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ELECTRON TRANSFER THROUGH MACROMOLECULAR SYSTEMS

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ABSTRACT

Electron transfer between metal complexes which can be in intimate contact has been the subject of systematic study for about four decades. A major conclusion of the vast amount of work which has been done with intermolecular reactions of ordinary metal complexes is that the reactions are adiabatic, or nearly so (i.e., the only barriers to the reactions are the work of bringing the reagents into contact and the work of exciting them to the isoergic state, which is the configuration reached after the nuclei have readjusted so that the energy of the system is independent of the alternate sites the electron occupies). In adiabatic transfer, the rate of chemical change does *not* depend on the frequency of electron transfer between the two sites in the isoergic state. The measurement of the rate of electron transfer over large distances, especially when the intervening matter is made up of protein, has been a matter of great interest. At present, it is a very active field of investigation and several different methods for making such measurements have been introduced. The results obtained with one such method, developed by S. S. Isied in 1973, are emphasized. A key feature of the method is that reactions are studied in the intramolecular mode. This is a great simplification because the work of assembling the precursor complex is no longer a factor, and the interesting effects which arise from nonadiabatic behavior are more directly exposed. The method was first applied to simple bridging groups such as 4,4′-bipyridine, which tie the metal-contain-

ing moieties $(NH_3)_5Co(III)$ and $(NH_3)_5Ru(III)$ together. An external reducing agent reduces Ru(III) in preference to Co(III), and the subsequent chemical change, which involves reduction of Co(III) by Ru(II) by an intramolecular process, can be followed spectrophotometrically. The work with these simple bridging ligands showed that unless measures are taken to uncouple the two centers electronically, electron transfer in these systems is adiabatic, a conclusion confirmed by studies of the properties of mixed valence molecules with the same bridging groups. Isied has gone on to study electron transfer through polyprolines using the same general kind of technique. Even with the simplest bridging group of the series, the reactions are nonadiabatic. They become quite slow as the length of the polypeptide chain increases, and with longer chains a conformation change in which the metal centers are brought closer together precedes electron transfer. A similar technique has also been applied by Isied and others to studying the rate of electron transfer between the iron center of cytochrome C and a ruthenium complex attached to a histidine diametrically opposite the heme group.

INTRODUCTION

Perhaps because electron transfer by tunneling is a nonclassical phenomenon, the possibility of measuring the rate of electron transfer in chemical reactions over distances of many atomic diameters has long had a special appeal for many. While much research has been done on electron transfer reactions in the bimolecular mode, and as a result of this effort, several basic ideas on the factors that govern the rates of such reactions have been validated, these systems do not readily lend themselves to a systematic study of the rate of electron transfer as a function of the properties of the matter which separates the two sites. The association of the reactants in the encounter (precursor) complex is usually labile, and such variables as the relative orientation of the partners, which can be important in determining the extent of electron delocalization and thus the rate, cannot be defined, nor for that matter can the optimum distance of approach be experimentally determined in such systems. These difficulties can be eliminated or, at worst, greatly alleviated when the reactions are studied in the intramolecular mode, if the condition is imposed that the reactant metal centers are attached to suitable bridging groups by kinetically robust bonds.

A recurrent basic issue in trying to understand the rate patterns in electron transfer reactions is the degree of nonadiabaticity. In principle, this can be extracted from the rate as a function of temperature. In the Eyring equation,

$$k_1 = \kappa \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta H^{\ddagger}/RT}.$$

The transmission coefficient, κ, has the maximum value of unity when reactions are adiabatic. In this limit the only barriers to electron transfer are (a) the work of assembling the partners into the precursor complex and (b) the work of reorganizing the inner and outer coordination shells about the partners so that the energy of the system is independent of which of two sites the electron occupies [1]. When site-to-site electron delocalization in this isoergic condition is great enough so that the electron jumps at least once while the isoergic condition is maintained, the reaction rate is independent of the electron jump frequency. In the absence of delocalization the system is twofold degenerate. With the onset of delocalization the degeneracy is removed and a symmetric (bonding) and antisymmetric (antibonding) state rises. It is common to express the degree of delocalization in terms of the energy separation ($2H_{AB}$) between the two states. According to estimates by Sutin [2] for ordinary transition-metal complexes when $2H_{AB}$ is 1 kcal or greater, the reactions will be adiabatic. In the nonadiabatic regime ($2H_{AB}$ small), the system passes through the isoergic condition many times before an electron jump occurs to consummate reaction, and in this regime the electron jump (tunneling) frequency between the sites is also a factor in determining rates. The distinction is clearly an important one because, in order to calculate electron-transfer rates for adiabatic reactions, $2H_{AB}$, which is difficult to measure, need not be known.

