Calculation of the Cytochrome P-450 Redox Potential from Kinetic Data by the Marcus Treatment; Feasible or Not?
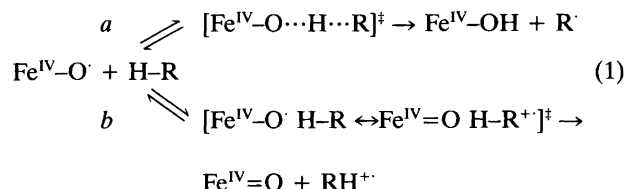
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Using calculations based on the Marcus theory (or linear approximations thereof) and existing rate constant/oxidation potential data, an attempt has been made to estimate the effective standard potential and reorganization energy of the P-450/(P-450)$^-$ couple. Due to the limited data basis, the results only qualitatively indicate that P-450 either must possess an extremely high $E^{\circ\prime}_{Fe(V)/Fe(IV)}$ value (>2.5 V!) or not act as an electron transfer oxidant at all. By similar calculations, it was found that model alkene epoxidation reactions do not follow an ET mechanism.
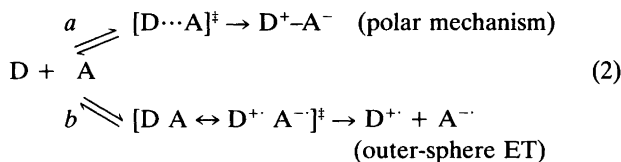
The oxidative reactivity of the cytochrome P-450 family of enzymes is discussed[1–3] in terms of either hydrogen atom [a in eqn. (1)] or electron [b in eqn. (1)] transfer to the perferryl oxygen center, written as Fe^{IV}–O· or Fe^V=O. In the former case, the oxenoid species has been compared to an iron(IV) substituted hydroxyl radical.[4] In the latter, it is inferred that a neutral substrate molecule is transformed into a radical cation[5,6] of finite life-time, capable of under-

$$
\begin{aligned}
&a \quad\quad\quad [Fe^{IV}\text{–}O\cdots H\cdots R]^\ddagger \to Fe^{IV}\text{–}OH + R^\cdot \\
Fe^{IV}\text{–}O^\cdot + H\text{–}R&\\
&b \quad\quad [Fe^{IV}\text{–}O^\cdot\ H\text{–}R \leftrightarrow Fe^{IV}\text{=}O\ H\text{–}R^{+\cdot}]^\ddagger \to
\end{aligned}
$$
(1)
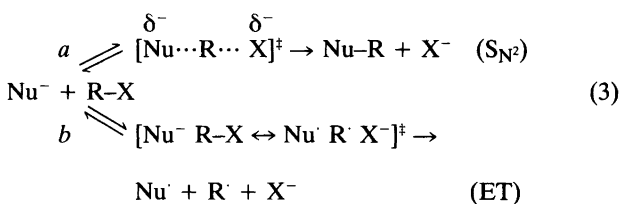
$$
Fe^{IV}\text{=}O + RH^{+\cdot}
$$

going the characteristic reactions of the system at hand, e.g., ring-opening of heteroatom-substituted cyclopropane radical cations with subsequent suicidal inactivation of the enzyme,[7–9] rearrangement of the quadricyclane radical cation to that of norbornadiene,[10] ring expansion of a cyclobutylamine radical cation,[11] α-proton abstraction from amine radical cations,[12,13] and $S_{ON}2$ substitution (a diagnostically useful mechanism for substitution of fluorine in F-containing aromatic radical cations[14]) of fluorine against hydroxyl in 6-fluorobenzo[a]pyrene.[15]

In recent years, it has been widely recognized[16–19] that an analogous mechanistic dichotomy exists for any bimolecular step that involves a pair of molecules with donor (D)/acceptor (A) complementarity [eqn. (2)]. Depending on the redox reactivity of D and A, a spectrum of mechanisms ranging from outer-sphere electron transfer (ET) to a

genuine polar mechanism can be envisaged, and it becomes a major experimental and interpretational problem to deal with borderline cases. An example of a universally accepted mechanism that has been re-evaluated along these

$$
\begin{aligned}
&a \quad\quad [D\cdots A]^\ddagger \to D^+\text{–}A^- \quad \text{(polar mechanism)} \\
D + A&\\
&b \quad\quad [D\ A \leftrightarrow D^{+\cdot}\ A^{-\cdot}]^\ddagger \to D^{+\cdot} + A^{-\cdot} \\
&\quad\quad\quad\quad\quad\quad\quad\quad \text{(outer-sphere ET)}
\end{aligned}
$$
(2)

lines is the S_N2 mechanism;[20] when the attacking nucleophile is strong enough as a reductant, outer-sphere ET (possibly of the dissociative type) will take place [eqn. (3)].

$$
\begin{aligned}
&a \quad\quad \overset{\delta^-}{[Nu}\cdots R\cdots \overset{\delta^-}{X]^\ddagger} \to Nu\text{–}R + X^- \quad (S_N2) \\
Nu^- + R\text{–}X&\\
&b \quad\quad [Nu^-\ R\text{–}X \leftrightarrow Nu^\cdot\ R^\cdot\ X^-]^\ddagger \to \\
&\quad\quad\quad\quad Nu^\cdot + R^\cdot + X^- \quad\quad\quad\quad \text{(ET)}
\end{aligned}
$$
(3)

