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# PULSE RADIOLYSIS KINETICS OF THE REACTION OF THE HYDRATED ELECTRON AND THE CARBOXYL ANION RADICAL WITH *PSEUDOMONAS AERUGINOSA* CYTOCHROME *c*-551

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**The kinetics of the reaction of hydrated electron ($e_{aq}^-$) and carboxyl anion radical ($CO_2^-$) with *Pseudomonas aeruginosa* ferricytochrome *c*-551 were studied by pulse radiolysis. The rate of reaction of $e_{aq}^-$ with the negatively charged ferricytochrome *c*-551 (17 $nM^{-1} \cdot s^{-1}$) is significantly slower than the larger positively charged horse heart ferricytochrome *c* (70 $nM^{-1} \cdot s^{-1}$). This difference cannot be explained solely by electrostatic effects on the diffusion-controlled reactions. After the initial encounter of $e_{aq}^-$ with the protein, ferricytochrome *c*-551 is less effective in transferring an electron to the heme which may be due to the negative charge on the protein. The charge on ferricytochrome *c*-551 is estimated to be −5 at pH 7 from the effect of ionic strength on the reaction rate. A slower relaxation ($2 \cdot 10^4$ $s^{-1}$) observed after fast $e_{aq}^-$ reduction is attributed to a small conformational change. The rate of reaction of $CO_2^-$ with ferricytochrome *c*-551 (0.7 $nM^{-1} \cdot s^{-1}$) is, after electrostatic correction, the same as ferricytochrome *c*, indicating that the steric requirements for reaction are similar. This reaction probably takes place through the exposed heme edge.**

## Introduction

The kinetics of the reactions of hydrated electrons ($e_{aq}^-$) and carboxyl anion radicals ($CO_2^-$) with cytochrome *c* and other heme proteins have been well studied because of their biological redox properties [1–11]. Most studies have used horse heart ferrocytochrome *c* [1–4,6–10] though *Pseudomonas fluorescens* ferricytochrome *c*-551 [4] and *Rhodospirillum rubrum* ferricytochrome $c_2$ [5] have been briefly examined. The cytochrome *c*-551 of *Pseudomonas aeruginosa* is a relatively small ($M_r$ 8700) cytochrome of the bacterial respiratory chain and the X-ray structure of both reduced and oxidized forms [12] is known. Cytochrome *c*-551 differs from horse heart cytochrome *c* in that it is negatively charged (p$I$ = 4.7) [13] at neutral pH, whereas horse heart cytochrome *c* is positively charged (p$I$ = 10.5). Also, cytochrome *c*-551 is smaller and consequently could have a more exposed heme. Thus, electrostatic effects and the efficiency of direct heme reduction compared to intramolecular electron transfer may be examined. The large second-order rate constants for the reaction of $e_{aq}^-$ with the heme proteins are indicative of diffusion-controlled reactions. Recent developments in the treatment of diffusion-controlled reactions of protein-small molecule reactions provide a basis for the interpretation of these rate constants [14–17].

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## Experimental procedures

*Materials*

The growth of the bacteria and the extraction and purification of cytochrome *c*-551 from *P. aeruginosa* were, with some modifications, as previously described [18,19]. The purified ferricytochrome *c*-551 had a molar absorption coefficient ratio $\epsilon_{red}^{551}/\epsilon_{ox}^{280} = 1.11$, simular to that reported previously [18,19]. Monomeric horse heart cytochrome *c* (Sigma, type VI) was obtained by chromatography [20]. The ferricytochromes were obtained by oxidation with $K_3Fe(CN)_6$ followed by desalting on Sephadex G-25. Their concentrations were determined spectrophotometrically. NaCl was used to achieve the desired ionic strength. The reaction solutions were buffered only by the protein or formic acid-formate.

