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# Biological electron transfer: progress and future directions

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**The rich diversity among bacterial cytochromes has played a key role in the development of our understanding of biological electron transfer. Although studies to date have allowed the elucidation of the contributions of driving force, electrostatics interactions and surface topology to electron transfer kinetics in collision-dependent reactions, much remains to be learned. Little is known about intramolecular and intracomplex electron transfer. Several factors controlling intramolecular and intracomplex electron transfer can be defined. These include driving force, the distance between redox centers, the relative orientation of prosthetic groups, the nature of the intervening media and the molecular dynamics within the electron transfer complex. However, at the present time, we have only a limited understanding of the contribution of these factors to electron transfer kinetics in biologically relevant systems. Nevertheless, a wide range of techniques are now available which should soon provide the information necessary to describe in molecular terms the mechanism of intramolecular and intracomplex electron transfer. Principal among these new approaches are site-directed mutagenesis and NMR spectroscopy.**

It has been almost 40 years since Kamen and coworkers demonstrated that high-potential *c*-type cytochromes were present in anaerobically grown photosynthetic bacteria. During the ensuing period, a great deal of progress has been made in defining the various classes of *c*-type cytochromes as well as in elucidating their mechanisms of electron transfer. It is now clear that the high potential bacterial cytochromes *c* are members of the Class-I *c*-type cytochrome family which include examples from almost all known organisms [1]. Although eukaryotic cytochromes *c* have been the most extensively studied examples – in particular horse and tuna cytochromes *c* – it is the rich diversity among the bacterial cytochromes *c* that have contributed most extensively to our understanding of biological electron transfer. The Class-I *c*-type cytochromes are structurally homologous. As a subfamily, the eukaryotic cytochromes *c* are substantially similar in terms of their physicochemical properties. Midpoint potentials are relatively constant (260 ± 5 mV), and isoelectric points are consistently greater than 9. This is in sharp contrast to the prokaryotic cytochromes *c*, where the midpoint potentials range from 20 to 450 mV and the isoelectric points from approx. 4 to 10 [2]. These diverse properties led to the application of Marcus theory, thus allowing the separation of the contributions of (1) driving force (the difference in redox potential between reactants), (2) electrostatics and (3) surface topography in the cytochrome interaction domain to the kinetics of electron transfer [3,4]. To date, the principal focus has been on the interaction of soluble redox reactants acting via second-order collision-dependent processes. Marcus theory describes electron transfer and, hence, would not be expected to apply to second-order reactions. However, collision dependent reactions consist of two steps (Eqn. 1): transient complex formation ($K$) followed by intracomplex electron transfer ($k_{et}$). Since Marcus theory is applicable, it follows that the observed second-order rate constant must be $Kk_{et}$ as the result of a rapid equilibrium between reactants.

$$\text{oxidant} + \text{reductant} \overset{K}{\rightleftharpoons} \text{oxidant} - \text{reductant} \xrightarrow{k_{et}} \text{reductant} - \text{oxidant} \quad (1)$$

Marcus theory has been shown to apply to a variety of redox proteins (Class-I *c*-type cytochromes, Class-II *c*-type cytochromes, copper proteins and high-potential iron-sulfur proteins) when they react with free flavin semiquinones and flavodoxin semiquinones. This application has established that, within this structural family, $K$ is approximately constant for most individual cytochromes [3,4]. This is a reasonable conclusion, since prosthetic group exposure and topology in the interac-

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tion domain should be approximately the same in view of the structural similarities among the cytochromes. However, deviations from Marcus theory are sometimes observed for individual cytochromes within structural families [3]. The deviations result from differences in the electrostatics and topology of the interaction domain. Analysis of data from several structural families indicates that driving force, electrostatics and topology can each contribute up to a factor of a thousand to the observed kinetics [3]. Thus, by varying the redox potential, interaction domain charge and topology, nature can modulate the kinetics of electron transfer up to nine orders of magnitude. This is the basis for biological specificity in redox systems and provides variation in mechanisms for the flow of electrons through functional pathways at rates which are optimal for individual pathways while minimizing thermodynamically allowable but nonproductive side-reactions.