A few important points should be emphasized with regard to eqn. (2) in order to delineate the difference between the ET and polar transition state. In the former, the electronic interaction between D and A *by definition* is very small, normally with the upper limit placed at 1 kcal mol^{-1}, whereas in the purely polar transition state the electronic interaction between D and A is large, as exemplified by the S_N2 transition state.[21] Another consequence of eqn. (2) is that the two radical ions of the ET step might couple to give D^+–A^-. Even more complicated is the case in which the

polar step of eqn. (2) is succeeded by fast dissociation to form $D^{+\cdot}$ and $A^{-\cdot}$; this constitutes an inner-sphere electron transfer process, of which radical cation formation in the reaction between easily oxidizable aromatic hydrocarbons and nitronium ion (NO_2^+) is a possible example.[22] Others have recently been published by Steenken et al.[23,24]

It is clear that the discussion around the oxidation mechanism(s) of P-450 is of similar nature as the examples referred to above, except that one of the reaction partners contains a metal center. For this situation, outer-sphere ET chemistry has been unequivocally demonstrated in several model reactions, involving for example Fe(III),[25,26] Co(III)[27,28] and Ce(IV)[29,30] complexes in reactions with organic molecules of different types. Linear free-energy relationships (LFER) of the $\log(k)/E$ type are often used to show that ET reactivity (k = rate constant) is well correlated with the redox reactivity of the substrates, as expressed in a redox potential, E (E^0 = the standard potential under the prevailing reaction conditions, or, if this is not available, the reversible $[E_{rev}]$, peak $[E_p]$ or half-wave $[E_{1/2}]$ potentials from electrochemical measurements). However, such correlations have their drawbacks. Generally, oxidative and electrophilic reactivities tend to run in parallel, so that in any particular case a $\log(k)/\sigma^+$ might look equally good or even better. In order to distinguish between such cases, a deeper analysis is necessary, based upon the fact that a $\log(k)/E^0$ relationship, if treated as a linear one, should be not only statistically well behaved but also have a unique value of its slope, depending in the first approximation upon the difference in redox potentials between the participating species. Alternatively, one can treat $\log(k)/E^0$ data by a quadratic (parabolic) equation, which forms the basis of the Marcus theory[31] of outer-sphere electron transfer.[32-34]

As a prelude to the discussion of the Marcus treatment, one should note that several studies have related P-450 reactivity to the redox potentials of series of substrates by LFERs. As an example, the oxidation of sulfides and sulfoxides by P-450 generated $\log(V_{max})/E_p$ LFERs with slopes $= -0.35$ and -0.64 eV^{-1}, respectively,[35,36] which numerically are very *low* in the context of the Marcus theory. In a preliminary study[34] it was noted that one way to reconcile these values with the Marcus treatment is to assign a very high, perhaps unrealistically so, redox potential to P-450, >2 V vs. NHE (normal hydrogen electrode; all potentials given here are given vs. NHE). Recently, the same conclusion was reached by an analysis of rate data for the oxidation of substituted N,N-dimethylanilines by P-450[37] according to the Rehm-Weller and Agmon–Levine equations (hyperbolic expressions that are sometimes used in place of the Marcus equation; see below). These estimates placed the 'apparent $E_{1/2}$' for the oxidized enzyme in the range of 1.9–2.2 V.[37]

It is the purpose of this paper to analyze available $\log(k)/E$ data in terms of the Marcus theory in order to test whether it can be applied and if so, which physically realistic situations are feasible to account for the observed

reactivities. For an organic chemist, the qualitative experimental evidence in favor of an ET mechanism referred to above[7-15] would seem to be quite compelling, even though many of the substrates are very difficult to oxidize (like most of the cyclopropanes of Ref. 9, with $E_{1/2}$ values in the range 2.5–3.5 V) and thus require that E^0_{P-450} be extremely high.

The Marcus theory

This theory and its application to outer-sphere ET reactions has been described in detail for inorganic,[38] organic[18,33,34] and biological[32] systems. Therefore, it will only be necessary to reiterate features of particular relevance for the discussion to follow.[39] The model of the ET process is simple: the two redox species are viewed as spheres of charge Z_1 and Z_2 and radii r_1 and r_2 [subscripts 1 and 2 refer to the oxidant and reductant, A and D of eqn. (2), respectively], embedded in a continuous medium of dielectric constant D. The spheres collide and form a collision complex with distance $r_{12} = r_1 + r_2$ between the spheres. For the outer-sphere ET case, the electronic interaction between D and A in the transition state is by definition put at <1 kcal mol^{-1}.

