Biological Electron Transport Systems

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Abstract. The solid-state electrical conductivities of a number of ferredoxin model compounds are reported. For one of these compounds, (KFeS)₄, an electron transfer rate for a 25 Å unit is shown to be at least 1 × 10⁴ electrons sec⁻¹. The rate becomes proportionally larger for smaller molecular units. This rapid rate is consistent with a short pipe model for electron transport between two reaction sites. Some of the factors leading to this rapid transfer rate are considered.

We would like to propose that mixed-valence organometallic compounds may play a vital role in the short-range₁ electron transfer occurring via the electron transfer intermediates on the inner membranes of chloroplasts and mitochondria.⁵ This electron transfer, occurring in the course of biological oxidation reactions, can be very rapid. Actively respiring mitochondria have relatively slow electron turnover rates; for example, a typical turnover rate of cytochrome c is 30 electrons/sec.⁶ However, a number of investigators have shown that the rate constants for electron transfer between members of the electron transfer chain are very large—from 10⁴ to 5 × 10⁸ M⁻¹ sec⁻¹.⁷ This indicates that while the average rate (over seconds or minutes) is relatively slow, the rate of each individual electron transfer is fast because of the large rate constants and high effective carrier concentrations on the membrane. Inasmuch as electron transport is very rapid in chloroplasts and mitochondria, it is important to construct and study model systems which may exhibit similar characteristics. The preparation and solid-state conductivities of several biological electron transfer model compounds are described here.

Materials and Methods. (a) Roussin’s red ethyl ester, [Fe(NO)₂SC₂H₅]₂, II, was prepared by bubbling NO through a basic solution of FeSO₄ and ethyl mercaptan. The product was recrystallized from ethanol, mp 78–79°C.⁸

(b) Potassium dithioferrate (III), (KFeS)₄, III, was prepared by the reaction of iron and sulfur in a melt (900°C) of anhydrous sodium and potassium carbonate.⁹ After the melt was allowed to cool slowly, the solid was washed with water, ethanol, and ether, and was dried in a vacuum desiccator. The final product consisted of long, fibrous, permanganate-colored crystals.

(c) (C₅H₅FeS)₄, IV, was prepared by heating sulfur and [C₅H₅Fe(CO)₂]₂ to reflux in toluene. The product was recrystallized from bromobenzene in a nitrogen atmosphere, dec 230–240°C.⁷

(d) Conductivity measurements on single crystals of II, III, and IV were performed in a nitrogen atmosphere, using the two probe technique, with a Keithley 241 regulated direct current power supply and a Keithley 602 electrometer in series with the crystal. The size of the crystals precluded the use of four-probe and guard ring methods. When
using the two-probe method, the conductivity was measured for a number of crystals from several preparations. In addition, the conductivity was measured with current flow in both directions (polarity reversed). The electrode material used was DuPont silver preparation no. 4817. The conductivity ($\sigma$) was determined, at a given temperature, from the slope of a plot of current ($I$) versus voltage ($V$) or log $I$ versus log $V$ when the current and voltage range was very large.

$$\sigma = IL/Va.$$  

The crystal length $L$ and area $a$ were determined by microscopic measurement.

**Results and Discussion.** While the electron transfer between members of the electron transport chain is very rapid ($k = 10^4$ to $5 \times 10^8$ M$^{-1}$ sec$^{-1}$), many solution-phase inorganic systems have relatively slow electron transfer rates ($k = 10^{-3}$ to $10^2$ M$^{-1}$ sec$^{-1}$). An example is the electron exchange between Fe$^{+2}$ and Fe$^{+3}$ in aqueous solution:

$$\text{Fe}^{+2}(\text{H}_2\text{O})_6 + \text{Fe}^{+3}(\text{H}_2\text{O})_6 \rightleftharpoons \text{Fe}^{+2}(\text{H}_2\text{O})_6 + \text{Fe}^{+3}(\text{H}_2\text{O})_6 \; k = 0.87 \text{ M}^{-1} \text{ sec}^{-1}.$$  