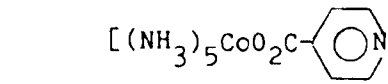
In practice κ and $\Delta S^{\ddagger}$ cannot be determined separately, and κ is included in the apparent entropy of activation, which is determined experimentally by measuring the rate of reaction as a function of temperature. In bimolecular processes, particularly when both reactants are charged, there are such large contributions to the apparent entropy of activation associated with forming the precursor complex that those from nonadiabaticity are masked. When the reactions are studied in the intramolecular mode, this complication is eliminated, and as we shall see, the effects of nonadiabaticity are often clearly exposed.

In 1973 a procedure [3] was described which makes it possible to study in a systematic way the rates of intramolecular electron transfer between transition-metal complexes. Since that time, a number of other procedures

have been developed for the same purpose, some [4] involving electron transfer to or from electronically excited states, and as described in the first of a particularly notable series of publications, for measuring intramolecular electron transfer rates within wholly organic molecules [5]. The literature on measurements of this kind is too extensive for all the measurements to be discussed in an article of the present length, and I shall, therefore, deal mainly with observations which have been made with the method described in Ref. 3, or with slight variations of it. This is all the more justified because, even within the limited scope, a number of important aspects of the subjects are being addressed.

ELECTRON TRANSFER THROUGH SIMPLE BRIDGING MOLECULES

In designing systems that would retain their integrity in solution, because of earlier experience, the choice of the amminecobalt(III) moiety as the oxidizing agent and the ammineruthenium(II) moiety as the reducing agent came readily to mind. Early attempts [6] to produce the precursor to intramolecular transfer by introducing an oxidizing agent such as

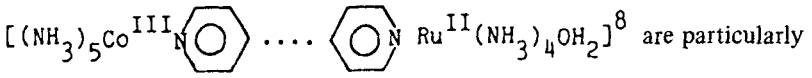


to a reducing agent such as $[(NH_3)_5RuOH_2]^{2+}$ failed in their purpose. In this approach, intramolecular electron transfer proved to be rapid compared to the rate at which the Ru(II)-N-heterocyclic bond is formed by substitution, and the rate-determining step in the sequence proved to be the latter process. An alternate strategy in which the binuclear complex was assembled with each metal ion in the oxidation state (III) led to success [3]. When the fully oxidized molecule is reduced, Ru(III) reacts preferentially to Co(III); the resultant Co(III)-Ru(II) species shows strong absorption [7] in the visible [metal-to-ligand charge transfer at the Ru(II) center], which disappears when the Ru(II) is oxidized and which provides a convenient and sensitive method of measuring the rate of intramolecular electron transfer.

In the present case, because of the difference in electronic structure, the rate of reduction of Ru(III) (πd^5) is very much more rapid than is that of Co(III) (πd^6) (only in the latter case is the entering electron antibonding and, as a result, there is a large inner shell reorganizational barrier to reduction) so that the reduction of Ru(II) by the external reductant is essentially quantitative. This is not a necessary condition for the success of the method. Since the reaction of interest is a first-order process, the half-life is indepen-

dent of concentration of the reactant. Interference from a parallel intermolecular process is easily avoided by dilution and is not a significant complication in any of the experiments which will be described.

To validate the approach, data for simple bridging groups will be described before the observations for macromolecular bridging groups, which can introduce additional complications, are presented. Of the measurements which have been made with the simple bridging groups, those for the series

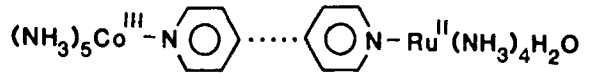
 are particularly

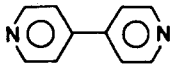
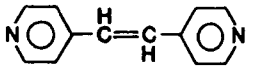
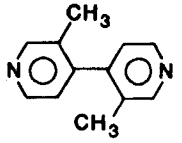
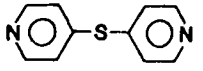
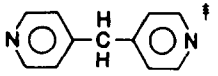
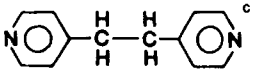
extensive and instructive. Within the series the first coordination sphere about each metal, and thus the driving force, remains almost constant, and the extent of electron delocalization between the metal ions is changed by changing the connections between the pyridine rings (indicated by the dotted line in the formula), and is the major variable. Most of the data which were obtained with this series of molecules are summarized in Table 1. The bridging molecule featured in the last entry is flexible and is treated as a special case, to be described last.