In order to transfer the electron from D to A, the energy of (D A) must be raised until it reaches a level of the same energy as a similarly excited $(D^{+\cdot}A^{-\cdot})$ pair (Fig. 1). At this point the transition state for ET has been reached, the electron can jump from D to A and the system descends along the $(D^{+\cdot}A^{-\cdot})$ configuration curve. This process is symbolized by the ET transition states of eqns. (2a), (2a) and (3a) which are resonance hybrids of vibrationally excited states. The energy required to reach the TS along the (DA) and $(D^{+\cdot}A^{-\cdot})$ configuration curves is denoted *reorganization energy* (symbolized by λ) and stems from changes in bond lengths, bond angles and torsional angles (bond reorganization energy, λ_i after *inner*) and solvation around the TS (solvent reorganization energy, λ_o after *outer*). These energy changes are classical in nature, in keeping with the requirement that the electronic interaction in the TS should be negligible. The total reorganization energy, λ, is then equal to ($\lambda_i + \lambda_o$).
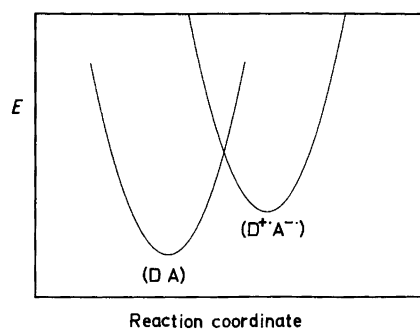

Fig. 1. Potential energy (E) curves for (D A) and $(D^{+\cdot}A^{-\cdot})$ states. The transition state for ET is located at the (forbidden) crossing between the two curves.

In any situation where there is a choice, the outer-sphere pathway is the least favorable one. A polar transition state always has a significant electronic interaction between the reactants, which lowers its energy with respect to that of the outer-sphere ET transition state. Therefore, the occurrence of the outer-sphere mechanism often is dependent upon prevention of the polar pathway, e.g., by steric factors.[16] Only when outer-sphere ET is energetically favored, as normally is the case when ΔG^0 of eqn. (2a) is ≤ 10 kcal mol^{-1}, do we frequently encounter reactions mediated by ET steps.

Marcus quantified the spherical model by the parabolic expression of eqn. (4) for $\Delta G^\ddagger$, the free energy of activation of the ET step. In eqn. (4) the first term, denoted by W

$$\Delta G^\ddagger = Z_1 Z_2 e^2 f/(D\ r_{12}) + \lambda(1 + \Delta G^{0'}/\lambda)^2/4 \qquad (4)$$

$$\Delta G^{0'} = \Delta G^0 + (Z_1 - Z_2 - 1)e^2 f/(D\ r_{12}) \qquad (5)$$

in the following, describes the electrostatic free-energy change when the reactants are brought from infinity to distance r_{12}, with e = the electronic charge and f a factor expressing the influence of ionic strength (μ). With r_{12} expressed in Å, the introduction of suitable conversion factors for e^2 in order to give W in kcal mol^{-1} puts W equal to 331.2 $Z_1 Z_2 f/(r_{12}\ D)$. With typical values of $Z_1 Z_2 = 1, f =$ 1 ($\mu = 0$), $D = 37$ (acetonitrile) and $r_{12} = 6$ Å, W comes out at 1.5 kcal mol^{-1}. With an inert salt present so as to make f smaller, the W term often becomes negligibly small. Electrostatic terms usually become important only for highly charged ions and/or in low-D environments, such as dichloromethane and acetic acid ($D = 11$ and 6, respectively). For most organic ET reactions, one or both reactants is neutral, in which case $W = 0$.

The second term of eqn. (4) is the non-trivial one, giving the activation-controlled part of $\Delta G^\ddagger$ as a quadratic function of λ and $\Delta G^{0'}$. The latter quantity is in turn given by eqn. (5), in which an electrostatic correction term is applied to the standard free-energy change of the ET step. This term expresses the electrostatic consequence of the change
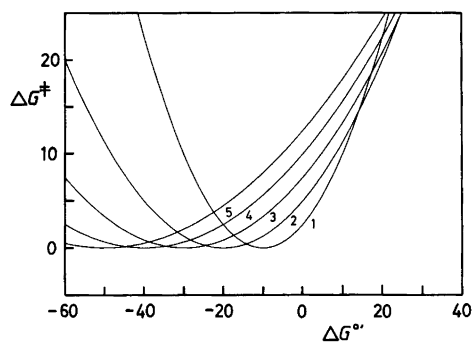
in $Z_1 Z_2$ upon ET, as for example from $(0) \times (-3) = 0$ to $(+1) \times (-4) = -4$ in the ET step between $Fe^{III}(CN)_6^{3-}$ and a neutral organic substrate RH to give $Fe^{II}(CN)_6^{4-}$ and $RH^{+\cdot}$. This term only becomes equal to zero when $Z_1 = Z_2 + 1$, and is therefore likely to be of significance more often than W.

If W is put equal to zero, a plot of $\Delta G^\ddagger$ vs. $\Delta G^{0'}$ at constant λ is a parabola with its vertex at $-\lambda/0$ (Fig. 2). For larger values of λ, the parabola becomes flatter and the vertex moves in the exergonic direction. In a sufficiently narrow range of $\Delta G^{0'}$, the parabola can be approximated as a straight line with a slope equal to that of the tangent to the parabola at the midpoint of the $\Delta G^{0'}$ range. The slope of the tangent in any point c is given by 0.5 $[1 + \Delta G^{0'}(c)/\lambda]$ which varies between 0 and 1 in the interval of $-\lambda \leq \Delta G^{0'} \leq \lambda$.