The study of collision-dependent processes also has the fringe benefit of defining what we term 'kinetic taxonomy'. This means by using free flavin semiquinones or flavodoxin semiquinones as reductants, the structural family to which a redox protein of unknown structure belongs can be determined. Moreover, estimates of prosthetic group exposure to solvent and of the topology and of electrostatics of the interaction domain can be obtained from comparison with values established for known families. Thus, in these cases structural information can be inferred from kinetic data.

Irrespective of the success at elucidating collision dependent processes, much remains to be established concerning the mechanism of biological electron transfer. This is particularly true for intracomplex and intramolecular electron transfer. Marcus theory relates the rate constants for electron transfer ($k_{et}$) to three fundamental parameters (Eqn. 2): $\Delta G°$, the driving force energy; $\lambda$, the reorganizational energy; and $\nu_{et}$, the preexponential term [5,6].

$$k_{et} = \nu_{et} \exp\left(-\frac{(\Delta G° + \lambda)^2}{4\lambda RT}\right) \quad (2)$$

In short-range electron transfer, $\nu_{et}$ is dependent on the distance between redox centers and the electronic coupling. However, in long range electron transfer, $\nu_{et}$ is described by a tunnelling matrix which will be quite dependent on (1) intervening media (that is, the amino-acid side-chains and solvent between redox centers), (2) orientation (the relative orientation of prosthetic groups), (3) the distance between redox centers and (4) likely, the molecular dynamics within the electron transfer complex. Thus, in general terms, the rate constant for electron transfer will depend on driving force ($\Delta G°$), orientation ($\lambda$, $\nu_{et}$), distance ($\nu_{et}$), intervening media ($\nu_{et}$) and dynamics within the complex ($\lambda$, $\nu_{et}$). The issue at hand and for the future is how to resolve the contributions to the kinetics of electron transfer of each of the factors discussed.

The current challenges are illustrated in Table I. Here we have presented the measured rate-limiting kinetics ($k_{et}$) for a number of biological reactions. In these examples, relatively long-lived complexes are formed and, hence, $k_{et}$ has been measured directly. A number of important conclusions can be drawn from the data given in Table I. First, there is no clear relationship between driving force ($\Delta E_0'$) and $k_{et}$. For example, the cytochrome *c*-cytochrome *c* peroxidase system has an extremely large driving force ($\approx$ 750 mV), yet a relatively small $k_{et}$. Moreover, the cytochrome *c*-cytochrome *c* peroxidase system is quite sensitive to ionic strength, a result not expected on the basis of Marcus Theory, since although ionic strength influences complex formation ($K$ in Eqn. 1), it should not affect

TABLE I

*Intracomplex and intramolecular electron transfer*

| System | $d$ (Å) [a] | Angle | $\Delta E_0'$ (mV) | $k_{et}$ ($s^{-1}$) |
|---|---|---|---|---|
| Flavodoxin → cyto. *c* | 3.5 | coplanar | ~ 300 | 85 |
| Cyto. $b_5$ → cyto. *c* | 3.5 | coplanar | ~ 250 | 1400 |
| Trimethylamine dehydrogenase (flavin → Fe-S) | 3.5 | n.a. | ~ 60 | ~ 60 |
| Cytochrome $b_2$ (flavin → heme) | 10 | coplanar | ~ 60 | > 600 $s^{-1}$ |
| Cyto. $c_2$ → *R. sphaeroides* reaction center | 11 | coplanar | ~ 100 | 500–13000 [c] |
| Cyto. *c* → cyto. *c* peroxidase | 17 | coplanar | ~ 750 | 150–2300 [c] |
| *R. viridis* reaction center [b] | | | | |
| heme 1(*c*-559) → $BChl^+$ | ~ 15 | perpendicular | ~ 70 | $3.7 \cdot 10^6$ |
| heme 2(*c*-553) → $BChl^+$ | ~ 25 | coplanar | ~ 530 | $6.0 \cdot 10^6$ |

[a] Distance edge to edge where planar chromophores are involved.