In comparing the results for the first five entries, it is to be noted that the values of $\Delta H^{\ddagger}$ remain essentially constant. This is the result expected when the differences in rates are mainly reflected in the value of the apparent entropy of activation, $\Delta S^{\ddagger}$, which in turn reflects variations in the extent of electronic coupling. The general insensitivity of rates to the degree of electronic coupling suggests that the most rapid (see first two entries) are in, or at least close to, the adiabatic limit. The small difference in rate which is observed between the first two entries can be attributed in large part to the variation in intersite separation, which does affect the rates of electron transfer even in the adiabatic limit; an increase in distances increased the barrier to electron transfer attributed to charge trapping by the solvent [11, 12]. The decreases in rate which are observed, culminating with the slowest, as recorded for the fifth entry, are qualitatively in accordance with expectations based on taking note of bond conjugation. Though qualitative arguments are inadequate to account for the order observed, they suffice to account for the differences in pairwise comparisons. Thus note Entry 1 compared to Entry 3: insertion of the methyl groups in the 3,3′ positions forces the rings out of the planar structure even more than is the case for the unsubstituted bridging ligand; insertion of CH_2 between the rings, Entry 5 compared to 1, is expected greatly to decrease resonance interaction.

The results for the last entry in Table 1 compared to the others introduces a feature which will be of particular concern when we consider data for macro-

TABLE 1. Rates of Intramolecular Electron Transfer for the Series



	$10^3 k$, s^{-1} at 25°C	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$
	44[a]	20.1 ± 0.3	+3
	19	20.2	+1
	5.5	20.2	-1
	4.9	20.0	-2
	2.1	18.4 ± 0.5	-9[b]
	1.0	19.5	-6.5

[a]For 3,3′ Isomer: $10^3 k = 4.2\ s^{-1}$.
[b]Reference 10; all other data from Ref. 9.
[c]Flexible bridging group.

molecular systems. Though some flexibility which can affect the distance of approach of the metal centers is present in –S– and $-CH_2-$ linked bridging molecules, it is much less than it is for the $-CH_2-CH_2-$ linked molecule where rotation about the bonds of the linking function can bring the metal ions into close proximity. In this case there is no reason to suppose that the detailed mechanism of electron transfer is any different than it is for outer-sphere electron transfer in the intermolecular mode, at least in the sense that the "bridging group" plays no role in facilitating electron delocalization, though it may

play a role in restricting the geometries accessible to the precursor complex.

The issue of flexibility in the bridging group takes on added interest when the results just described are compared with those reported by Haim and coworkers [13] for analogous studies with $[Fe(CN)_5]^{3-}$, in place of $[Ru(NH_3)_4H_2O]^{2+}$, as the reducing agent. When the bridging molecules are rigid, the results obtained in the two sets of studies run closely parallel. However, when the rate observed for the rigid bridging molecule 1,2-bis(4-pyridyl)-ethylene for each reducing agent is compared to that with the flexible bridging molecule, 1,2-bis(4-pyridyl)ethane, an interesting difference is seen. Whereas the change from rigid to flexible for $Ru(NH_3)_5^{2+}$ as the reducing agent leads to a decrease in rate by a factor of 19, when $Fe(CN)_5^{3-}$ is the reducing agent, the introduction of flexibility leads to a (slight) rate increase. The difference in behavior on changing the reducing agent is readily understandable. The flexible link in the bridging molecule admits of intramolecular ion-pair formation and, as a result, the $[Co(NH_3)_5]^{3+}$ moiety comes into close contact with the $[Fe(CN)_5]^{3-}$ moiety. When $[Ru(NH_3)_4H_2O]^{2+}$ is the reductant, close approach, though possible, is disfavored by ion-ion repulsion. It is of interest to note that when $[Co(NH_3)_5L]^{3+}$ (where L is a ligand of the general type used as bridging molecules) is brought into reaction, ion pairs form readily, and the rates of intramolecular electron transfer within the ion pairs are observed [14] to be of the order of 3 to 9×10^{-3} s^{-1}, compared to the value of 2.0×10^{-3} s^{-1} which was measured in the intramolecular mode when $[Co(NH_3)_5]^{3+}$ and $[Fe(CN)_5]^{3-}$ are joined by 1,2-bis(4-pyridyl)ethane. The agreement of the results supports the conclusion that, when the latter molecule is the bridging group, it plays no special role in electron transport.

