Oxidation–Reduction Chemistry of DL-α-Lipoic Acid, Propanedithiol, and Trimethylene Disulfide in Aprotic and in Aqueous Media

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Abstract: The electrochemistry of propanedithiol, trimethylene disulfide (TMDS), and DL-α-lipoic acid has been studied in water and acetonitrile solutions by use of cyclic voltammetry and controlled potential coulometry at a gold electrode. Redox mechanisms are proposed on the basis of the electrochemical data. TMDS and oxidized lipoic acid are electrochemically reduced at extremely negative potentials in acetonitrile solution (−1.77 and −1.92 V vs. SCE, respectively). Oxidized lipoic acid cannot be electrochemically reduced in water. The molecule also does not react with singly reduced methyl viologen in acetonitrile solution, which indicates that reduction cannot take place by a sequential one-electron transfer mechanism. In contrast, both TMDS and oxidized lipoic acid are readily reduced by electrochemically generated molecular hydrogen, which confirms that reduction of the disulfide linkage must occur by an atom transfer mechanism.

Lipoic acid is a cofactor for a number of enzyme-catalyzed reactions, including those of pyruvate dehydrogenase, α-oxoglutarate dehydrogenase, and lipoamide oxido reductase.[1] In these systems its primary function is oxidation and acyl group transfer during oxidative decarboxylation of α-keto acids. This function is dependent upon the chemical oxidation and reduction of the sulfur groups in lipoic acid.

Although the chemical reactivity of the lipoic acid disulfide group has been studied extensively,[2,3] previous electrochemical studies of lipoic acid are limited to polarographic investigations in aqueous media.[4] This and a continuing controversy with respect to its biological redox mechanism[5,6] has prompted the present electrochemical study of DL-α-lipoic acid in both aprotic and aqueous media.

The electrochemical behavior of alkyl thiols and disulfides at a dropping mercury electrode in aqueous media has been the subject of several polarographic investigations.[7-10] An oxidation wave is observed for thiols which corresponds to the formation of a mercurous thiolate complex which involves oxidation of the mercury rather than oxidation of the thiol to a disulfide.

$$RSH + Hg \rightarrow RSHg^{I} + H^{+} + e^{-} \quad (1)$$

The electrochemical reduction of disulfides at a mercury electrode also may involve complex formation, but the thiols are the final product.

$$RSSR + Hg \rightarrow RSHg^{I} + RS\cdot \rightarrow (RS)_2Hg^{II} \quad (2)$$

$$Hg^{II}(RS)_2 + 2e^{-} + 2H^{+} \rightarrow Hg + 2RSH \quad (3)$$

An alternative mechanism has been proposed whereby reduction occurs in two successive one-electron steps.[9]

$$RSSR + e^{-} + H^{+} \rightarrow RS\cdot + RSH \quad (4)$$

$$RS\cdot + e^{-} + H^{+} \rightarrow RSH \quad (5)$$

Electrochemical investigations at other electrodes or in aprotic solvents have not been reported.

Experimental Section

Materials and Measurements. The cyclic voltammetric experiments were accomplished by use of a solid-state potentiostat constructed from Philbrick operational amplifiers.[11] A Princeton Applied Research Model 1730/179 potentiostat/digital coulometer was used for controlled-potential electrolysis studies.

A gold-inlay electrode was used as the working electrode for cyclic voltammetry and a gold foil electrode was employed in the coulometric experiments. The reference electrode consisted of a Ag/AgCl electrode in aqueous tetramethylammonium chloride solution (0.000 V vs. SCE) placed in a glass tube which made contact through a cracked glass bead junction with bulk solution in a Luggin capillary. The platinum-flag auxiliary electrode was isolated from the bulk solution by a fine-porosity frit. For cyclic voltammetry experiments, the auxiliary compartment was filled with the bulk solution; for coulometric experiments, the auxiliary compartment was filled with solvent and supporting electrolyte only.