We now see that linear $\Delta G^\ddagger/\Delta G^{0'}$ or, which is the same apart from some constant factor, $\Delta G^\ddagger/E^{0'}$ or $\log(k)/E^0$ plots of ET reactions present problems which are not normally taken into account for ordinary LFERs. Since kinetic studies are often performed for reaction series of limited reactivity range, such plots should be linear as a good approximation, provided that all reactions of the series have the same λ value. However, the slope of the correlation must also fit with the value expected in the particular range of $\Delta G^{0'}$ involved.[33] Numerically small slopes are expected near the vertex of the parabola, whereas high values should be found in the endergonic region.

Before we continue, the meaning of λ needs to be expanded upon. As seen from Fig. 2, a higher λ in most cases leads to a higher $\Delta G^\ddagger$ at a given value of $\Delta G^{0'}$ and hence to lower redox reactivity. What, then, does λ signify and how can one get a grasp of this parameter?

It can be shown that λ of any hetero-exchange reaction between D and A can be related to those of the corresponding homo-exchange reactions between D and $D^{+\cdot}$ and A and $A^{-\cdot}$, respectively, according to eqn. (6). The relationship is not exact but is sufficiently accurate for most

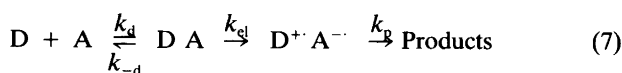$$\lambda_{D/A} = (\lambda_{D/D^+} + \lambda_{A/A^-})/2 \qquad (6)$$

purposes. The strength of this approximation lies in the fact that λ of homoexchange reactions, generally denoted $\lambda(0)$, can be experimentally determined $[\lambda(0) = 4 \times \Delta G^\ddagger(0)$, where $\Delta G^\ddagger(0)$ is the activation free energy of the homoexchange reaction which can be measured by, e.g., ESR or NMR spectroscopic line exchange broadening or isotope methods] or calculated by simple mathematical expressions. With a list of n $\lambda(0)$ values of ET reagents, one immediately has access to n^2 λ values of all the possible combinations of heteroexchange processes.[18,33] In general, small $\lambda(0)$ values are associated with good charge delocalization possibilities, small conformational and/or other changes associated with bond angles, weak solvation and/or ion-pairing. In contrast, a high $\lambda(0)$ is dependent upon high degree of charge localization, extensive conformational

Fig. 2. Plots of $\Delta G^\ddagger$/kcal mol^{-1} vs. $\Delta G^{0'}$/kcal mol^{-1} for $\lambda = 10$ (1), 20 (2), 30 (3), 40 (4) and 50 (5) kcal mol^{-1} according to eqn. (4) ($W = 0$).

change and/or bond angle deformation, strong solvation and/or ion pairing.

An example of a low $\lambda(0)$ value is that of the $\mathrm{ArH^+/ArH}$ couple (where ArH denotes an unsubstituted aromatic compound), ca. 10 kcal mol^{-1}. In this system the bond length/angle changes necessary to reach the transition state can be accommodated as many minute ones over the whole aromatic system and thus λ_i is almost negligible. Since the solvent (acetonitrile) is weakly solvating, λ_0 is small and the total sum small. For an alkyl-substituted aromatic system, i.e., $\mathrm{ArCH_3^+/ArCH_3}$, a considerably higher $\lambda(0)$, 40–50 kcal mol^{-1}, has been found due to the localization of charge into the α-C-H bonds of the radical cation.[26,40,41] High values are also found for non-aromatic couples, such as $\mathrm{R_4Sn^+/R_4Sn}$, where charge localization into the Sn-C bonds leads to significant changes in geometry upon ET.[25] Ultimately high $\lambda(0)$ are found for systems in which one bond breaks in the ET event [dissociative ET, exemplified by the ET reduction of alkyl halides shown in eqn. (3b)]; with this concept the use of the Marcus theory is extended outside the domain originally defined, but nevertheless it seems to work in a consistent manner.[18,42]

Kinetic models

Before one can apply the Marcus theory to experimental rate constants (k_{obs}), it is necessary to establish a realistic kinetic model for the reaction at hand. Cusanovich et al.[43] have used the simple model of eqn. (7) for reactions of

$$\mathrm{D + A} \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} \mathrm{D A} \overset{k_{el}}{\rightarrow} \mathrm{D^+A^-} \overset{k_p}{\rightarrow} \text{Products} \qquad (7)$$

redox proteins where the ET step is assumed to be rate-determining. Here D and A diffuse together with rate constant k_d (reverse rate constant k_{-d}) to give an encounter complex which undergoes *irreversible* intramolecular ET

$$\log k_{obs} = \log(v_{ET}) - \lambda(1 + \Delta G^{0\prime}/\lambda)^2/(4\cdot2.303RT) \qquad (8)$$

$$\log k_{obs} = \log(k_d) - $$
$$\log\{1 + k_{-d}/Z \exp[\lambda(1 + \Delta G^{0\prime}/\lambda)^2/(4\cdot2.303RT)]\} \qquad (8a)$$

with rate constant k_{el} to give $(\mathrm{D^+A^-})$. The latter in its turn is assumed to be irreversibly converted into products. After the simplifying approximation that $k_d \gg k_{el}$ in the interval studied, this treatment eventually led to eqn. (8) to which k_{obs} were fitted by a non-linear regression method, with $\log(v_{ET})$ and λ as disposable parameters. The parameter v_{ET} is equal to ZK_d, where $K_d = k_d/k_{-d}$ and $Z = kT/h = 6\times10^{12}$ s^{-1} at room temperature. Without the approximation that $k_d \gg k_{el}$, the slightly more complicated expression of eqn. (8a) results. Eqn. (8) worked well for a number of reactions[43] which were exergonic enough to fill the requirement of irreversibility in the k_{el} and k_p steps (Table 1).