ELECTRON TRANSFER THROUGH OLIGOPEPTIDES

In an effort to study the effect on the rate of intramolecular electron transfer with oligopeptides as spacers (bridging groups), Isied elected to use polyprolines, this choice being made because these are the most conformationally rigid of all the oligopeptides. The simplest molecule of the proline series is portrayed in Fig. 1 to show how the attachment of the metal ions to the amino acid is made. There is no difficulty with the direct attachment of $Co(NH_3)_5^{3+}$ to the carboxylate function of the proline anion. The bond of Ru(II) to the secondary nitrogen of the proline is too labile for present purposes, but robust molecules result when, as indicated in Fig. 1, this nitrogen is converted to a pyridine amide.

Figure 2 is a representation of the molecule when 4 proline units are inserted as spacers. In all cases the polypeptide segments are in the *trans-trans*

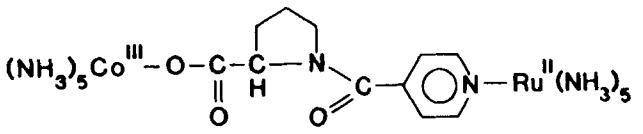


FIG. 1. Representation of the redox-active species with a single proline spacer.

conformation, which is the most stable under the experimental conditions, but it must be recognized that *trans-cis* isomerization can occur. The specific rate for this type of change has been measured [15, 16] as 0.5×10^{-2} to 1×10^{-2} s^{-1}, and as shall become apparent, can play a role in electron transport.

The results obtained with molecules [17] in which $[Ru(NH_3)_5]^{2+}$ is the reducing agent are summarized in Table 2. The studies have been extended [18] to $[Os(NH_3)_5]^{2+}$, which is a much stronger reducing agent, and these are included for ready comparison.

Let us consider first the rates observed for Ru(II) as the reducing agent and take note of the marked decrease in rate—by a factor in excess of 100—when the first spacer proline is inserted. There is known to be electron delocalization from an occupied t_{2g} orbital of Ru(II) to the LUMO of the isonicotinamide ligand and if, as part of the activation process, the Co(III) complex undergoes a suitable distortion, the electron delocalization between the metal

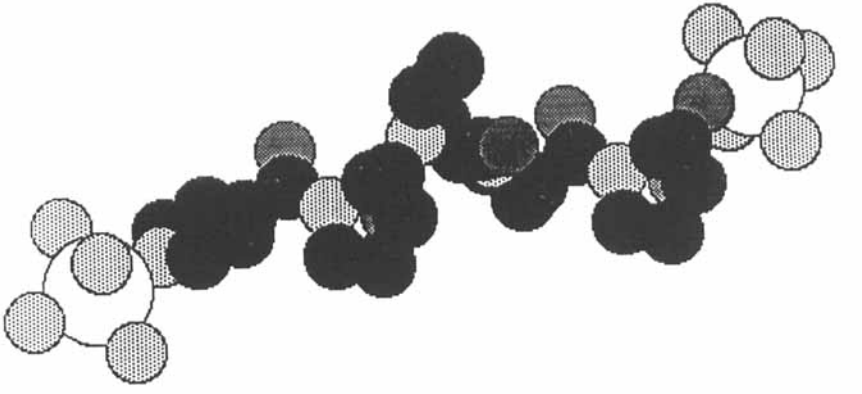


FIG. 2. Representation of the redox-active species with 4 proline spacers.