Acetonitrile (Burdick and Jackson) was obtained in quart bottles to minimize water contamination; the water content as specified by the source was 0.013%. Acetonitrile and aqueous solutions were degassed with Ar in the electrochemical cell prior to the addition of the compound to be studied. Tetraethylammonium perchlorate (TEAP) was used as the supporting electrolyte in all of the electrochemical studies in a 50-100-fold excess over the concentration of the electroactive species. TEAP was prepared according to the procedure of House et al.[12]

Oxidized and reduced DL-α-lipoic acid was obtained from Sigma (minimum purity 99 and 90%, respectively), and 1,3-propanedithiol was obtained from Aldrich. These compounds were used without further purification. Lead acetate trihydrate and elemental sulfur were obtained from the Mallinckrodt Chemical Works; the sulfur was recrystallized from toluene. Trimethylene disulfide was prepared by the method of Cragg and Weston.[13] The compound was isolated directly from the reaction mixture and used immediately (minimum purity was judged to be 95% on the basis of its UV-visible spectrum[3] and cyclic voltammograms). Benzene was reagent grade and used without further purification.

Results

In biological systems the carboxylic acid group of lipoic acid forms a peptide linkage to the ε amino group of protein bound lysine,[14] and therefore has no direct effect on the redox behavior of the disulfide or sulfhydril groups. To distinguish the electrochemical properties of the disulfide group from the carboxylic acid group of lipoic acid, the electrochemical behavior of 1,3-propanedithiol (H_2PDT) and trimethylene disulfide (TMDS) has been studied in acetonitrile at a gold electrode.

The cyclic voltammograms for H_2PDT and TMDS in acetonitrile are shown in Figure 1. For an initial negative scan, H_2PDT has reduction peaks at −1.53 V vs. SCE and −1.81 V. On the basis of peak currents both reductions probably are one electron per molecule of H_2PDT processes.[15] Although the use of peak currents as a measure of electron stoichiometry is subject to error, in the absence of meaningful controlled potential coulometric data peak currents can provide insight to the probable electron ratios if corrected for the background currents due to neighboring peaks. Reversal of the voltage-scan direction results in an anodic peak at −0.62 V. For an initial

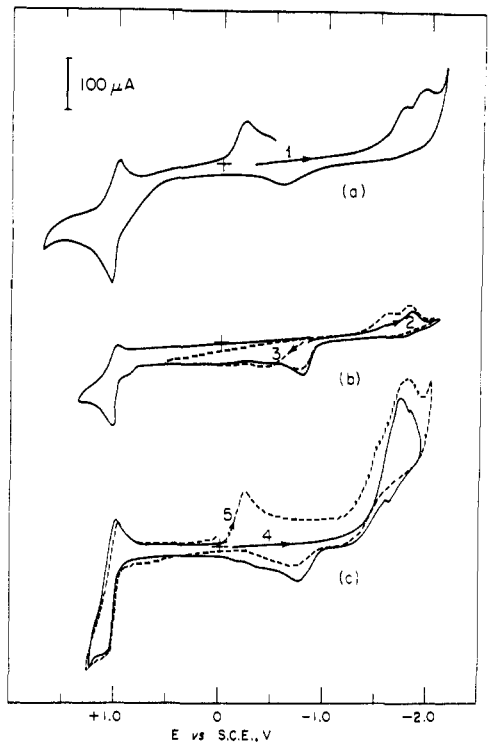


Figure 1. Cyclic voltammograms of 1 mM solutions in 0.1 M TEAP in acetonitrile at a gold electrode, scan rate 0.1 V/s: (a) H_2PDT; (b) H_2PDT plus 2 equiv of OH^-; (c) 4, TMDS; 5, TMDS plus 2 equiv of H^+; 1, 2, 4, and 5, initial negative scans; 3, initial positive scan.

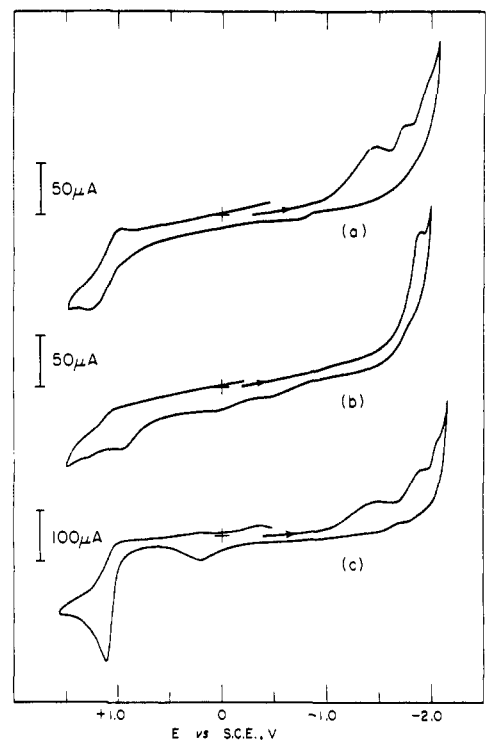


Figure 2. Cyclic voltammograms of 1 mM solutions in 0.1 M TEAP in acetonitrile at a gold electrode, scan rate 0.1 V/s: (a) oxidized lipoic acid; (b) oxidized lipoic acid plus 1 equiv of OH^-; (c) reduced lipoic acid.