*Apparatus and methods*

The reactant $e_{aq}^-$ is produced upon pulse radiolysis of purified Ar-saturated solutions in the presence of 0.02 M of the hydroxy radical scavenger *t*-butanol (Fluka, puriss.). The reactant $CO_2^-$ was produced upon pulse radiolysis of purified $N_2O$-saturated solutions in the presence of sodium formate. The Varian linear accelerator at the Hebrew University, the spectrophotometric detection system, the on-line computer analysis and the sample handling and loading were as described [8,11]. The optical path of the flow-through quartz cell was 4 or 12 cm as conditions warranted. The $e_{aq}^-$ reactions were followed at wavelengths of 650 nm (absorption change due to $e_{aq}^-$) and at 550, 450, 440 and 417.5 nm (absorption change is due primarily to ferricytochrome *c*-551 reduction). The $CO_2^-$ reaction was followed at a wavelength of 550 nm. The reactions were studied under pseudo-first-order conditions with an excess of the cytochrome. Under these conditions [11] a minimum reaction scheme is:

$$\text{Cyt(III)} + e_{aq}^- \xrightarrow{k_1} \text{Cyt(II)} \quad (1)$$

$$\text{Cyt(III)} + e_{aq}^- \xrightarrow{k_2} \text{Cyt(III)}\cdot \quad (2)$$

$$e_{aq}^- \xrightarrow{k_3} \text{decay products} \quad (3)$$

Reaction 1 results in a Fe(II) heme and reaction 2 results in the formation of a free radical on sites on the protein other than the heme and reaction 3 is loss of $e_{aq}^-$ through reaction of $H^+$, $e_{aq}^-$, $H_2O$ and any other adventitious impurities. The observed pseudo-first-order rate constant $k_{ob}$ is:

$$k_{obs} = (k_1 + k_2)[\text{Cyt(III)}]_0 + k_3 \quad (4)$$

$$k = k_1 + k_2 \quad (5)$$

$$[e_{aq}^-] = [e_{aq}^-]_0 e^{-k_{obs}t} \quad (6)$$

$$[\text{Cyt(III)}] = \frac{k_1[\text{Cyt(III)}]_0[e_{aq}^-]_0}{k_{obs}} \cdot (1 - e^{-k_{obs}t}) \quad (7)$$

$$\text{heme reduction yield} = k_1/(k_1 + k_2) = k_1/k \quad (8)$$

Regardless of whether the reaction is followed with absorption changes in ferricytochrome or $e_{aq}^-$, the same $k_{obs}$ is observed.

For $CO_2^-$ reduction, the minimum reaction scheme is:

$$\text{Cyt(III)} + CO_2^- \xrightarrow{k_4} \text{Cyt(II)} + CO_2$$

$$CO_2^- + CO_2^- \rightarrow (CO_2)_2^{2-} \quad (9)$$

This scheme differs in that a reaction analogous to 2 is not very significant [9]. The second-order dimerization of $CO_2^-$ (reaction 9) can be shown not to be kinetically significant at low $[CO_2^-]$.

## Results and Discussion

*Kinetics of reaction with $e_{aq}^-$*

The reaction of ferricytochrome *c*-551 with $e_{aq}^-$ exhibited fast and slow components similar to that amply demonstrated in horse heart cytochrome *c* [1,2,8]. The pseudo-first-order rate constant for the fast reaction of ferricytochrome *c*-551 was measured as a function of $[\text{Cyt(III)}]_0$ to obtain *k* from Eqns. 4 and 5. From Fig. 1, the apparent second-order rate constant $k = 17 \pm 5\ \text{nM}^{-1} \cdot \text{s}^{-1}$. The rate constant $k_3$ was determined directly in the absence of any protein. The other values of *k* in Table I were calculated from Eqn. 4 at a single $[\text{Cyt(III)}]_0$, generally 5 $\mu$M. The changes in *k* with pH are consistent with a decrease in the negative