The large difference in rate constants could be caused by (a) the lack of short-range order, in the above example, that is achieved in biological systems via the membrane structure; (b) the rather large bond length and geometrical changes required in the hydration spheres; or (c) a combination of both factors. Libby has proposed that because electron transfer is fast compared to nuclear motions (Franck-Condon principle), rapid electron transfer reactions will require the initial and final states to have similar geometries and bond lengths. The fact that considerable reorganization of the surrounding ligands is required, either before, during, or after electron transfer in the Fe$^{2+}$/Fe$^{3+}$ system, is the reason often used to explain why that exchange is slow compared to some other inorganic systems.

We have recently shown that the solid-state electrical conductivity of the mixed-valence compound biferrocene [Fe (II) Fe (III)] picrate I is six orders of magnitude larger than the conductivity of either ferrocene or ferrocenium picrate (see Table 1).

Thus it appears that mixed-valence organometallic compounds may have much larger conductivities, that is, short-range hopping is facilitated, than their monovalent counterparts. In addition, we have observed an electron transfer transition band at 1900 nm. Using the position of this band and Hush's theoretical model, it is possible to calculate that the activation energy for intramolecular electron hopping is 0.16 eV (3.78 kcal) and that the rate constant characterizing
this process is $1.3 \times 10^{10}$ sec$^{-1}$. This first-order rate constant is consistent with
the second-order rate constant (at 25°C) of the ferrocene-ferrocenium ion electron exchange
reaction. From low temperature data, we estimate the latter to be about $3 \times 10^9$ M$^{-1}$ sec$^{-1}$. To compare the two rate constants, we calculate
that a total concentration of 12 M (Fe and Fe$^+$) is required to produce a solution
where the average distance between the ions is 4.1 Å (ref. 13), which is in the
range of iron-iron distance of 1, 3.8–5.1 Å. This concentration, when multiplied
by the bimolecular rate constant, gives a pseudo-first-order rate constant of
$4 \times 10^9$ sec$^{-1}$.

It is important to note that the geometry and bond lengths in ferrocene and
in the ferrocenium ion are almost identical. For example, the metal-ring plane
distance in ferrocene$^{14}$ is 1.63–1.66 Å, while the corresponding distance in fer-
croenium picrate$^5$ is 1.68 ± 0.005 Å and in ferrocenium triiodide$^{16}$ it is 1.65 ±
0.1 Å. Probably the most dramatic biological example of this geometrical re-
requirement for fast electron transfer is in the copper enzymes (ceruloplasmin),
where the geometry and bond lengths are believed to be distorted from either the
preferred Cu(II) or Cu(I) geometry so as to achieve a compromise geometry
suitable for fast electron transfer between the two metal centers.$^{17}$ Williams has
presented evidence that a similar, though less dramatic, compromise is observed
in the cytochromes,$^{17}$ cc', o, a$s$, a, c, b. It is also possible that the rapid confor-
mational changes in the membrane produce relatively small changes in the short-range
order, geometry, and bond lengths in order to facilitate electron transport. Three
factors that may lead to rapid electron transport have been discussed. These
are summarized below along with several other important factors: (1) Short-
range order (membrane structure), (2) mixed-valence compounds or mixed
valence arrays of carriers, (3) little required geometrical or bond length changes
in going from the initial to the final state, (4) linear path (electrons hate to go
around corners),$^{18}$ (5) small change in spin quantum numbers of the complexes,$^{19}$
(6) exothermic reactions,$^{19c}$ (7) matched redox potential of donor and acceptor,$^{20}$
(8) low charged complexes to reduce coulombic repulsions,$^{19c}$ and (9) delocalized
ligands or highly polarizable ligands.$^{20,21}$

**Ferredoxin.** In light of these postulates, we would like to report the solid-
state electrical conductivities of several compounds that have been proposed
as models for the active site in ferredoxin. Despite the extensive study of the
nonheme iron proteins (with a variety of physical techniques over a wide
temperature range), there are still several plausible geometrical arrangements
for the metal-ligand systems.$^{20,22}$ Two of the models require that from 2 to 7
iron-ligand centers be isolated or noninteracting (tetrahedral field and distorted
octahedral field models), while the third requires the iron to be in close proximity
with at least one other iron complex. The possible implication of this third model
is that the arrangement is ideally suited for electron transport between two
distant reaction sites. If this "short pipe" model is correct, we would expect
intramolecular electron transfer to be rapid.