positive scan, H_2PDT has a small, broad, irreversible oxidation peak at +0.89 V, and a reversible one at +1.10 V. Controlled potential coulometry at +1.20 V indicates that 6 mol of electrons is transferred per mol of H_2PDT. In the presence of 2 mol of hydroxide ion per mol of H_2PDT the initial cathodic peak currents are much smaller. Reversal of the voltage-scan direction yields an anodic peak at −0.73 V. Initial positive scans of this alkaline solution yield an anodic peak at −0.44 V. In the presence of 3 or more equiv of base, all of the anodic peaks attributable to propanedithiol vanish (the electrochemical behavior of hydroxide ion in acetonitrile is complicated and has been described elsewhere).[16,17]

For initial negative scans, TMDS has an irreversible reduction peak at −1.77 V which, on the basis of peak currents, is a two electron per TMDS molecule process.[15] Reversal of the scan direction yields anodic peaks at −0.73 and −0.44 V. Initial positive scans result in a reversible anodic peak at +1.13 V. Controlled potential coulometry at +1.20 V indicates that this is a four electron per molecule process. In the presence of 2 or more mol of H^+ per mol of TMDS, initial negative scans result in the reduction of protons at −0.24 V; the cathodic peak at −1.77 V has been replaced by two new peaks at −1.58 and −1.83 V. These peaks are qualitatively similar in shape to those observed for initial negative scans of H_2PDT.

Cyclic voltammograms for both oxidized and reduced lipoic acid in acetonitrile are illustrated in Figure 2. For initial negative scans, oxidized lipoic acid has reduction peaks at −1.38, −1.83, and −2.00 V. The broad reduction peak at −1.38 V is due to a one electron per lipoic acid molecule process on the basis of controlled potential coulometry. The currents for the peaks at −1.83 and −2.00 V indicate that these also are one electron per molecule reductions. The peaks are too close to the edge of the solvent window to allow meaningful controlled potential coulometric measurements. For initial positive scans, oxidized lipoic acid has a quasi-reversible oxidation peak at +1.17 V. In the presence of 1 mol of hydroxide ion per mol of lipoic acid, the cathodic peak at −1.38 V disappears and the two peaks at −1.83 and −2.00 V are replaced by a single peak at −1.92 V with roughly twice the peak current. Reversal of the scan direction yields small anodic peaks at −0.50, +0.05, and +0.95 V.

The cyclic voltammograms for reduced lipoic acid are qualitatively similar to those for the oxidized form. There are three reduction peaks at −1.55, −1.83, and −2.00 V for an initial negative scan. Reversal of scan direction results in an anodic peak at +0.24 V. On the basis of peak currents each of the three cathodic peaks is a one electron per molecule process. Addition of 1 mol of hydroxide ion per mol of lipoic acid removes the initial cathodic peak at −1.55 V. For initial positive scans, a quasi-reversible oxidation peak occurs at +1.18 V.

Cyclic voltammograms of both oxidized and reduced lipoic acid in H_2O are shown in Figure 3. For initial negative scans, oxidized lipoic acid has a reduction peak at −0.72 V. Reversal of the scan direction at −1.0 V results in an anodic peak at +0.11 V. On the basis of controlled potential coulometry at −0.75 V the reduction is a one electron per molecule process. The initial cathodic peak current at −0.72 V is greatly diminished by the addition of 1 equiv of hydroxide ion.

For initial positive scans, reduced lipoic acid in aqueous solutions has oxidation peaks at +0.20 and +0.84 V; oxidized lipoic acid only has the peak at +0.84 V. Controlled potential coulometry indicates that the oxidation at +0.20 V is a two-electron process. A UV spectrum of this solution after controlled potential electrolysis confirms the formation of the ozidized form of the molecule.[3] Controlled potential coulometry at +0.90 V confirms that the oxidation at +0.84 V is a two-electron process for oxidized lipoic acid and a four-electron process for reduced lipoic acid.