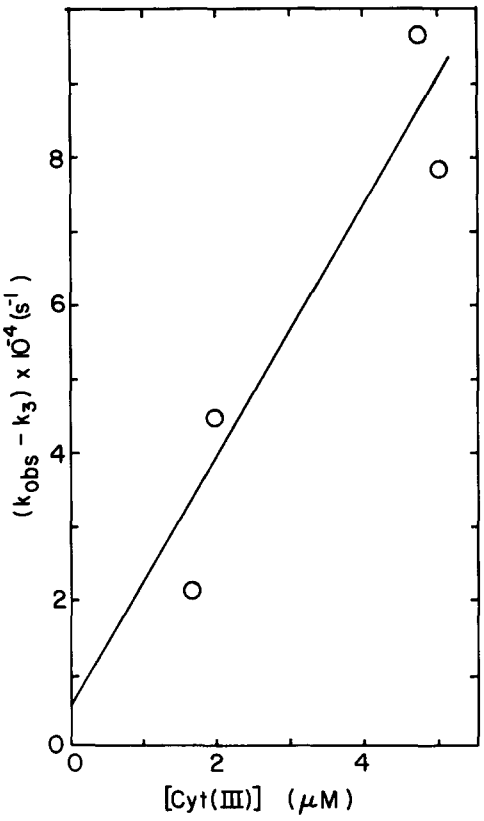


Fig. 1. Plot of $k_{obs} - k_3$ vs. [ferricytochrome *c*-551] for the reaction with $e_{aq}^-$ at pH 7.0 and 10 mM NaCl. The straight line is weighted linear least squares calculated. The intercept is zero within 1 S.D. $T = 18°C$.

charge on the protein. The $k$ of 39 $nM^{-1} \cdot s^{-1}$ compares well with a previous determination of 40 $nM^{-1} \cdot s^{-1}$ in salt-free pH 6.4 solution at the lower wavelength of 370 nm [4]. The value of $k$ for horse heart ferricytochrome *c* is in good agreement with a value of $k$ of 60 $nM^{-1} \cdot s^{-1}$ [8] in neutral salt-free solution and 66 $nM^{-1} \cdot s^{-1}$ [5] at pH 6.3.

A slower subsequent first-order process of smaller amplitude was observed (Fig. 2B–C) that was independent of a 3-fold change in ferricytochrome *c*-551 concentration and gave a $k_{obs} \simeq 2 \cdot 10^4\ s^{-1}$. This can be clearly seen in Fig. 2B where the fast initial change in transmission in the first several microseconds is due to the fast second-order reaction and the slower relaxation in the time region 20–240 $\mu$s is due mainly to the slower first-order process. The changes in the absorption spectrum associated with the slow relaxation could not be determined due to the small amplitude of the slow relaxation and also its lack of separation in time from the fast relaxation. This value compares to a slow process after $e_{aq}^-$ reduction of horse heart ferricytochrome *c* of $1.2 \cdot 10^5\ s^{-1}$ [8] which has been ascribed to a conformational relaxation. In the temperature jump relaxation study of the electron transfer reaction between ferricytochrome *c*-551 and the blue copper protein *P. aeruginosa* azurin, an isomerization of zero amplitude of ferricytochrome *c*-551 that was uncoupled from the

TABLE I

REACTION OF $e_{aq}^-$ WITH FERRICYTOCHROME *c*-551

Reactions were studied at 18 ± 2°C, λ = 650 nm with a 500 ns pulse.

| Reactant | $k$ [a] ($nM^{-1} \cdot s^{-1}$) | $k_1$ [b] ($nM^{-1} \cdot s^{-1}$) | $f$ [c] | pH | [NaCl] (mM) |
|---|---|---|---|---|---|
| Ferricytochrome *c*-551 [d] | 17 | 8 | 0.26 | 7.0 | 10 |
| Ferricytochrome *c*-551 | 15 | 7 | 0.24 | 7.0 | 4 |
| Ferricytochrome *c*-551 | 13 | 6 | 0.19 | 7.0 | 2.4 |
| Ferricytochrome *c*-551 | 11 | 5 | 0.17 | 7.0 | 1.0 |
| Ferricytochrome *c*-551 | 39 | 17 | 0.60 | 6.4 | 4 |
| Ferricytochrome *c* [e] | 70 | 49 | 0.95 | 7.0 | 4 |
| Ferricytochrome $c_2$ [f] | 66 | 46 | 0.91 | 6.3 | 0 |