[b] Hemes are numbered according to their distance from the special pair bacteriochlorophyll ($BChl^+$).

[c] $k_{et}$ is dependent on ionic strength.

$k_{et}$. This result suggests that the nature of the complex is altered by ionic strength affecting either orientation or distance or both and resulting in a change in $k_{et}$ [5,6]. Second, no obvious correlation exists between distance and $k_{et}$. Indeed, some of the shortest electron transfer distances are among the slowest (flavodoxin → cytochrome *c*) and the most distant among the fastest (heme 2 (*c*-553) → $BChl^+$). Third, from the limited data available, it is difficult to quantify the contribution of orientations. In most systems the chromophores are coplanar. However, in the case of *Rhodopseudomonas viridis* reaction centers, both coplanar and perpendicular orientations have similar rate constants. By deduction, it follows from the results presented in Table I that the through protein pathways, that is, intervening media, must be important. However, to date, very little information is available which can be used to analyze in molecular terms the role of intervening media in the naturally occurring systems presented here.

The issue of molecular dynamics within the electron transfer complex is complex and requires further discussion. For example, for the cytochrome *c*-flavodoxin complex, it has been shown that the complex 'breathes', that is, moves sufficiently to allow penetration of free flavin semiquinones into the complex interface [7]. This is a first-order process with a limiting rate constant (upwards of 2500 $s^{-1}$, depending on the ionic strength) which is independent of the chemistry of the flavin semiquinone. This breathing apparently results from large motions on a relatively rapid time-scale and serves to illustrate the possible situation within naturally occurring complexes. The concept of rate-limiting dynamic motions has been termed 'conformational gating' and has been clearly shown in the cytochrome *c*-cytochrome $b_2$ complex [8]. In this example $k_{et}$ is found to be independent of driving force. Thus, it is difficult, if not impossible, to resolve 'gating' from rate-limiting electron transfer in the naturally occurring systems given in Table I without having additional information.

To summarize to this point, it is quite apparent that at least five variables – driving force, distance, orientation, intervening media and molecular dynamics – can contribute to, and/or control, measured rate-limiting electron transfer processes. The clear mandate for the future is to develop structurally defined systems which allow the individual variables to be perturbed and analyzed. Fortunately, a number of technologies are now available which should allow us to address the important questions in biological electron transfer. For example, X-ray crystallography is now being applied to systems containing multiple redox centers (for example, *R. viridis* reaction centers) and will provide information on distance, orientation and intervening media. New NMR techniques permit the study of the solution structure of sufficiently small systems and provide direct measurements of molecular dynamics. Finally, site directed mutagenesis provides the means to modify specific amino-acid side-chains and to alter directly one or more of the variables important in electron transfer. In this context, a number of systems are now available for the mutation of *c*-type cytochromes, including yeast cytochrome *c*, rat cytochrome *c* and *Rhodobacter capsulatus* cytochrome $c_2$.

TABLE II

*R. capsulatus cytochrome $c_2$ mutants*

| Mutant [a] | Expression [b] | Growth [c] (colony size in mm) | $E_0'$ (mV) [d] |
|---|---|---|---|
| Wild-type | 1.0 | 1.0–1.2 | 367(416) [e] |
| K12D | 2.6 | 1.0–1.2 | (394) [e] |
| K14E | 2.2 | 1.0–1.1 | (390) [e] |
| K32E | 2.5 | 1.1–1.2 | (388) [e] |
| K14E/K32E | 2.1 | 1.1–1.2 | (363) [e] |
| P35A | 4.4 | 0.9–1.1 | 359 |
| W67Y | 4.4 | 1.0–1.2 | 369 |
| Y75F | 5.8 | 1.2–1.3 | 308 |
| Y75C | 0.2 | 0.6–0.7 | 348 |
| Y75S | 0.2 | 0.6–0.8 | ND |

[a] Notation for mutants is wild-type amino acid/position/mutation. For example, K12D represents the replacement of lysine at position 12 by aspartic acid.