Given that the requirements (irreversible ET, $k_d \gg k_{el}$) above are met, eqn. (8) can be used with advantage to correlate second-order rate parameters with $\Delta G^{0\prime}$. It can also be used for first-order rate parameters, whereby v_{ET} of course has the dimension time^{-1}. In any case where it is suspected that the ET rate constants k_{el} are close to k_d (which for an enzyme summarizes all rate processes leading up to the rate-determining ET step[37]), the full expression of eqn. (8a) should be used.

If the ET step is not irreversible, the influence of k_{-el} has to be taken into account. This problem has been treated by Rehm and Weller[44] and gives slightly more complicated $\log(k_{obs})/\Delta G^{0\prime}$ expressions than those of eqns. (8) and (8a).[33] In the context of P-450 oxidation, this complication need not be taken into account, as was checked for all calculations reported in this paper.

We have already indicated that for P-450 oxidation, the experimental region is close to the vertex region, i.e., k_{el} is approximately of the same magnitude as k_d. Thus the approximative eqn. (8) should be avoided and eqn. (8a) used. The essential difference is that the resulting $\log(k_{obs})/\Delta G^{0\prime}$ curves become truncated parabolas because of the limit imposed by the magnitude of k_d.

Fig. 3 shows $\log k_{obs}/\Delta G^{0\prime}$ curves for different values of λ, using eqn. (8) for their construction. Again it should be noted that linear approximations of limited data sets in different regions of $\Delta G^{0\prime}$ will have vastly differing slopes,[33] in $\log(k_{obs})/E^{0\prime}$ terms varying between 0 at the vertex of the parabola and ca. -17 eV^{-1} in the endergonic region at $\Delta G^{0\prime} = \lambda$. Low values are only found near the vertex of the parabola.

Table 1. Examples of $\log(v_{ET})/\lambda$ parameters for reactions between redox proteins and small organic species, as estimated from eqn. (8) by a non-linear regression method.[43]

| Reaction	Range of $\Delta G^{0\prime}$/kcal mol^{-1}	$\log(v_{ET}/\mathrm{M^{-1}s^{-1}})$	λ/kcal mol^{-1}
Cytochromes + 5-deazariboflavin semiquinone	$-(19.6–24)$	3.0×10^9	11.2
HiPIPs + 5-deazariboflavin semiquinone	$-(16.2–18)$	1.0×10^9	14.8
Cytochromes + lumiflavin semiquinone	$-(6–16)$	1.0×10^8	11.2
HiPIPs + lumiflavin semiquinone	$-(6.5–15.7)$	9.6×10^7	14.8
HiPIPs + fully reduced lumiflavin	$-(4.0–12.2)$	9.2×10^6	12.0
Blue copper proteins + lumiflavin semiquinone	$-(9.5–13.9)$	4.4×10^7	14.0
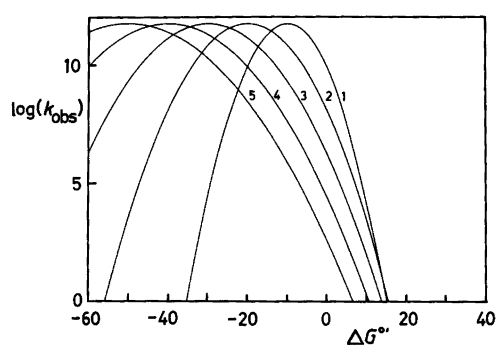
Fig. 3. Plots of $\log(k_{obs})/M^{-1}\,s^{-1}$ vs. $\Delta G^{0\prime}/\text{kcal mol}^{-1}$ for $\lambda = 10$ (1), 20 (2), 30 (3), 40 (4) and 50 (5) kcal mol^{-1} according to eqn. (8).

Results and discussion

Assumptions. The underlying assumption[37] in using the Marcus theory on P-450 reactions is that the measured rate contants reflect the reactivity of the perferryl center in the sense of eqn. (1) and not any of the other reactions in the catalytic cycle, nor any physico-chemical process engaged in bringing the substrate up to the active site of the enzyme or dissociation of products from it.[45] Rate data will be expressed as both first- (k_{cat}) and second-order (k_{cat}/K_m) quantities, if possible. The quantity k_{cat}/K_m is considered to be the best measure of the catalytic power of enzymes.[46]

Linear approximations. As already mentioned, $\log k_{obs}/E^{0\prime}$ data can be treated according to a linear approximation in sufficiently narrow ranges of potential and the slopes used

for certain mechanistic assessments. Table 2 shows the slopes of available linear correlations of this type involving P-450, together with those of two model systems which simulate the P-450 mechanism [epoxidation of alkenes by oxometal(V) species].