[a] $k$ is the observed second-order rate constant calculated from Eqns. 4 and 5.
[b] $k_1$ is the second-order rate constant for heme reduction calculated from $k$ and the heme reduction yield (Eqn. 8).
[c] $f$ is calculated from $k/k_D$ where $k_D$ is the diffusion-controlled rate constant in the absence of any electrostatic or steric factors.
[d] From *P. aeruginosa*.
[e] From horse heart.
[f] From *Rhodospirillum rubrum* [5], assumed reduction yield of 70%.

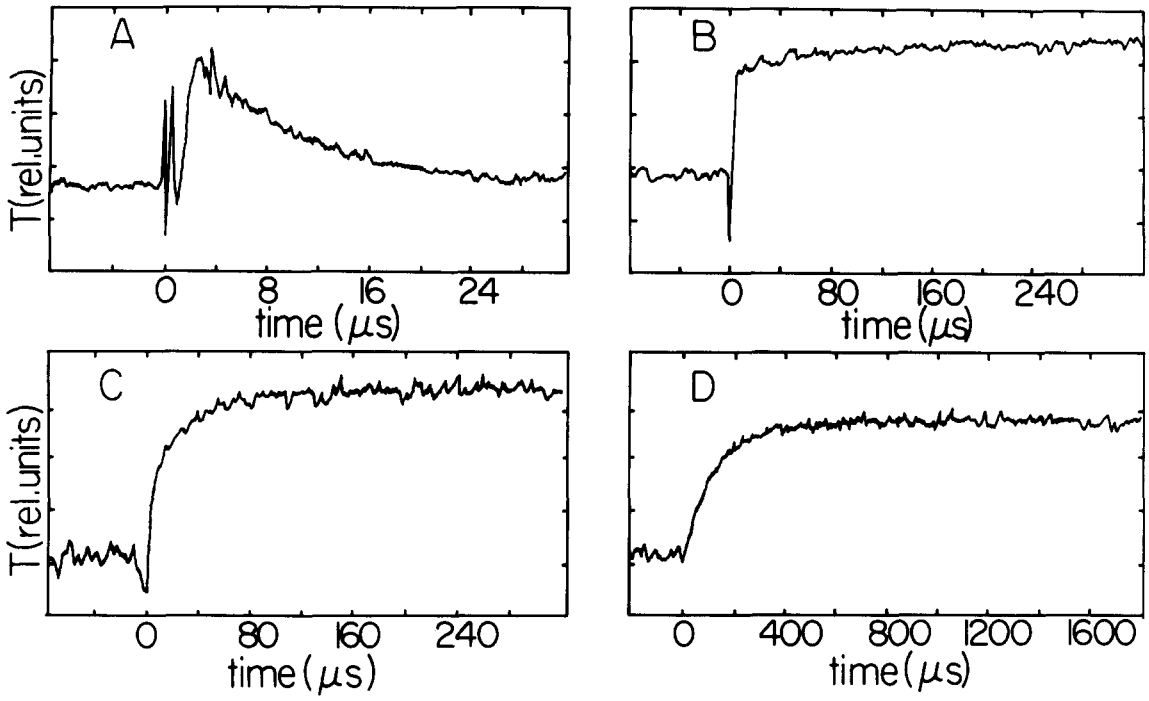


Fig. 2. (A–C) Relative light transmission changes ($T$) as a function of time for the reaction of $e_{aq}^-$ with ferricytochrome *c*-551, [NaCl] = 10 mM, pH 7.0, $T$ = 18°C. (A) $[Cyt(III)]_0 = 5.2$ μM, wavelength = 650 nm. Absorption change due mainly to fast second-order $e_{aq}^-$ reaction. (B) $[Cyt(III)]_0 = 5.2$ μM, wavelength = 550 nm. Fast initial absorption change due both to ferricytochrome *c*-551 reduction and $e_{aq}^-$ reaction. The slower relaxation ($k_{obs} \simeq 2 \cdot 10^4$ $s^{-1}$) is due to a conformational isomerization of the ferrocytochrome *c*-551 product obtained upon fast $e_{aq}^-$ reduction. (C) $[Cyt(III)]_0 = 1.6$ μM, wavelength = 417 nm. Absorption change is due primarily to ferricytochrome *c*-551 reduction. Fast initial second-order process and slower first-order conformational isomerization is shown. (D) Relative light transmission changes ($T$) as a function of time for the reaction of $CO_2^-$ with ferricytochrome *c*-551. $[Cyt(III)]_0 = 12.8$ μM, wavelength = 550 nm, [NaCOOH] = 0.10 M, pH 7.0, $N_2O$ saturated, $T$ = 17°C.

electron transfer was observed with a $k_{obs} \simeq 10^4$ $s^{-1}$ [19]. Also with *Alcaligenes faecalis* azurin as the reductant, the results could only be explained on the basis of a conformational isomerization of ferricytochrome *c*-551 [18]. It would appear that a conformational change with a similar relaxation time also occurs in ferrocytochrome *c*-551 produced by $e_{aq}^-$ reduction of ferricytochrome *c*-551 and hence is probably due to the same process. The first-order process is probably the relaxation of reduced ferrocytochrome *c*-551. After the fast transfer of the electron to the iron(III) of ferricytochrome *c*-551, the reduced protein probably still retains