For reduced lipoic acid, the initial anodic peak shifts to more positive potentials in the presence of 1 equiv of hydroxide ion. When 2 or more equiv is added, the first anodic peak disappears.

Finally, oxidized lipoic acid does not react chemically with singly reduced methyl viologen in acetonitrile. Reduced methyl viologen (generated electrochemically in situ by controlled

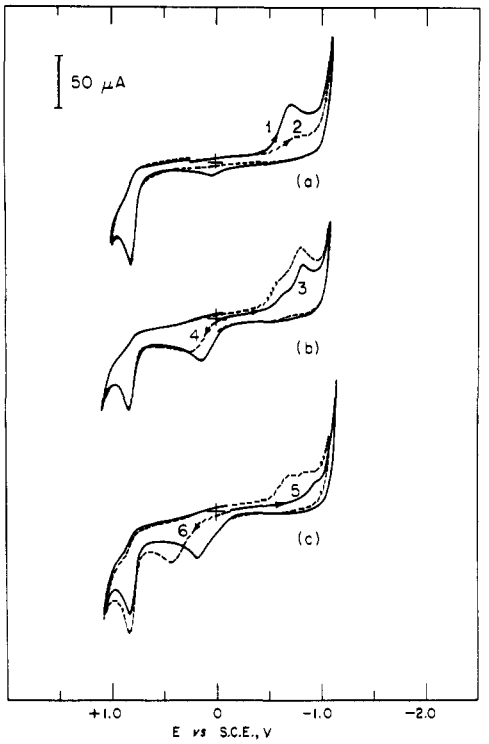


Figure 3. Cyclic voltammograms of 1 mM solutions in 0.1 M TEAP in water at a gold electrode, scan rate 0.1 V/s: (a) 1, oxidized lipoic acid; 2, oxidized lipoic acid plus 1 equiv of OH^-; (b) reduced lipoic acid; (c) reduced lipoic acid plus 1 equiv of OH^-; 1, 2, 3, and 5, initial negative scans; 4 and 6, initial positive scans.

potential electrolysis of methyl viologen at −0.50 V) has a formal reduction potential in acetonitrile of −0.45 V vs. SCE. In contrast, reduced lipoic acid reacts rapidly and stoichiometrically with riboflavin, which has a formal redox potential in aprotic solvents of −0.80 V vs. SCE.[18]

Discussion and Conclusions

The electrochemical behavior of oxidized and reduced propanedithiol in acetonitrile solution is summarized by Figure 4. The cathodic peaks at −1.53 and −1.81 V for the reduced form of the molecule correspond to the one electron per molecule reduction of the protons of the sulfhydryl groups to form hydrogen gas and the dithiolate dianion. The dianion undergoes hydrolytic reactions with the residual water present in the solvent to form a mixture of the monoanion and the neutral reduced molecule again. The anodic peak observed at −0.44 V after reversal of the scan direction corresponds to the oxidation of the monoanion to the disulfide and H^+ ion. This oxidation also occurs for solutions of the reduced molecule that contain 2 equiv of base.

The potential for a given redox process in aprotic solvents usually shifts by −0.3 to −0.5 V per increase in negative charge.[19] Hence, because of its more negative charge the dianion of H_2PDT should be more easily oxidized than the monoanion. However, in the presence of 3 or more equiv of base, new anodic peaks do not appear at more negative potentials. A peak attributable to the oxidation of the dianion is observed at −0.73 V after reduction of the sulfhydryl proton in solutions that contain 2 mol of hydroxide ion per mol of H_2PDT, and after reduction of TMDS.

The anodic peak at +0.89 V for H_2PDT probably corresponds to the oxidation of the neutral molecule to the disulfide. The peak is poorly defined, which may indicate that the oxidation actually is a one-electron process, followed by a slow disproportionation reaction. Oxidation of the mono- and dianions of H_2PDT may proceed by similar mechanisms.

