First Determination of the Standard Potential for the Dissociative Reduction of the Antimalarial Agent Artemisinin

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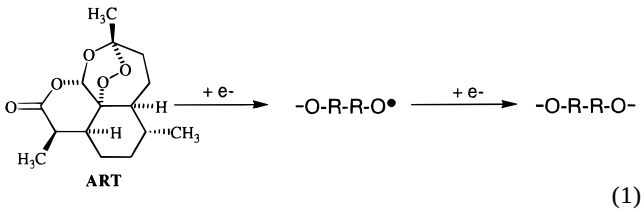
The reduction of the known antimalarial agent Artemisinin (**ART**) has been studied in *N*,*N*-dimethylformamide (DMF) by cyclic voltammetry and other electrochemical techniques. **ART** undergoes an irreversible, dissociative reduction with an anodic peak potential (E_p) that varies with scan rate and is −1.68 V vs SCE at 1 V/s. Direct electrochemical reduction of **ART** is subject to a large activation overpotential, and thus the E_p's do not provide an accurate value of the standard reduction potential ($E°_{diss(\mathbf{ART})}$) required for the determination of the free energy of electron transfer from possible biological electron donors ($\Delta G°_{ET} = F(E°_{D^{\bullet+}/D} - E°_{diss(\mathbf{ART})})$). Using careful heterogeneous electrochemical methods with convolution analysis, the standard potential of the dissociative reduction of **ART** ($E°_{diss(\mathbf{ART})}$) has been determined for the first time to be −0.89 V versus SCE in DMF. This value is ca. 0.8 V more positive than the irreversible direct reduction observed using cyclic voltammetric measurements. In addition, the value $E°_{diss(\mathbf{ART})}$ allows the estimation of the O−O bond dissociation energy. The thermochemical values determined are important to understanding **ART**'s biological activity and investigating its potential for undergoing electron-transfer-initiated processes with biological donors.

Introduction

Artemisinin (**ART**) and its derivatives are a relatively new and potent class of antimalarial agents that possess an endoperoxide function as the critical structural component in their activity.[1,2] While the mechanism of action of these drugs is not completely understood, there is growing evidence supporting the idea that the initial key step is the reductive cleavage of the O−O bond of the endoperoxide moiety, presumably by hemin, leading to oxygen- and then carbon-centered radicals that subsequently lead to the biologically relevant damage to the malarial parasite.[3−5] A few recent reports have described that the electrochemical reduction of **ART** and some derivatives leads to irreversible, heterolytic cleavage of the O−O bond.[6−8] A few have further shown that this cleavage can be catalyzed by hemin as the mediator, supporting the role of ET in its activity.[6,8] Our own work on the reduction of other endoperoxides suggests that the reduction of the O−O bond in **ART** is dissociative.[9] In such cases the measured reduction potentials from cyclic voltammetry (E_p's) are not an accurate measure of the true standard potential, since the direct reduction is subject to a large overpotential owing to slow heterogeneous electron transfer.[9−12]



ART (1)

While peak potentials may provide a qualitative estimate of the ease of reduction relative to similar systems, it is the standard reduction potential that is critical in the evaluation of electron-transfer kinetics with possible donors. In this paper we describe the use of electrochemical methods to study the heterogeneous electron-transfer kinetics to **ART**. Our analysis of the data provides the *first* determination of the standard potential for the dissociative reduction of **ART** ($E°_{diss(\mathbf{ART})}$), as outlined in step one in eq 1, and will allow for an accurate evaluation of the energetics of electron transfer from possible biological donors. In addition, our methods provide estimates of other thermochemical parameters, such as bond dissociation energies, that are not well-established and are critical to understanding the antimalarial activity of these endoperoxides.