Blomstrom and co-workers$^{22}$ proposed compounds II and III as models for
the iron-sulfur bonding in ferredoxin. It is interesting to note that the polymer
III and at least two nonheme Fe-S proteins, Clostridium ferredoxin and puti-
daredoxin, have similar Mossbauer spectral properties (isomer shift, quadrupole splitting, and magnitude of internal fields).23,24

Blomstrom’s original model for the active site of ferredoxin from *Clostridium pasteurianum* contains a linear array of seven iron atoms. Sieker and Jensen25 published preliminary x-ray data on the structure of ferredoxin isolated from *Micrococcus aerogenes*. Their work indicates that the Fe atoms are not arranged in a single linear array as proposed by Blomstrom. They state that their 5 Å data “is not incompatible with several short linear arrays of heavy atoms.” Eisenstein, and Wang’s20” and Orme-Johnson and Beinert’s261 data on the anaerobic reductive titration of *Clostridium pasteurianum* ferredoxin suggests that there must be at least two distinct paramagnetic iron complexes in the molecule. Bayer has argued that the iron atoms are not in a linear array but in a “multi-center three-dimensional iron sulfur cluster.”27 As a model for his suggested active site, he proposes the tetrameric cyclopentadienyl iron sulfide (C₅H₅FeS)₄ (IV).

To test the short pipe concept, the solid state electrical conductivities of II, III, and IV were measured. The conductivities of the ethyl ester of red Roussin’s compound II and the cluster compound IV are very small (see Table 1). The low conductivities of these compounds could be caused by slow intermolecular or intramolecular electron transfer. However, the KFeS₂ polymer shows a relatively high conductivity (5.0 × 10⁻⁴ Ω⁻¹ cm⁻¹) in the direction of

Table 1.  Electrical measurements on model compounds.

| Compound*          | σ at 25°C (Ω⁻¹ cm⁻¹) | Eₛ (eV)│ σₑ(Ω⁻¹cm⁻¹)† |
|--------------------|----------------------|-------|---------------|
| [Fe(II)⁻Fe(III)]⁺P⁻ | 2.3 × 10⁻⁸           | 0.43  | 0.4           |
| Fe                 | 1 × 10⁻¹⁴            |       |               |
| Fe⁺P⁻              | 7.3 × 10⁻¹⁴          |       |               |
| [(NO)₂Fe(SC₂H₅)I₂] | <1 × 10⁻¹⁴           |       |               |
| [C₅H₅FeS]₄        | 6.0 × 10⁻¹⁰          |       |               |
| (KFeS₂)n           | 5 × 10⁻¹⁰           | 0.28† | 40            |
|                    | 2.7 × 10⁻⁴**         | (6.4 ± 0.4 kcal/mole) |

* Fe = ferrocene, P = picrate.
† Values obtained from σ(Ω⁻¹cm⁻¹) = eₑ⁻Eₛ/kT.
‡ Average of five measurements.
§ Parallel to fibers.
** Perpendicular to fibers.
†† DeVault and Chance obtained an activation energy of 3.3 kcal/mole (temperature above 120 °K) for the rate of oxidation of cytochrome following absorption of a short pulse of light from a ruby laser in the photosynthetic bacterium *Chromatium*. Below 100 °K an activation energy of less than 80 kcal/mole was observed. While our activation energy for electron transport is of the same order of magnitude, it is not apparent that these values can be related.
the fiber chains and a low conductivity \((2.7 \times 10^{-9} \, \Omega^{-1} \, \text{cm}^{-1})\) across the chains. The crystal is reported to be made up of infinite chains of \(\text{FeS}_2^+\)-ions formed of iron tetrasulfide tetrahedra sharing opposite edges. Between these lie the potassium ions surrounded by eight sulfur atoms.\(^{28}\) All of the iron atoms in \((\text{KFeS}_2)_n\) are formally in the +3 oxidation state so that this linear array of iron atoms is not a mixed-valence system until electrons are injected during the conduction process.