some conformational features that it had in the oxidized state. This higher energy conformational state then relaxes to normal ferrocytochrome *c*-551. The X-ray structures of the reduced and oxidized forms [12] show very little tertiary differences. The most significant structural changes are the movement of a couple of water molecules away from the heme iron on going from oxidized to the reduced form due to the decrease in attraction of the water dipole to $Fe^{2+}$. Also, very small conformational changes in the heme group might be taking place as the thioether-heme bonds showed small changes in position. How these small changes cumulatively act to cause the spectral changes observed upon $e_{aq}^-$ reduction is unclear.

*Reduction yield of ferricytochrome c-551*

From the total absorbance change (Fig. 2B and C) occurring at the completion of both fast and slow processes (approx. 300 μs), the concentration of ferrocytochrome *c*-551 produced could be estimated knowing the changes in the molar absorption coefficients between oxidized and re-

duced forms. The ratio of the concentration of ferrocytochrome *c*-551 produced to $[e_{aq}^-]_0$, estimated from the absorption changes occurring at 650 nm (Fig. 2A), which are due almost completely to the hydrated electron, gives the minimum reduction yield [11] of ferricytochrome *c*-551. The minimum reduction yields estimated are 40% at 440 nm, 45% at 450 nm and 45% at 550 nm. These values are lower than for horse heart ferricytochrome *c*, recently determined to be 70% [8]. The absorption changes observed corresponded well in both relative magnitude and sign to that expected from the difference spectrum.

*Diffusion control of the $e_{aq}^-$ reaction*

Simple Von Smoluchowski theory [23] predicts that for a diffusion-controlled reaction:

$$k_D = f4\pi RDN/1000 \quad (10)$$

where $k_D$ (in $M^{-1} \cdot s^{-1}$) is the diffusion-controlled rate constant, $R$ (in cm) is the encounter distance (usually taken as the sum of the radii of the two reacting species, $D$ (in $cm^2 \cdot s^{-1}$) is the sum of the translational diffusion coefficients, $N$ is Avogadro's number and the added $f$ may include electrostatic and steric factors. For noninteracting uniformly reactive spheres, $f = 1$ (Table II).

