution results in the blue color of the radical cation of MV. This solid is not what is being formed on the gold surface. Further work is being performed in an attempt to isolate the electrochemically generated solid, likely to be the salt of the cation radical of MV or neutral MV. The experiments which have been performed seem to preclude the existence of a salt on the electrode surface based on the following: (1) the electrodes may be profusely washed with water after preparation with no effect, (2) exposure to air does not result in the loss of reducing power, (3) the SEM results indicate an amorphous deposit on the electrode which would be consistent with a polymeric vs. a salt product, and (4) the bulk electrolysis results lead to a solution which has characteristics consistent with the work of Simon and Moore.[18]

The work of Simon and Moore[18] showed that their viologen polymers were photolytically reduced to the blue color characteristic of the cation radical of MV. This result suggested that the proposed polymer film formed on the gold minigrid electrode might have the capability of reducing photosynthetic redox components via a *direct* reduction at the modified surface without the need of solution mediators. This possibility was investigated in the following way. A clean gold minigrid electrode was assembled in the cell with a 1.0 mM solution of MV^{2+}. A cyclic voltammogram was run on this solution (see Figure 4a). This voltammogram gave the typical result obtained for the reduction of MV^{2+} at gold at this solution pH. The potential was then set at −0.950 V for 10 min at which time the current had decayed to the constant value of background levels and no blue color due to the radical cation remained. The cell was then rinsed with distilled water and only the buffer medium was introduced into the cell. The cyclic voltammogram for buffer without MV^{2+} is shown in Figure 4b. A solution of 0.30 mM spinach ferredoxin was then introduced into the cell and the cyclic voltammogram shown in Figure 4b was obtained. The location of the peak indicating the heterogeneous reduction of a species is consistent with the formal potential of ferredoxin, −0.428 vs. NHE.[20] An explanation for the increase in the background current shown in the cyclic voltammogram of ferredoxin, Figure 4b, was not found. The cell was mounted in the sample compartment of a Cary 17 spectrometer and spectropotentiostatic experiments were performed monitoring the change in absorbance at 420 nm vs. time. The results of this experiment are shown in Figure 5. The reversible reduction and oxidation of spinach ferredoxin is indicated. No change was found after four cycles of complete reduction and oxidation. These results show a significant rate of electron transfer between the surface-modified gold minigrid electrode and the ferredoxin.[24] In the study of Rickard et al.[19] direct heterogeneous reduction of ferredoxin by gold required over 1.5 h for reductions and oxidations. No heterogeneous reduction occurred at SnO and ferredoxin was rapidly denatured by Hg|Au minigrid electrodes.

Conclusions

The results of this investigation indicate that a chemical reaction between the radical cation of MV, or possibly between the dimer of the radical cation and itself or monomer, occurs beyond the potential range of the first reduction step, eq 1, but before the potential range of the second step, eq 2. The polymer film formed by this reaction is mechanically stable on the surface of a gold minigrid electrode with respect to the application of potential between 0.500 and −0.950 V, reaction with molecular oxygen, reaction with MV^{2+}, or reaction with Fe(III). Also, once the polymer is formed the MV dication cannot be electrochemically generated. This surface-modified gold minigrid electrode has the properties of being able to hold reducing power greater than that of sodium dithionite and of permitting direct electron transfer between itself and spinach ferredoxin, a rather large molecule with a molecular weight of 11 000.[25]

The characteristics of the electrode described in this paper may permit optical studies of components of the plant photosynthetic apparatus without the need for added mediators. Current work in this laboratory is directed at determining whether other metal surfaces (i.e., nickel and platinum) behave similarly with MV and into the possible analogous behavior of 1,1-trimethylene-2,2′-dipyridyl dibromide, 4,4′-dimethyl-1,1′-trimethylene-2,2′-dipyridyl dibromide, and 5,5′-dimethyl-1,1′-trimethylene-2,2′-dipyridyl dibromide. These viologen compounds have formal potentials of −0.520, −0.670, and −0.740 V, respectively,[15] for their first reduction potentials.

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