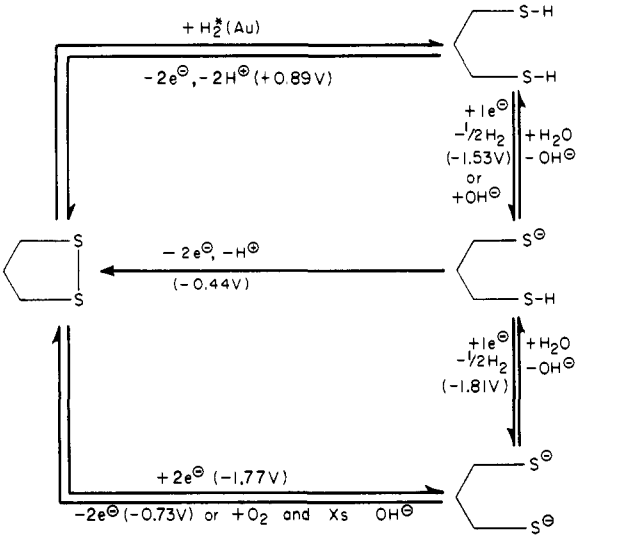
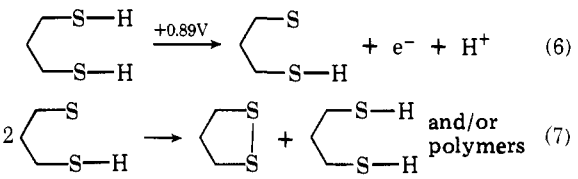


Figure 4. Redox mechanism for H_2PDT and TMDS in acetonitrile solution at a gold electrode.



The second oxidation peak at +1.1 V is a multielectron process. The identity of this highly oxidized species is unknown, but the product species undoubtedly undergoes post-electron-transfer chemical reactions with the solvent or with residual water present in the solvent. Several "superoxidized" disulfide structures have been proposed.[3,14]

The cathodic peak at −1.77 V for TMDS corresponds to the reduction of the disulfide to form the reduced dianion. The dianion undergoes hydrolytic reactions to form a mixture of reduced species; the dianion is reoxidized at −0.73 V and the monoanion at −0.44 V. The initial anodic peak at +1.13 V for TMDS corresponds to the oxidation of the molecule to a "superoxidized" form. The oxidations that occur at +1.10 V for H_2PDT and +1.13 V for TMDS probably are the same process.

When 2 equiv of protons is added to solutions of oxidized propanedithiol and the protons subsequently electrochemically reduced at −0.24 V, the cyclic voltammograms indicate that propanedithiol is formed. The ease of chemical reduction of TMDS by hydrogen on a gold surface is in sharp contrast to the difficulty of electrochemical reduction.

The electrochemical behavior of oxidized and reduced lipoic acid in acetonitrile solution is summarized in Figure 5. The initial reductions at −1.38 (oxidized form) and −1.55 V (reduced form) have nothing to do with the sulfur-containing part of the molecule, but correspond to the reduction of the carboxylic acid protons to form H_2 and carboxylate anions. This assignment is confirmed by the electron stoichiometry and by the fact that these cathodic peaks vanish in the presence of 1 mol of hydroxide ion per mol of lipoic acid. Other than the difference in potential of the carboxylic acid proton reduction peaks, the cyclic voltammograms for oxidized and reduced lipoic acid are virtually identical, and similar to those of reduced propanedithiol. This implies that after reduction of its carboxylic acid proton, oxidized lipoic acid is converted to the reduced form. This can be rationalized on the basis of the reactivity of electrochemically generated hydrogen toward oxidized propanedithiol. When 1 mol of hydrogen atoms per mol of oxidized lipoic acid is generated by the reduction at −1.38

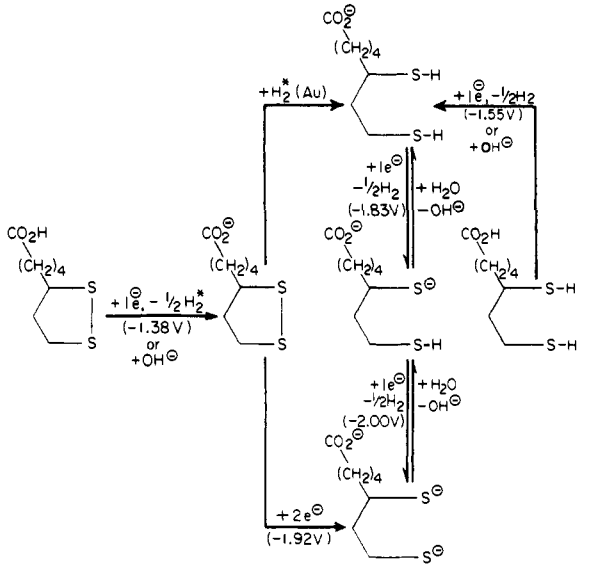


Figure 5. Redox mechanism for DL-α-lipoic acid in acetonitrile solution at a gold electrode.