TABLE II

RADII ($r$) AND DIFFUSION COEFFICIENTS ($D$) FOR THE CALCULATION OF THE DIFFUSION-CONTROLLED RATE CONSTANT, $k_D$, FOR REACTION OF $e_{aq}^-$ WITH HEME PROTEINS

The theoretical diffusion-controlled rate constant, $k_D$, was calculated from $k_D = 4\pi RDN/1000$ with $f = 1$ in Eqn. 10.

| Reactant | $r$ (nm) | $D$ ($cm^2 \cdot s^{-1}$)($\times 10^5$) | $k_D$ ($nM^{-1} \cdot s^{-1}$) |
|---|---|---|---|
| $e_{aq}^-$ | 0.28 [a] | 4.8 [b] | – |
| Ferricytochrome *c*-551 | 1.45 [c] | 0.148 [d] | 65 |
| Ferricytochrome *c* | 1.66 [e] | 0.101 [e] | 73 |
| Ferricytochrome $c_2$ | 1.66 [e] | 0.101 [e] | 73 |
| Ferrimyoglobin | 2.03 [f] | 0.10 [f] | 86 |

[a] Ref. 24.
[b] Ref. 25.
[c] Estimated from the Stokes equation and $D_{Cyt\ c\text{-}551}$ [13].
[d] Ref. 13.
[e] Ref. 21.
[f] Ref. 11.

The size of the experimental $f$ factors from $k/k_D$ in Table I indicate that there is a considerable degree of diffusion control in these reactions. Two other corrections to the $f$ factors are necessary before the different proteins can be reasonably compared. Values of $k_1$ were calculated from Eqn. 8 and the spectrophotometric heme reduction yield (Table III). Similarly, the experimental $f_{heme}$, the diffusion factor for heme reduction, is calculated from $k_1/k_D$. The reduction yield corrected $f_{heme}$ can itself be factored into electrostatic and steric components:

$$f_{heme} = f_{elec} f_{steric} \quad (11)$$

to correct for the effect of different charges on the heme proteins. Using a numerical integration technique as described before [11,16,27], the charge on cytochrome *c*-551 was estimated to be approx. $-5$ from the dependence of $k$ on the ionic strength (Fig. 3). The Wherland-Gray equation [21,22] gives a protein charge of $-8 \pm 3$ for the same data. Both values are higher than the protein charge estimated from the primary amino acid sequence of $-2$ [21], indicating either that not all ionizable residues are exposed to the solvent or that there is some anion binding. The $f_{steric}$ of Table III are those obtained from Eqn. 11. Thus, after these corrections, ferricytochrome *c* still reacts more effectively than ferricytochrome *c*-551. On the basis that $f_{steric}$ represents the effective fraction of the

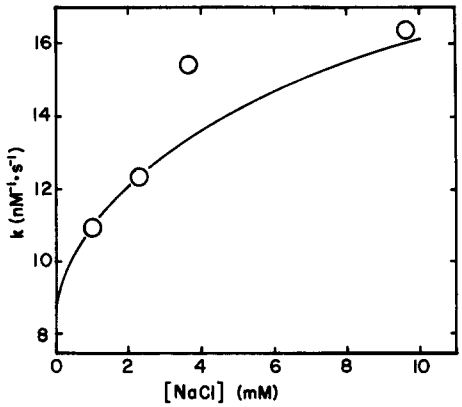


Fig. 3. Dependence of $k$ for the reaction of $e_{aq}^-$ with ferricytochrome *c*-551 on the ionic strength due to added NaCl. $T = 18°C$, pH 7.0. The solid line is that calculated for a protein charge of $-5$.