The high conductivity of the \(\text{KFeS}_2\) polymer in the direction of the polymer strands allows us to place a lower limit on the rate of intramolecular electron transfer. If we take the conductivity of an individual fiber to be \(10^{-3} \, \Omega^{-1} \, \text{cm}^{-1}\), then the resistance of a molecular unit \(5 \times 5 \times 25 \, \text{Å}\) can be readily estimated. This molecular unit could approximate the dimensions of the active site of ferredoxin from \textit{Clostridium pasteurianum} if \((\text{KFeS}_2)_n\) is a good model. The resistance of this unit is about \(1 \times 10^{11} \, \Omega\):

\[
R(\text{molecular unit}) \leq 10^2 \, \Omega \, \text{cm} \times \left( \frac{25 \, \text{Å}}{5 \times 5 \, \text{Å}} \right) \times \left( \frac{1 \, \text{Å}}{10^{-8} \, \text{cm}} \right) = 1 \times 10^{11} \, \Omega
\]

This resistance should be an upper limit since the polymer fibers are known to be very long but not infinite, thus results in a lower limit for the electron transfer rate. The resistance across the fiber is so large that electron hopping from one fiber to another should not make a very important contribution to the over-all conduction process. If we assume a 1 V potential difference\(^{29}\) between two iron atoms at the extremes of this molecular unit, then the current is calculated to be \(1.0 \times 10^{-11} \, \text{amp}\):

\[
I(\text{molecular unit}) = \frac{1 \, \text{V}}{1 \times 10^{11} \, \Omega} = 1 \times 10^{-11} \, \text{amp/molecular unit}.
\]

This corresponds to a minimum rate of \(1 \times 10^8\) electrons sec\(^{-1}\) molecular unit\(^{-1}\):

\[
i(\text{amp-unit}^{-1}) = q/t = 1 \times 10^{-11} \, \text{C sec}^{-1}\text{-unit}^{-1} = 1 \times 10^{-11} \, \text{C sec}^{-1}\text{-unit}^{-1}/1.6 \times 10^{-19} \, \text{C-electron}^{-1} = 10^8 \, \text{electrons sec}^{-1}\text{-unit}^{-1}.
\]

We have demonstrated that groups of Fe atoms tetrahedrally bound to sulfur with Fe-Fe distances of 2.7 Å can transfer electrons very rapidly between two distant sites. The model compound meets the criteria for fast electron transfer in that it has (a) short-range order; (b) becomes mixed valent upon charge injection; (c) provides a linear path for electron flow; (d) has a small change in spin-quantum number on electron migration; (e) has matched redox potentials \((\text{Fe}^{+2}\text{/Fe}^{+3}\) and \(\text{Fe}^{3+}\text{/Fe}^{2+}\)); (f) has little coulombic repulsion as a result of charge; and (g) contains polarizable ligands. The rate \((1 \times 10^8\) electrons sec\(^{-1}\)), calculated from the conductivity data, is consistent with the short-pipe model for rapid electron transport from reaction center to reaction center.

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1 The electron transfer on the inner membranes of chloroplasts and mitochondria is short in range if compared to electron transport via conduction bands in solids but long range if compared to solution electron transfer reactions. Possibly the best analogy is electron hopping in organic molecular crystals.


Even a potential difference of 10 mV will give a relatively large current through the molecular unit and a large rate constant ($5 \times 10^7$ electrons sec$^{-1}$ molecular unit$^{-1}$).