TABLE 2. Electron Transfer through Oligoprolines from Ru(II)[a] or Os(II)[b] to Co(III)

n[c]	Reductant	Distance,[b] Å	k, 25°C, s^{-1}	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal·mol^{-1}·K^{-1}
0	Ru(II)	9	1200×10^{-5}	19.7 ± 0.2	-1.0 ± 0.6
	Os(II)	9	1.7×10^{5}	10.2	0
1	Ru(II)	12	10.4×10^{-5}	18.0 ± 1	-16 ± 4
	Os(II)	12	270	11.2	-10
2	Ru(II)	15	0.6×10^{-5}	18.6 ± 2	-20 ± 6
	Os(II)	15	0.65	13.1	-16
3	Ru(II)	18	5.6×10^{-5}	14.5 ± 1	-29 ± 4
	Os(II)	18	0.4	11.9	-23
4	Ru(II)	20	14.0×10^{-5}	10.0 ± 1	-43 ± 3
	Os(II)	20	0.01	9.8	-30

[a] Ru ≡ *trans*-$Ru(NH_3)_4H_2O$.
[b] Os ≡ $Os(NH_3)_5$.
[c] Number of proline spacers in bridging molecule.

centers is, in all likelihood, sufficient to result in adiabatic transfer. Insertion of the first spacer–a saturated bond system–greatly reduces the value of $2H_{AB}$, and, as is indicated by the much reduced value of $\Delta S^{\ddagger}$, the reaction now appears to be nonadiabatic. Adding a second spacer further reduces the rate, with a concomitant reduction in $\Delta S^{\ddagger}$. It is surprising at first sight that introduction of the third spacer now leads to a rate increase, but it must be borne in mind that the *cis-trans* conformation changes in the oligoproline unit are rapid compared to the rates being measured. When enough prolines are inserted, the bridging molecules can adopt conformations that bring the oxidizing and reducing centers closer than is possible for the two shorter units, and it is reasonable to infer that this is the cause of the rate increase. On this interpretation, after two proline units have been added, the changes in the entropy of activation no longer provide a measure of nonadabaticity. We now have to reckon, as we do in the bimolecular case, with the entropy change associated with bringing the charged ions closer together. The changes in the

enthalpy of activation which result on inserting the third and fourth proline units can also be attributed to the change in character of the activation process, approach of the cations now being part of it. The bridging molecules for $n = 1$ or 2 are in effect rigid, while with $n = 3$ or 4, as already mentioned, account must be taken of configurations of the bridging molecule which, while not favored energetically, are favorable for electron transfer. This line of argument suggests that when n becomes large enough, a decrease in rate will ensue because of a dilution effect, that is, conformations suitable for electron transfer become a small fraction of the total which are accessible. Experiments to test this inference have not been done.

In the experiments with Ru(II) as the reducing center, reagents such as $Eu^{2+}(aq)$ or $Ru(NH_3)_6^{2+}$ were used to generate the reactive state [Co(III), Ru(II)] from [Co(III), Ru(III)]. Pentaammineosmium(II) is much more strongly reducing than is the ruthenium analog (by 0.65 V), and the rates of intramolecular electron transfer in the [Co(III), Os(II)] species are too rapid for the simple procedure followed in the [Co(III), Ru(III)] experiments to be effective. In the experiments with Os(II), organic radicals such as CO_2^- and $(CH_3)_2COH$, generated by pulse radiolysis, served for rapid reduction of [Co(III), Os(III)] to [Co(III), Os(II)].

As is the case when Ru(II) is the reducing agent, introduction of the first and second spacers leads to reductions in the rate, changes which are reflected in large part in decreases in the apparent entropies of activation. On adding additional spacers, conformational effects begin to play a role. When the experiments were done so that [Co(III), Os(III)] is almost fully reduced, the specific rates recorded in Table 2 were observed, but when a much higher concentration of [Co(III), Os(III)] was used, and only a small fraction of it was converted to [Co(III), Os(II)], the specific rates observed in each case are of the order of 0.09 s^{-1}.

The difference can be understood if the equilibrium

$$[\text{Co(III), Os(III)}]' + [\text{Co(III), Os(II)}] = [\text{Co(III), Os(II)}]' + [\text{Co(III), Os(III)}]$$

is considered. Here the prime indicates a conformer which, though not present in high concentration, provides the path for the oxidation of Os(II) to Os(III). The rapidly established equilibrium provides a means of harvesting the conformer more reactive for electron transfer, which is present in the large excess of [Co(III), Os(III)]. As a result of these complications, detailed discussion of the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ recorded for the bridging ligands with $n = 3$ and 4 is premature.