Typical of $\log(k_{obs})/E$ correlations involving P-450 are negative slopes close to zero. Such low values are only to be expected near the vertex of the Marcus parabola (Fig. 3), slightly to the endergonic side of it. Since most substrates are oxidized only with difficulty, this immediately tells us that an outer-sphere ET mechanism demands $E_0{'}_{P-450}$ to be very high. The same applies to the two model systems 7 and 8. Their extended potential ranges and small slopes are only compatible with tangents to shallow parabolas, indicative of a very high λ value and consequently a physically unrealistic situation (see below) as far as ET goes.

Calculations according to the Marcus treatment. Already reasoning based upon linear approximations tells us that the available experimental region lies close to the plateau region of the Marcus parabola. This means that attempts to fit $\log k_{obs}/\Delta G^{0\prime}$ data to the equations discussed above will give parameters of low accuracy only. In particular, when it is necessary to use three disposable parameters [$\log(v_{ET})$, λ, and $E^{0\prime}$] the values obtained will presumably only be of semiquantitative significance, more indicative of trends than possessing any quantitative reliability. It must also be stressed that the use of $\log(k_{cat}/K_m)$ values for k_{obs} also introduces considerable error due to the difficulties involved in obtaining accurate K_m values. The effect of these sources of error is amply borne out by the calculations reported below and the reader is warned that little significance can be attached to the absolute values of λ and $E^{0\prime}$.

Table 2. Linear correlations of $\log(k)/E_p$ or $E_{1/2}$ data for reactions of P-450 and model systems.

Reaction No. and type (No. of data pairs)	Oxidant (Temp./°C)	Range of E_p or $E_{1/2}$/V	Slope/eV^{-1} (std. dev.)	Rate parameter[a]
1: 4-XC$_6$H$_4$SCH$_3$ to 4-XC$_6$H$_4$SOCH$_3$ (4)[35]	P-450 (ca. 20)	1.50–2.09	−1.1(4) −0.39(6)	A B
2: 4-XC$_6$H$_4$SOCH$_3$ to 4-XC$_6$H$_4$SO$_2$CH$_3$ (4)[35]	P-450 (ca. 20)	1.99–2.29	0(3) −0.63(4)	A B
3: 1 and 2 combined (8)	P-450 (ca. 20)	1.50–2.29	−1.3(7) −1.3(3)	A B
4: Inactivation by heteroatom substituted cyclopropanes (14)[9]	P-450 (37)	1.66–3.53	−1.2(2)	C
5: Demethylation of N,N-dimethylanilines, NADPH/P-450 reductase supported (9)[37]	P-450 (37)	0.98–1.51	−0.9(6) −1.7(2)	A B
6: Demethylation of N,N-dimethylanilines, iodosylbenzene supported (5)[37]	P-450 (37)	0.98–1.51	0.6(10) −1.2(1)	A B
7: Alkenes, epoxidation (13)[47]	Fe^V=O^b (20?)	0.75–3.0	−1.7(3)	D
8: Alkenes, epoxidation (16)[48]	Cr^V=O^c (30)	1.38–3.04	−3.0(2)	D

[a]A $\log(k_{cat}/K_m)/M^{-1}\,s^{-1}$; B$\log(k_{cat}/\text{min}^{-1})$; C$\log(k_{inact}/\text{min}^{-1})$; D$\log(k_2/M^{-1}\,s^{-1})$. ^{b}Oxo[*meso*-tetrakis(2,6-dichlorophenyl)porphinato]iron(V); E^0[Fe(V)/(IV)] = 1.53 V. ^{c}Oxo[*meso*-tetrakis(2,6-dibromophenyl)porphinato]chromium(V); E^0[Cr(V)/Cr(IV)] = 1.03 V.

In most cases, these values are physically unrealistic and strongly emphasize that P-450 must either have some exceptional features as an ET reagent or not act as such at all.

Before proceeding to the P-450 data, the feasibility of extracting the three desired parameters from eqn. (8) or (8a) was tested on two data sets representing well-established outer-sphere ET processes, namely oxidation of alkylmetals by $Fe(phen)_3^{3+}$ [25,49] and quenching of excited $Cr(bpy)_3^{3+}$ by aromatic amines and methoxybenzenes in acetonitrile,[50] covering different ranges of $\Delta G^{0\prime}$. The first set comprises 22 data pairs of high quality, located in the steep region of the Marcus parabola, relatively remote from its vertex. Even in an ideal case, the curvature would be very slight and with real data presumably never defined with high enough precision to allow fitting to a three-parameter version of eqn. (8) or eqn. (8a). This expectation turned out to be correct, in that $\log(\nu_{ET})$, λ and $E^{0\prime}_{Fe(III)/Fe(II)}$ came out via eqn. (8a) (k_{-d}/Z was put equal to 0.1 in these and other calculations by this equation[33]) at $34(1)^*$ (impossible for a diffusion-controlled process or indeed in any other context), $1.3(2)10^2$ kcal mol^{-1} and $0.4(3)$ V, respectively, to be compared with the authentic values of 10.3, 41 kcal mol^{-1} and 1.22 V.[25] With eqn. (8), convergence was not obtained. On the other hand, with a fixed value of $\log(\nu_{ET}) = 10.3$, λ and $E^{0\prime}_{Fe(III)/Fe(II)}$ were estimated to be 42(5) kcal mol^{-1} and 1.21(8) V from a two-parameter version of eqn. (8a), in excellent agreement with the literature values.[25]

The second test set[50] comprised 16 data pairs situated in or close to the diffusion-controlled region [the vertex of the truncated parabola; $\log(k_{obs})$ varied between 10.1 and 7.3] and was considerably better suited for evaluation of all three parameters. Thus $\log(k_d)$, λ, and $E^{0\prime}_{Cr(III)^*/Cr(II)}$ were calculated to be 10.0(1), 18(5) kcal mol^{-1} and 1.7(1) V, cf. the authentic values of 10.2 and 12 kcal mol^{-1} and 1.7 V.