V, it chemically reduces one-half of the oxidized lipoic acid. More hydrogen is generated by reduction at −1.83 V of the newly formed sulfhydryl protons, and this hydrogen in turn further reduces the remaining oxidized lipoic acid. The potentials for the reduction of the sulfhydryl protons of reduced lipoic acid (−1.83 and −2.00 V) are more negative than those for propanedithiol (−1.53 and −1.81 V) because of the negative charge from the deprotonated carboxylic acid group of the lipoic acid.

If the carboxylic acid proton of oxidized lipoic acid is removed by the addition of 1 equiv of base, the disulfide linkage can be electrochemically reduced at −1.92 V. The potential is again more negative than that observed for TMDS because of the overall negative charge of the molecule.

The anodic peaks at +1.17 and +1.18 V for oxidized and reduced lipoic acid correspond to the same process that is observed for oxidized and reduced propanedithiol at +1.13 and +1.10 V. When base is present in solutions of oxidized or reduced lipoic acid, interpretation of the anodic processes becomes impossible. In addition to the "superoxidation" of the disulfide linkage, oxidation of the carboxylate anions[20] and of hydroxide ion[16,17] can occur as competing parallel processes.

Reduced lipoic acid can be oxidized at +0.20 V to form the disulfide. The electron stoichiometry has been confirmed by controlled potential electrolysis, and the identity of the oxidation product has been confirmed by its UV spectrum. The oxidation may proceed by a one-electron mechanism (reactions 6 and 7).

The initial cathodic peaks in water for both oxidized and reduced lipoic acid are due to reduction of the carboxylic acid protons and decrease in intensity in the presence of 1 equiv of hydroxide ion. Controlled potential coulometry has confirmed that these are one electron per molecule reductions. Hence, electrochemical reduction of the disulfide linkage is not possible in water at a gold electrode.

Both oxidized and reduced lipoic acid in H_2O are oxidized at +0.84 V. This process is analogous to the oxidations in acetonitrile solution at +1.17 and +1.18 V to form some "superoxidized" form of the molecule.

Biological Considerations. The electrochemical studies of lipoic acid in acetonitrile and aqueous solutions indicate that electrochemical reduction of the disulfide linkage in oxidized lipoic acid is extremely difficult. The process occurs at −1.92 V in acetonitrile and does not occur at all in water. We suspect that the difficulty arises because oxidized lipoic acid does not undergo a one-electron reduction to form a stable radical species. Furthermore, the reduction in acetonitrile may not be a two-electron transfer at all, but rather involve the concomitant reduction of trace water to form hydrogen atoms on the gold surface, which then chemically reduce the oxidized species in much the same way as electrochemically reduced protons reduce oxidized lipoic acid.

The fact that activated hydrogen readily reduces oxidized lipoic acid confirms that the reduction is not difficult thermodynamically; the difficulty arises from mechanistic considerations. Oxidized lipoic acid probably cannot be reduced at an electrode surface by successive one-electron transfers. Singly reduced methyl viologen (a one-electron reducing agent) does not react with oxidized lipoic acid in acetonitrile solution, even though it is a strong enough reducing agent (−0.45 V) to reduce protons to hydrogen gas (which does reduce lipoic acid). Although thermodynamically possible, the reaction does not occur because lipoic acid cannot be reduced by two successive one-electron transfers.

These results indicate that oxidized lipoic acid is reduced by an atom transfer mechanism. The reaction of reduced lipoic acid with riboflavin probably also occurs by an atom transfer mechanism. However, reduced lipoic acid can be oxidized by one-electron transfer processes (reactions 6 and 7).

The reported ease of electrochemical reduction of oxidized lipoic acid in water at a dropping mercury electrode[4] is in sharp contrast to the present negative results for a gold electrode (H_2O is more easily reduced than lipoic acid). The reaction at a mercury electrode probably is not a simple electron transfer, but must involve formation of mercury complexes of the type observed for other alkyl disulfides.

The results support the general hypothesis that one-electron oxidations and reductions can occur by electron transfer, but multielectron redox reactions only occur via pure electron transfer if the process involves successive one-electron steps.[21] Successive electron transfer requires the formation of relatively stable radical intermediates. In cases where such intermediates are of high energy, redox reactions will not occur via pure electron transfer, but rather by atom transfer.

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