The much higher rates recorded for Os(II) than for Ru(II) are attributable in large part to the much larger driving force for the reactions with the former reagent. In all likelihood the reorganizational barrier to electron transfer for the two couples is much the same, and therefore, on the basis of the Marcus-Hush correlation, the rate ratio is expected to vary approximately with the square root of the ratio of the equilibrium quotients, that is, the rates for Os(II) are expected to be approximately 10^5 times more rapid than those for Ru(II). The observed rate ratios for $n = 0$, 1, and 2 are 1.4×10^7, 2.7×10^6, and 0.9×10^5, respectively. The Marcus-Hush correlation was developed for adiabatic reactions, and it is therefore not too astonishing that the rate ratio changes as the degree of nonadiabaticity increases.

ELECTRON TRANSFER THROUGH PROTEIN

A modification of the strategy used in the experiments which have been described has led to interesting results on electron transfer through proteins. The first experiments along this line were done by Gray and coworkers [19]. Treatment of horse-heart cytochrome C with $Ru(NH_3)_5OH_2^{2+}$ leads to a kinetically stable attachment of the residue $Ru(NH_3)_5^{2+}$ to an exposed histidine, Hist 33, which is located almost diametrically opposite the heme, the distance between the ruthenium and iron sites being ~15 Å, as inferred from the crystal structure of oxidized tuna cytochrome C. Gray et al. used flash photolysis to generate the redox-active species [Fe(III), Ru(II)] and observed changes in the spectrum which followed first-order kinetics and which were, altogether reasonably, interpreted as arising from the intramolecular electron transfer process

$$[Fe(III), Ru(II)] \longrightarrow [Fe(II), Ru(III)].$$

The specific rate was reported as 22 s^{-1} at 25°C, and from experiments over a range of temperature between 0.5 and 60°C, it was concluded that $\Delta H^{\ddagger} = 0$ although the data themselves do show some scatter. In independent work, Isied and coworkers [20a] investigated the same system, but used pulse radiolysis to generate highly reactive reducing radicals. They reported for the specific rate a value of 82 ± 20 at 25°C. In a later, very thorough investigation, Isied et al. [20b] studied the rate as a function of temperature (40°C range) and reported k at 25°C to be 53 ± s^{-1} (conditions somewhat different from those in the earlier work), $\Delta H^{\ddagger}$ as 3.5 ± 0.2 kcal/mol and $\Delta S^{\ddagger}$ as -39 ± cal·deg^{-1}·mol^{-1}. The differences between the results of the two labora-

tories at this stage, considering that the experiments described were the first of their kind to be done, are of minor consequence. The follow-up of the work in the two laboratories, however, does reveal differences, not because the results are necessarily in conflict, but rather that different facets of the system were explored.

The issue of the temperature coefficients for reactions of this kind was pursued further in Gray's laboratories by resort to a different system. A factor in the weak temperature dependence for the ruthenium-modified horse-heart cytochrome C is the low inner sphere barrier to electron transfer in the coordination spheres of the two centers. This itself is a consequence of the fact that metal ions comprising the couples are in low spin states (πd^5,πd^6) and thus lack antibonding d electrons. Gray and coworkers [21] searched for and found evidence of high inner-shell reorganization energies when they turned their attention to myoglobin, in which the iron ions in both the oxidation states are of high spin. The results of the study of the dynamics of this molecule, modified by attaching $Ru(NH_3)_5$ to Hist 48, are altogether satisfying. The system reaches a state of measurable equilibrium between the electronic isomers [Ru(II), Fe(III)] and [Ru(III), Fe(II)], and the rates of both the forward (Ru(II) → Ru(III)) and reverse reactions, which maintain the equilibrium, were measured. The specific rates at 25°C are 0.019 and 0.041 s^{-1}, and the corresponding values of $\Delta H^{\ddagger}$ are 7.4 ± 0.5 and 19.5 ± 0.5 kcal/mol. The value of ΔH° for the process is (7.4-19.5), namely -12.1 kcal/mol. Most of the enthalpy change of the overall reaction is associated with the Fe(III)/Fe(II) couple.

A basic concern in interpreting the results on intramolecular electron transfer for proteins is whether the protein acts as a rigid body, and the possibility must be considered that some change in the protein, which prepares the system for electron transfer, is itself rate determining. Just this kind of effect has been documented in early work [22] on the redox properties of metal-containing proteins. This study provided evidence that, in the reduction of Fe(III) in cytochrome C by Cr^{2+} (a reducing agent which has a marked preference for reacting by an inner-sphere mechanism), a conformation change in the protein, presumably the opening of a crevice adjacent to the Fe(III), was under certain circumstances the rate-determining step in the reduction. More recently [23], experiments in which the circular dichroism of ferricytochrome C was studied in the stopped flow mode indicated that on its reduction by hemin Fe(II), a transient species is formed which decays at a rate of 17 s^{-1} (28°C).