These results show that three-parameter fits should be attempted with caution and include data covering both the diffusion and activation-controlled regions. As indicated by the linear approximations above, the P-450 rate data appear to be satisfactory from this point of view.

With these reservations in mind, the data sets of Table 2 were used to estimate $\log(\nu_{ET})$, λ and $E^{0\prime}_{P-450}$. The difficulties alluded to above were abundantly demonstrated, as seen from the results given in Table 3. It is for example obvious that the second-order quantity k_{cat}/K_m gives parameters with very large standard deviations and thus is too prone to error for use in this context.

Data set 5, demethylation of N,N-dimethylanilines by P-450 supported by NADPH/P-450 reductase, is the only one to give anything resembling physically realistic values of λ and $E^{0\prime}_{P-450}$, but only when eqn. (8) is used. These compounds are relatively easily oxidized compared with the others and thus are a priori better suited as substrates for ET oxidation. The $E^{0\prime}_{P-450}$ value, 2.6 V, is high and on

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* The number in parentheses represents one standard deviation.

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Table 3. Estimates[a] of $\log(\nu_{ET})$ [or $\log(k_d)$], λ, and $E^{0\prime}_{P-450}$ from $\log k_{obs}/E$ data.

Data set No. (Table 2)	Rate parameter[b] (eqn.)	$\log(\nu_{ET}/M^{-1} s^{-1})$	λ/kcal mol^{-1}	$E^{0\prime}_{P-450}$/V
3	B (8)	2(1)	$1(4)\times10^2$	7(13)
4	C (8)	2(7)	$7(19)\times10^2$	$3(7)\times10^1$
5	A (8a)	3(1)	$1(7)\times10^2$	5(25)
5	B (8)	1.1(1)	$4(1)\times10^1$	2.6(4)
6	A (8a)		No convergence	
6	B (8)	2.4(3)	$7(8)10^1$	4(3)

[a]By a non-linear regression program (Marquardt algorithm) developed for PCs by P.-O. Eriksson, University of Umeå. All results were checked by examination of data plots and comparison of statistics. [b]A, $\log(k_{cat}/K_m/M^{-1} s^{-1})$; B, $\log(k_{cat}/min^{-1})$; C, $\log(k_{inact}/min^{-1})$.

the verge of being physically unrealistic (see discussion below).

Macdonald et al.[37] used data set 5 (k_{cat}) in conjunction with eqn. (8) and noted that 'attempted fittings to the Marcus curve are unsatisfactory' since very high λ and $\Delta E^{0\prime}_{P-450}$ were obtained (70–100 kcal mol^{-1} and 4–5 V, respectively). This statement does not agree with the results reported for data set 5 in Table 3, using the same mathematical expression. Presumably the discrepancy is due to the tendency of calculations of three-parameter fits to produce false minima which have to be excluded by multiple calculations and combined by examination of plots and comparison of statistics.

Seemingly more realistic results were obtained by Macdonald et al.[37] by substituting the parabolic Marcus equation for the hyperbolic Rehm–Weller or Agmon–Levine equation. These are semiempirical expressions, designed to avoid the complication of the so-called 'inverted region' of the Marcus parabola, i.e., that ET rate constants are predicted to decrease in the strongly exergonic region.[18,33] By a three-parameter fit of data set 5 to the Rehm–Weller version of eqn. (8), $\log(\nu_{ET}) = 2.1(1)$, $\lambda = 22(2)$ kcal mol^{-1} and $E^{0\prime}_{P-450} = 1.94(6)$ were obtained; the Agmon–Levine expression gave $\log(\nu_{ET}) = 1.4(1)$, $\lambda = 22(1)$ and $E^{0\prime}_{P-450} = 2.02(4)$ V. The iodosylbenzene supported system (6) gave similar results. Even if the Rehm–Weller and Agmon–Levine equations produce superficially better results than the Marcus equation, there is really no reason to use them in this context. The data sets are located in the range around the vertex of the Marcus parabola, as seen already from linear correlations, and the mathematical expediency of these equations only produces a false indication of correctness and accuracy.

The remaining data sets (3, 4) either gave physically impossible values or no fit at all. It made no change if log (k_{cat}/K_m) values were replaced by at least superficially more well-behaved $\log(V_{max})$ values. The reason for this failure lies in the very high oxidation potentials of these substrate types; for outer–sphere ET to be feasible a very strong

Table 4. Estimates of $\log(k_d)$, λ and $E^{0\prime}$ for the non-P-450 data sets of Table 2.