Results and Discussion

The reduction of **ART** was studied by cyclic voltammetry in dimethylformamide (DMF) containing 0.1M tetraethylammonium perchlorate (TEAP) at 25 °C at a glassy carbon electrode.[13] Characteristic voltammetric behavior of a dissociative reduction[14,15] (where electron uptake and bond fragmentation are concerted) were observed: (a) its reduction appears as a single, broad, irreversible peak at all scan rates, (b) the peak widths, $\Delta E_{p/2}$, increase with increasing scan rate (e.g., 182, 200, and 212 mV at 0.5, 2, and 20 V/s, respectively), and (c) the peak potential E_p, which is −1.68 V versus SCE at 1 V/s, shifts to more negative values as a function of scan rate (ν) by an average of 51 mV/ln ν. A representative normalized cyclic voltammogram for the reduction of 2 mM **ART** in DMF/0.1M TEAP measured at 1 V/s is shown in Figure 1; the voltammogram corresponds to a two-electron reduction,[16a] presumably by initial dissociative O−O bond fragmentation followed by reduction of an incipient reducable species (eq 1).[16b] The results indicate that the heterogeneous reduction of **ART** cannot be described by Butler−Volmer kinetics but suggest that the transfer coefficient, α, varies with respect to potential. The transfer coefficient (or symmetry factor) is defined as $\partial\Delta G^{\ddagger}/\partial\Delta G°$, where

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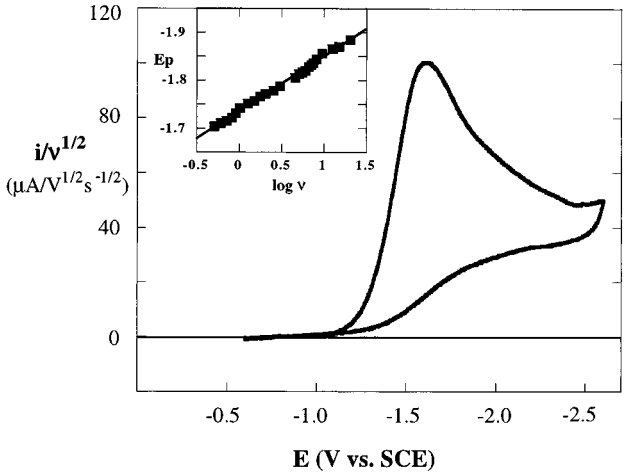

Figure 1. Cyclic voltammogram (normalized for scan rate) for the reduction of 2 mM **ART** in DMF/0.1M TEAP at a glassy carbon electrode at 25 °C at $\nu = 1$ V/s. Inset: Scan rate dependence of the E_p for the reduction of 2 mM **ART** in 0.1M TEAP/DMF at a glassy carbon electrode.

$\Delta G^{\ddagger}$ is the free energy of activation and $\Delta G°$ is the free energy of the ET. Values of α, estimated from the $\Delta E_{p/2}$ values according to the equation $\alpha = 1.857(RT/F)\Delta E_{p/2}$,[17] vary from 0.26 at 0.5V/s to 0.21 at 40 V/s, and its average value estimated from the E_p versus ln(ν) data (vide supra) is 0.255. Interestingly, the reduction was particularly sensitive to extraneous oxygen, and special precautions had to be taken to exclude O_2 from solution.[18]

In this study a convolution analysis approach was used to study the heterogeneous electron-transfer kinetics (k_{het}) of this dissociative reduction.[19] This method has recently been used successfully to study the reduction of a number of acyclic peroxides to provide valuable thermochemical information.[9−12] Convolution of background-subtracted voltammetric curves measured at scan rates between 0.5 and 20 V/s yield limiting current value, I_{lim}, that were independent of scan rate and correspond to two electrons, consistent with the two-step mechanism of cleavage in eq 1.[20] For a totally irreversible process, as is the case for the reduction of **ART**, and at constant I_{lim}, the limiting current can be related to the rate constant for the heterogeneous electron transfer k_{het} using eq 2, where D is the diffusion coefficient, calculated to be 4.1×10^{-10} m^2 s^{-1}.[21]

$$\ln k_{het} = \ln D^{1/2} - \ln \frac{I_t - I(t)}{I(t)} \qquad (2)$$

The resulting log (k_{het}) plot obtained by convolution analysis of 20 sets of data at 1−2 mV resolution is shown in Figure 2a. The plot illustrates the parabolic relationship between log k_{HET} and the driving force (E) expected from theories of ET.[22] As a result of the observed activation-driving force relationship, the data in Figure 2 can be related to the apparent transfer coefficient α_{app} using eq 3. Values for α_{app} are obtained by derivatization of the curve in Figure 2a by linear regression of

$$\alpha_{app} = -\frac{RT\ \mathrm{d} \ln k}{F\ \mathrm{d}E} \qquad (3)$$

the experimental data with small potential intervals (21 mV).[23] The resulting plot, which contains over 400 α values, is shown in Figure 2b, and a linear regression analysis of this data gives $\alpha_{app} = 0.781 + 0.342E$. A similar value for α_{app} can be obtained by fitting the log k data to a quadratic equation. By definition,