TABLE III

STERIC FACTORS FOR REACTION OF $e_{aq}^-$ WITH HEME PROTEINS

| Reactant | $k_1$ ($nM^{-1} \cdot s^{-1}$) | $f_{steric}$ [a] | Heme reactive site area $A_r$ [b] ($nm^2$) | Heme half solid angle $\theta$ [c] (°) |
|---|---|---|---|---|
| Ferricytochrome *c*-551 [d] | 7.5 | 0.20 | 5.2 | 81 |
| Ferricytochrome *c* [e] | 49 | 0.40 | 14.0 | 114 |
| Ferricytochrome $c_2$ [f] | 46 | 0.53 | 18.0 | 129 |
| Ferrimyoglobin [g] | 18 | 0.17 | 9.6 | 86 |

[a] $f_{steric}$ is the ratio of the rate constant for heme reduction, $k_1$, to $k_D$, after correction for electrostatic effects (Eqn. 11).
[b] $A_r$ is the effective area of the reactive site on the protein.
[c] $\theta$ is the minimum half-cone solid angle of entry necessary for reaction.
[d] pH 7.0, 10 mM NaCl, $Z_{protein} = -5$.
[e] pH 7.0, 4 mM NaCl, $Z_{protein} = +6.3$.
[f] pH 6.3, no added salt, $Z_{protein} = +1$, assumed 70% reduction [5].
[g] pH 7.0, 10 mM NaCl, $Z_{protein} = +2$ [11].

surface area of the protein molecule that is reactive, an effective reactive site area $A_r$ can be estimated from $f_{steric} 4\pi r_{Cyt}^2$ (Table III). On this basis, cytochrome *c*-551 is now even less reactive than the larger sperm whale ferrimyoglobin [11]. This area is much larger than the area of an exposed heme edge of 0.8 nm × 0.15 nm = 0.12 $nm^2$ and thus leads to the conclusion that there must be fast migration of the electron to the heme from the original site of attachment [12,2].

The Schurr and Schmitz [17] model for protein-small molecule diffusion-controlled reactions which considered both rotational and translational diffusion gives $f_{steric} = (1 - \cos\theta)\theta r_{e_{aq}^-}/(r_{Cyt} + r_{e_{aq}^-})$ where $\theta$ is the minimum half-cone solid angle (in rad) necessary for reaction at a hemispherical target. While all heme proteins studied have large half-cone angles, ferricytochrome *c*-551 is only about as reactive as ferrimyoglobin.

The cytochromes seem to require the presence of nearby aromaticity [28] to the heme either for structural or functional reasons. Especially striking [28] is the presence of tryptophan 59 (tuna numbering) hydrogen bonded to the inner propionic acid group of the heme in cytochromes *c*, *c*-550 and $c_2$. The role of this tryptophan in cytochrome *c*-551 is performed by the sequentially unrelated tryptophan 56 [28].

*Kinetics of reaction with $CO_2^-$*

The kinetics of the reduction of ferricytochrome *c*-551 (Fig. 1D) with the more selective and less reactive $CO_2^-$ was measured as a function of $[Cyt(III)]_0$, pH and ionic strength (Table IV). As might be expected for a negatively charged protein, $k_4$ decreases upon lowering the ionic strength. Also, $k_4$ increases (as did $k$) as the charge on the protein is increased at lower pH. These results show that ferricytochrome *c*-551 and ferricytochrome *c* react at nearly equal rates at pH 7.0. It is not clear from the magnitude of $k_4$ if the reactions are totally diffusion-controlled. However, the activation energies for the ferricytochrome *c* reaction have been measured [7] and are small as might be expected for a diffusion-controlled reaction. On the basis that the reactions are diffusion-controlled, the small values of $f$ indicate that the $CO_2^-$ reduces ferricytochrome *c*-551 largely via the exposed heme edge. These values of $f$ are still some 10-times larger than the $f$ of $1.4 \cdot 10^{-3}$ for diffusion-controlled CO binding to myoglobin [27] where the CO must combine directly to the $Fe^{2+}$ of the heme. The charge on ferricytochrome *c*-551 was determined from the ionic strength dependence to be −6 using the numerical integration method [11,16,27] and −6.2 with the Wherland-Gray equation [21] which is in reasonable agreement with the $e_{aq}^-$ data. Thus, with Eqn. 11, the electrostatically corrected $f_{steric}$ can be estimated. These values are some 10–50-times smaller than for $e_{aq}^-$, indicating the steric requirements for reaction are much less strict. Ferricytochrome *c*-551