Data set No. (Table 2)	Rate parameter[a] (eqn.)	$\log(k_d)$	λ/kcal mol^{-1}	$E^{0\prime}$/V
8	RR[b] (8)	$1.4(3) \times 10^2$	$1.9(6) \times 10^4$	$7(2) \times 10^2$
9	A (8a)	1.2(1.1)	159(56)	6(2)
9	A (8)	2.1(1.8)	155(77)	6(2)

[a] A $\log(k_2/M^{-1}\ s^{-1})$; RR relative rate. [b] In this case only eqn. (8) is applicable and '$\log(k_d)$' takes a different meaning.

oxidant is necessary and this requirement then automatically pushes the λ value sky-high. Differently phrased, the absurd parameters retrieved from data sets 3 and 4 strongly point to another mechanism for oxidation, namely hydrogen atom abstraction or, if we prefer the ET notation, a synchronous electron/proton transfer from substrate to the perferryl oxygen.

The remaining, non-P-450 data sets of Table 2 were also used to estimate rate parameters by fitting to eqn. (8a), as shown in Table 4. Data set 7 simulates P-450 epoxidation of alkenes by the system pentafluoroiodosylbenzene/iron(III) tetrakis(2,6-dichlorophenyl)porphyrin chloride.[47,51] This reaction has been claimed to follow an ET mechanism, supported by the good correlation between $\log k$ and $E_{1/2}$ (Table 2). It was already pointed out above that the structure of this data set, with a very wide range of E, is only compatible with a very high λ and $E^{0\prime}$[Fe(III)/(II)]. This is amply demonstrated by the calculation of these parameters reported in Table 4; an ET mechanism does not seem to be feasible. Data set 8 pertains to alkene epoxidation by oxo[*meso*-tetrakis(2,6-dibromophenyl)porphinato]Cr(V), formally a weaker oxidant than the Fe^V=O species (E^0 = 1.03 vs. 1.53 V). From an elaborate analysis of these data, it was concluded that an ET mechanism does not operate for most of the substrates, something that is easily verifiable by an analysis according to eqn. (8) or (8a) (Table 4). The absurd parameters retrieved strongly suggest that ET cannot be involved. Since this is a homogeneous solution reaction (in dichloromethane) $\log(k_d)$ is known to be = 10.4 and thus a two-parameter fit to eqn. (8a) can be used to give even more absurd values of $\lambda = 5.6(6) \times 10^2$ kcal mol^{-1} and $E^0_{Fe(V)/IV)} = 17(2)$ V!

Conclusions

Unfortunately, the analysis above does not bring out much in terms of positive results. One must first note the paucity of good kinetic data for P-450 oxidations, available in a form that lends itself to analysis by the Marcus equation (reactive substrates of similar type, well spread over a not-too-extended potential region, and with easily measurable E^0 or at least E_{rev} values). Data set 5 comes reasonably close to this ideal. Needless to say, more data of the desired characteristics are urgently needed.

Secondly, the P-450 reaction rates are remarkably insensitive to changes in E of the substrate. Interpreted negatively, one might surmise that the effect of E on ET reactivity is masked by reactions preceding the ET step and thus that Marcus calculations cannot be used at all to extract the redox parameters of P-450. Interpreted positively, the numerically small $\log(k_{obs})/E$ slopes in combination with the difficulty in oxidizing the substrates indicate that the $E^{0\prime}_{P-450}$ value must be very high. Data set 5, the only one approaching the ideal sought in this context, suggests a value of 2.6(4) V, by all standards an extreme one, on a par with that of the strongest known oxidants! In the remaining cases, the unrealistic parameter values must reflect the operation of a non-ET mechanism, such as hydrogen atom transfer.

Iron-based redox systems with very high redox potentials are known, so that at least in principle the very high effective $E^{0\prime}_{P-450}$ calculated above need not be impossible. Thus redox potentials around 3.5 V were determined for Fe(bpy)$_3^{4+/3+}$ and Fe(bpy)$_3^{5+/4+}$,[52] 2.0 V was found for Fe(CH$_3$CN)$_6^{3+/2+}$[53] and 1.8 V for a model of P-450, the perferryl complex, tetrakis(2,6-dichlorophenyl)porphinato]iron-oxene$^{3+/2+}$.[51] As discussed by Macdonald *et al.*,[37] it is a possibility that the gain of electrostatic free energy upon ET [eqn. (10)] contributes appreciably to increase the effective $E^{0\prime}_{P-450}$. With an active site dielectric constant of 3

$$[Fe^V=O\ RH \leftrightarrow Fe^{IV}=O^-\ RH^+]^{\ddagger} \tag{10}$$

and a distance between the reacting centres of 5 Å, the electrostatic term was estimated to be ca. 1 V, thus bringing E^0_{P-450} down to a more reasonable ca. 1.6 V. One difficulty with this idea is that the subsequent separation of the ion pair becomes correspondingly more difficult. It is then necessary to postulate a charge neutralization step at the active site, e.g., by radical cation deprotonation or its reaction with a mononegatively charged nucleophile. Either function can be effected by the perferryl oxygen, thus logically ending at our starting point, eqn. (1). It is tempting to assume that with decreasing reactivity of the substrate, we have a range of mechanisms beginning with fully developed ET and radical cation formation and ending with a synchronous electron/proton or electron/oxygen transfer step (or classically, a hydrogen or oxygen atom transfer step). Then it is only the timing between the successive steps that determines whether the typical reactions of radical cations will be exhibited or not.

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