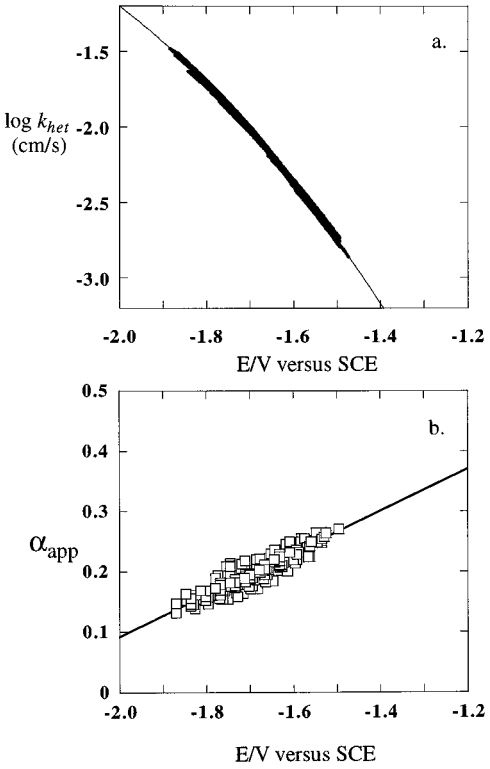

Figure 2. (a) Potential dependence of the log k_{het} for the reduction of **ART** at 25 °C. Plot is a composite of three separate experiments where the scan rate was varied between 0.5 and 40 V/s. (b) Potential dependence of the apparent transfer coefficient, α_{app}. Line is the best-fit linear regression through more than 400 data points.

a value of $E = E°_{diss(\mathbf{ART})}$ when $\alpha = 0.5$.[14] Setting α_{app} equal to 0.5 in the above expression leads to a value of $E°_{diss(\mathbf{ART})} = -0.82$ V versus SCE. Strictly speaking, the standard potential is found when the true transfer coefficient, α, is 0.5; the latter is related to α_{app}, through the double-layer correction.[24] To the best of our knowledge, no accurate determination of the double-layer potential has been determined for glassy carbon, but it has been estimated that the error in the $E°$ is smaller than the double-layer correction on Hg, estimated to be −0.07 V at this potential; this estimate is based on comparisons of data obtained for the reduction of acyclic peroxides using glassy carbon electrode with those obtained on a Hg electrode where the double-layer correction is more well-defined.[11,25] If we use this estimate for the double-layer correction, then the $E°_{diss(\mathbf{ART})}$ becomes −0.89 V vs SCE; we feel that this provides a reasonable estimate of the double-layer-corrected $E°$. In particular, any associated error is small relative to the error that would be introduced if the E_p was used as an estimate for $E°_{diss(\mathbf{ART})}$ for thermochemical estimates for the efficacy of ET to suitable donors ($\Delta G°_{ET} = F(E°_{D^{\bullet+}/D} - E°_{diss(\mathbf{ART})})$). The standard reduction potential determined for **ART** is ca. 0.8 V *more positive* than that observed for the direct reduction. Our results serve to exemplify the problem of using direct reduction potentials for thermochemical calculations; if the direct reduction peak potential is used as an estimate of its electron-accepting abilities, this difference would represent *at least* 16 kcal/mol in discrepancy.

The slope of the regression analysis of α_{app} vs E is related to the heterogeneous intrinsic free energy barrier, $\Delta G^{\ddagger}_0$, by eq 4 and leads to a value for $\Delta G^{\ddagger}_0 = 8.43$ kcal/mol for the reduction