[b] Expression refers to the relative amounts of *R. capsulatus* cytochrome $c_2$ expressed in whole cells of an *R. capsulatus* strain in which the wild-type gene has been deleted.

[c] The *R. capsulatus* cytochrome $c_2$ gene was expressed in an *R. sphaeroides* mutant which was unable to grow photosynthetically. The values given are colony sizes when grown under photosynthetic conditions.

[d] Measured in 50 mM potassium phosphate (pH 7.0) unless noted otherwise.

[e] $E_0'$ values extrapolated to zero ionic strength to illustrate the maximum effect of the net protein charge.

We have recently developed an expression system for cloned *R. capsulatus* cytochrome $c_2$ and have produced a first generation of mutants. To date, nine mutants have been prepared and sufficient quantities are available for X-ray, NMR and physical-chemical studies, including kinetics of electron transfer. One of our major goals is to investigate the effect of specific mutations of cytochrome $c_2$ on $k_{et}$ using *R. sphaeroides* reaction centers. This is an excellent system, since cytochrome $c_2$ and *R. sphaeroides* reaction centers form tight complexes at low ionic strength, and the reaction center structure is known. Table II summarizes the properties of the cytochrome $c_2$ mutants prepared to date. Although much work is still required to characterize fully the mutant proteins, general comments can be made which illustrate the approach. As shown in Table II, most of the mutants can be overproduced, making available large quantities for structural and kinetic studies. Moreover, by expressing the mutants in a *Rhodobacter sphaeroides* strain in which wild-type cytochrome

$c_2$ has been deleted and, thus, the strain is unable to grow photosynthetically, in vivo functionality of the mutant proteins can be determined (Table II). The mutants prepared to date can be divided into several different families which relate to the variables discussed above in reference to the factors controlling $k_{et}$. These families include mutants with altered redox potentials which will perturb the driving force (see Table II); charge mutants (K12D, K14E, K32E, K14E/K32E) which will effect orientation and, based on preliminary NMR studies, molecular dynamics; and structural mutants (for example, Y75F, P35A, W67Y) which may affect molecular dynamics and intervening media. Obviously, additional mutants can be made as the role of specific amino-acid side-chains becomes more defined.

It is quite apparent that the principal factors controlling biological electron transfer by *c*-type cytochromes can be described and that techniques required to understand electron transfer in molecular terms are in hand. Thus, we can look forward in the next few years to a new and more complete understanding of biological electron transfer.

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**References**

1 Meyer, T.E. and Kamen, M.D. (1982) Adv. Protein Chem. 35, 105–212.
2 Meyer, T.E. and Cusanovich, M.A. (1989) Biochim. Biophys. Acta 975, 1–28.
3 Tollin, G., Meyer, T.E. and Cusanovich, M.A. (1986) Biochim. Biophys. Acta 853, 29–41.
4 Cusanovich, M.A., Meyer, T.E. and Tollin, G. (1988) in Advances Inorganic Biochemistry, Vol. 7, Heme Proteins (Eichorn, G.L. and Marzilli, L.G. eds.), pp. 37–92, Elsevier, New York.
5 Cusanovich, M.A., Hazzard, J.T., Meyer, T.E. and Tollin, G. (1989) J. Macromol. Sci-Chem. A26, 433–443.
6 Cusanovich, M.A., Hazzard, J.T., Meyer, T.E. and Tollin, G. (1988) in Progress in Clinical and Biological Research, Vol. 24: Oxidases and Related Redox Systems (King, T.E., Mason, H.S. and Morrison, M. eds.), pp. 401–418, A.R. Liss, New York.
7 Hazzard, J.T., Cusanovich, M.A., Tainer, J.A., Getzoff, E.D. and Tollin, G. (1986) Biochemistry 25, 3318–3328.
8 McLendon, G., Pardue, K. and Bak, P. (1987) J. Am. Chem. Soc. 109, 7540–7541.