TABLE IV

REACTION OF $CO_2^-$ WITH CYTOCHROME *c*-551 AND CYTOCHROME *c*

Reactions were studied at 19 ± 3°C, λ = 550 nm.

| Reactant | $k_4$ [a] ($nM^{-1} \cdot s^{-1}$) | [NaCOOH] (mM) | pH | *f* | $f_{steric}$ | Heme reactive site area $A_r$ ($nm^2$) | Heme half solid angle θ (°) |
|---|---|---|---|---|---|---|---|
| Ferricytochrome *c*-551 | 0.74 | 100 | 7.0 | 0.011 | 0.013 | 3.5 | 32 |
| Ferricytochrome *c*-551 | 0.45 | 10 | 7.0 | 0.007 | 0.013 | 3.5 | 32 |
| Ferricytochrome *c*-551 | 3.7 | 90 | 5.6 | 0.057 | – | – | – |
| Ferricytochrome *c* [b] | 0.82 | 100 | 7.0 | 0.011 | 0.010 | 4.3 | 30 |
| Ferrimyoglobin [c] | 2.0 | 10 | 7.0 | 0.023 | 0.021 | 11.0 | 41 |

[a] $k_4$ is the rate constant for the reaction of $CO_2^-$ with heme protein.
[b] Interpolated $k_4$, [7]; $Z_{protein} = +6.3$.
[c] $Z_{protein} = +2$, [11].

has an $f_{steric}$ only slightly larger than ferricytochrome *c*, indicating that the degree of exposure of the heme edges are not much different. This is in contrast to the analogous $e_{aq}^-$ reactions in which ferricytochrome *c*-551 is significantly less effective in transferring electrons to the heme. It can be concluded that the manner in which electrons are transferred to the heme are somewhat different for $CO_2^-$ and $e_{aq}^-$. The effective heme-binding site areas for $CO_2^-$ are, nonetheless, several-times larger than the area of the exposed heme edge, possibly indicating an area larger than simply the exposed heme edge may be effective in collecting $CO_2^-$.

## Conclusions

The reaction of $e_{aq}^-$ with ferricytochrome *c*-551 proceeds at a rate that suggests that the electron migrates from its site of original attachment to the heme and, in parallel, to other reducible sites on the protein. The mode of electron migration is unknown [1,2] and proposals have been advanced for sequential stepping between aromatic amino acids, attachment to peptide carbonyls followed by migration via peptide hydrogen bonds and electron tunneling. Diffusive flow along the protein surface as a result of short-range van der Waal's attractions can theoretically increase $k_D$ [14]. Nonspecific binding followed by a two-dimensional random walk until a sink is encountered can also greatly increase the effective area of the reactive site [31]. Ferricytochrome *c*-551 in its diffusion-controlled limit reacts with $e_{aq}^-$ significantly slower than does ferricytochrome *c*. If $e_{aq}^-$ migrates over the protein surface then the net negative charge on ferricytochrome *c*-551 would result in decreased nonspecific binding and less effective transfer of $e_{aq}^-$ to the heme. This would result in both a decreased rate and lowered reduction yield for $e_{aq}^-$. The distribution of positive and negative charges on ferricytochrome *c*-551 is very asymmetric [12,28]. As is typical of cytochromes there is a ring of positive charges around the heme opening whereas the back hemisphere is largely negatively charged. In ferricytochrome *c*-551, a smaller part of the protein may be available for nonspecific binding.

The reaction of $CO_2^-$ may also be diffusion-controlled and the rate at which the reaction proceeds suggests that reaction is largely taking place directly with the heme. The larger $CO_2^-$ would bind nonspecifically less effectively than $e_{aq}^-$ also decreasing the rate.

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