$$\partial\alpha/\partial E = F/8\Delta G^{\ddagger}_0 \qquad (4)$$

$$\Delta G^{\ddagger}_0 = (\lambda_0 + \mathrm{BDE})/4 \qquad (5)$$

of **ART**. Using Savéant's theory for dissociative ET,[14,22] the intrinsic barrier is related to the reorganization energy (λ_0) and the bond dissociation energy (BDE) of the bond being fragmented, in our case the O−O bond, by eq 5. If we take as our estimate for a value of λ_0 the average value of those calculated and determined recently for a series of dialkyl peroxides using the Marcus approach (10.9 kcal/mol),[11] the estimated BDE for homolysis of the O−O bond in **ART** is ca. 23−24 kcal/mol. This value is significantly lower than the BDE in acyclic endoperoxides (ca. 37 kcal/mol).[10] While the λ_0 value for this dissociative reduction of **ART** leading to a distonic radical anion may not be the same when reduction leads to two fragments as it does for the acyclic peroxide, we believe the value used represents an upper limit. To test the validity of our BDE obtained using Savéant's approach and the approximation noted, we wished to compare the value determined with that calculated using thermochemical cycles and literature data. However, in the case of **ART** no thermochemical information is currently available, and thus this approach is not viable. One would expect that the BDE for these strained endoperoxides, in which the lone pairs on the oxygen are eclipsed, should be lower than the acyclic peroxides. Preliminary estimates of the BDE based on eq 6 and using heats of formation (ΔH_f) for model endoperoxides estimated from ab initio energies[26,27] are in the range of 24−28 kcal/mol and give support for our electrochemical determination of $E^\circ_{\mathrm{diss}(\mathbf{ART})}$ and the corresponding estimated BDE.

$$\mathrm{BDE} = \Delta H_f(^{\bullet}\mathrm{O{-}R{-}R{-}O}^{\bullet}) - \Delta H_f(\overline{\mathrm{R{-}O{-}O{-}R}}) \quad (6)$$

The determination of E°_{diss} for **ART** allows for a more accurate evaluation of the driving force for ET to **ART** from possible biological donors. In addition, our electrochemical studies in combination with Savéant's approach provide a route to obtain other thermochemical data unavailable previously. This information may be critical in delineating the modes of action of this trioxane against malarial parasites. We are currently using the methodology outlined above to study the thermochemical properties of other antimalarial active derivatives of **ART**, and the results will be reported shortly.

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(16) (a) Bulk electrolyses were carried out under constant potential conditions in a divided cell at both Hg and carbon cathodes, both in the presence and absence of a non-nucleophilic acid. (b) The first electron in the mechanism forms the distonic radical anion on dissociative reduction. The second electron can be due to reduction of $-\mathrm{O{-}R{-}R{-}O}^{\bullet}$ or another easily reduced species formed by reaction of the initially formed distonic radical anion.

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(21) Calculated from the value of the limiting current and the equation $I_l = nFAD^{1/2}C^*$, where n equals the number of electrons, C^* is the bulk concentration of the substrate, and using an electrode of known area (A). This value agrees with a value recently reported: Chen, Y.; Zhu, S. M.; Chen, H. Y.; Li, Y. *Acta Chim. Sin.* **1997**, *55*, 921−925.

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(24) The true α is related to α_{app} by the equation $\alpha = \alpha_{\mathrm{app}}/(1 - \partial\phi^{\ddagger}/\partial E)$, where $\phi^{\ddagger}$ is the difference between the potential of the bulk solution and the potential at the average distance at which **ART** is located when ET occurs.

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(26) Estimates of the bond dissociation energy in these endoperoxides using eq 6 require estimates of the heats of formation (ΔH_f) of the endoperoxide and the biradical formed on homolytic cleavage of the O−O bond. Values for the latter were estimated using Benson's rules.[27a,b] However, the ΔH_f values for the former are not available and cannot be estimated in the usual way.[27b] We have estimated the ΔH_f's of a number of model endoperoxides from ab initio energies following a group contribution procedure analogous to that of Schleyer and Ibrahim,[27c] with the following changes: (a) energies were calculated at the B3LYP/6-31G*//RHF/6-31G** level; (b) groups were defined following Benson. The method was checked by determining the ΔH_f's of acyclic peroxides (e.g., di-*tert*-butyl peroxide, $\Delta H_f = -79.8$ kcal/mol), where thermochemical data is available.[10] In these cases values agreed within 1 kcal/mol as did the BDE determined using the calculated ΔH_f. Model endoperoxides used and the ΔH_f's calculated: 2,3-dioxabicyclo[2.2.2] octane, $\Delta H_f = -27.15$ kcal mol^{-1}; 1,4-dimethyl-2,3-dioxabicyclo[2.2.2] octane, $\Delta H_f = -48.72$ kcal mol^{-1}; 2,3-dioxabicyclo[3.2.2] nonane, $\Delta H_f = -30.99$ kcal mol^{-1}; 1,4-dimethyl-2,3-dioxabicyclo[3.2.2]nonane, $\Delta H_f = -50.53$ kcal mol^{-1}. Full details of these calculations will be reported at a later date (Guthrie, J. P.; Workentin, M. S., work in progress).

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