This kind of concern has motivated much of the recent work in Isied's laboratory. One possibility for an alternative to rate-determining electron

transfer is that a change at the heme iron is rate determining. The rate of dissociation of Met 80 from the Fe(III) (in the range 30-60 s^{-1}) is close to that reported for intramolecular electron transfer, but the possibility that the rate of this change was being measured in the experiments on intramolecular electron transfer could be set aside because $\Delta H^{\ddagger}$ for substitution of Met 80 by imidazole was found [24] to be 13.9 kcal rather than 3.5 kcal, as determined for the oxidation of Ru(II) by Fe(III). In the same work the authors measured the specific rate of oxidation of Ru(II) by the imidazole complex of heme iron(III) to be 1.2 s^{-1}, the decrease in rate being attributable in large part to the decreased driving force for the modified heme. The rate of reaction was found to be independent of pH over the range 9 to 5, but to increase below this pH, attaining a value of 5.0×10^2 s^{-1} at pH 2.0.

A direct way of testing whether the rate of electron transfer is actually being measured in the experiments with horse-heart cytochrome C would be to change the driving force, keeping other variables constant. If electron transfer is involved in the rate-determining step, then according to the Marcus-Hush correlation, the rate should increase approximately with the square root of the equilibrium quotient for the reaction. In the first of a series of experiments designed to apply this test of mechanism, $Ru(NH_3)_5^{2+}$ was replaced by *trans*-$[Ru(NH_3)_4\ isn]^{2+}$ (isn represents isonicotinamide bound to Ru by the heterocyclic nitrogen). By happenstance, when this change is made, the magnitude of ΔG° is not substantially altered, though its sign is changed. That is, in the redox reactive species, electron transfer takes place from Fe(II) to Ru(III).

The astonishing outcome of these experiments [25] is that the rate of intramolecular electron transfer is now so slow that it does not compete with second-order processes for restoring the system to equilibrium. This result has been met with concern [26] as well as skepticism, but there is no basis for questioning the quality of the work, particularly since the authors themselves were not prepared for this unexpected outcome of their experiments. Nor does a recent report of reversible long-range electron transfer in ruthenium-modified sperm-whale myoglobin [27] invalidate the results reported in *Nature*. Different proteins are being studied, and moreover, the consequences of replacing NH_3 by the π-acid isonicotinamide must be examined. It is highly unlikely that this change, or the attendant redistribution in charge ([Fe(II), Ru(III)] rather than [Fe(III), Ru(II)]), can bring about a large change in metal-metal distance. What can be dramatically altered by a change of this kind is symmetry. In the pentaammine system, the imidazole has much the quality of NH_3, and the electron hole is expected to be distributed between the d_{xz}, d_{yz}, d_{zx} orbitals. On the other hand, when isonicotinamide is located *trans*

to imidazole, delocalization embracing imidazole, metal ion, and π-acid can reasonably be expected, with the result that the electron pairs in the orbital perpendicular to the plane of two ligands are stabilized, so that the electron hole is now restricted to the other two orbitals, i.e., the electron hole does not delocalize significantly onto the histidine. If this proves to be the correct explanation of the effect discovered by Isied et al. [25], it would suggest that the electron transfer for simple bridging ligands between the metals is through-bond rather than through-space. Just what the mechanism of electron transfer is in each particular case of nonadiabatic transfer is an issue of vital concern.

It is obvious that the extension of the approach to studying intramolecular electron transfer developed for simple bridging ligands to macromolecular systems has opened up an area which holds much interest. It has already led to significant, as well as unexpected, discoveries, and there is every reason to believe that the field will unfold further and that further investigation will improve our understanding of electron transfer in biological systems.

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Note Added in Proof. Experiments done after this paper was offered in Japan show that *cis* and *trans* complexes behave much the same. A full account of these results and their interpretation by R. Bechtold, M. Cho, M. Gardineer, and S. S. Isied is being submitted for publication in the *Journal of the American Chemical Society.*