The Role of Phenylalanine-82 in Electron-Exchange Reactions of Eukaryotic Cytochromes c

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Abstract: The role of the invariant Phe-82 residue in eukaryotic cytochrome *c* has been investigated using high-resolution ^{1}H NMR techniques. In particular, yeast iso-1-cytochrome *c* modified at residue 102 with a threonine residue replacing cysteine (Cys-102-Thr) and a second variant containing, in addition to the first mutation, glycine instead of phenylalanine at residue 82 (Phe-82-Gly, Cys-102-Thr) have been studied. Using saturation transfer we show that replacement of phenylalanine with glycine has negligible effect on the rate of bimolecular electron self-exchange when compared with that of Cys-102-Thr iso-1-cytochrome *c*. In the presence of ferricytochrome b_5, the oxidation-state lifetimes of ferrocytochrome *c* of both variants and horse heart cytochrome *c* are reduced. This process is concentration independent and leads to values for intracomplex electron exchange of 1.4, 0.5, and 0.7 s^{-1} for horse heart cytochrome *c*, Cys-102-Thr, and Phe-82-Gly, Cys-102-Thr variants of yeast iso-1-cytochrome *c*. The substitution of Phe-82 with glycine therefore has a negligible effect on the first-order rate of electron transfer.

Introduction

Our understanding of the structural factors governing rates of electron transfer between transition metal complexes in solution has benefited greatly from measurements of electron self-exchange rates.[1] Such measurements have featured less prominently in studying structural constraints on long-range electron transfer between metal centers buried within redox proteins, many of which remain ill-defined. The purpose of this paper is to show how equilibrium measurements of electron-exchange rates can provide insight into the part played by the medium in one prototypal long-range electron transfer reaction, namely, that between the redox proteins mitochondrial cytochrome *c* and bovine microsomal cytochrome b_5.

The availability of high-resolution X-ray crystallographic structures for several variants[2] of cytochrome *c* has encouraged much work on the protein's electron-transfer properties and how these are influenced by individual residues, both in the protein's interior and on its surface. One residue, phenylalanine-82, has been the subject of mutational analysis in an attempt to estimate its role in protein electron-transfer reactivity.[3] The invariance of this residue,[3b] its proximity to the edge of the protein's heme group, and its simulated dynamic properties have all supported a possible role in physiological electron transfer.[3c] Moreover, replacement of Phe-82 of yeast cytochrome *c* with the nonaromatic residues Gly, Ser, Leu, and Ile leads to a dramatic 10000-fold decrease in the rate of electron transfer from ferrocytochrome *c* to the π cation radical state of Zn-substituted cytochrome *c* peroxidase in the binary complex.[3d] Paradoxically, these same mutations do not appear to prevent the growth of yeast cells.

In this paper we describe the use of nuclear magnetic resonance (NMR) spectroscopy to determine the effect of substituting Phe-82 with Gly on the rates of two electron-exchange reactions involving yeast iso-1-cytochrome *c*: (1) conventional bimolecular electron self-exchange between ferrocytochrome *c* and ferricytochrome *c*, and (2) unimolecular "cross" electron exchange between ferrocytochrome *c* and the tryptic fragment of ferricytochrome b_5, a small (*M_r 11 500) heme protein with which cytochrome *c* forms a binary complex at low ionic strength. Our results show that in both cases the mutation has a negligible effect on the rate of electron exchange. We conclude that, although the evidence for Phe-82 modulating electron transfer in the context of a large and favorable thermodynamic driving force appears to have been established,[3e] this residue plays no direct role in electron transfer when the driving force is small, here approximately 250 mV.

Materials and Method

Cytochrome b_5 was isolated and purified as the tryptic fragment of the bovine microsomal protein using the previously published protocols, and for all NMR experiments the $A_{413/280}$ ratio was greater than 5.7.[4] Horse heart cytochrome *c* (Sigma Chemical Co. Type VI) was purified by chromatography on CM-cellulose to remove deamidated forms of the protein.[4d] Mutants of yeast *Saccharomyces cerevisiae* iso-1-cytochrome *c* were obtained by site-directed mutagenesis and purified as described previously.[5] Protein concentrations were determined spectrophotometrically at 413 and 556 nm for cytochrome b_5 assuming extinction coefficients of 117 and 19 mM^{-1} cm^{-1}, respectively, while for cytochrome *c* extinction coefficients at 550 nm of 29 and 9 mM^{-1} cm^{-1} were used for ferro- and ferricytochrome *c*, respectively. Mixtures of ferrocytochrome *c*, ferricytochrome *c*, and ferricytochrome b_5 were obtained by adding appropriate volumes of known stock concentrations to an NMR tube containing a total sample volume of 400 μL. Fully reduced samples were obtained by adding a minimal quantity of solid sodium dithionate to a deoxygenated sample immediately prior to data collection. The fully reduced sample was kept under an atmosphere of N_2 and remained fully reduced for approximately 10 h under these conditions.

^{1}H NMR spectra were recorded on a Bruker AM600 FT spectrometer equipped with an Aspect 3000 computer. Free induction decays (FID's) in saturation transfer experiments were accumulated with a sweep width

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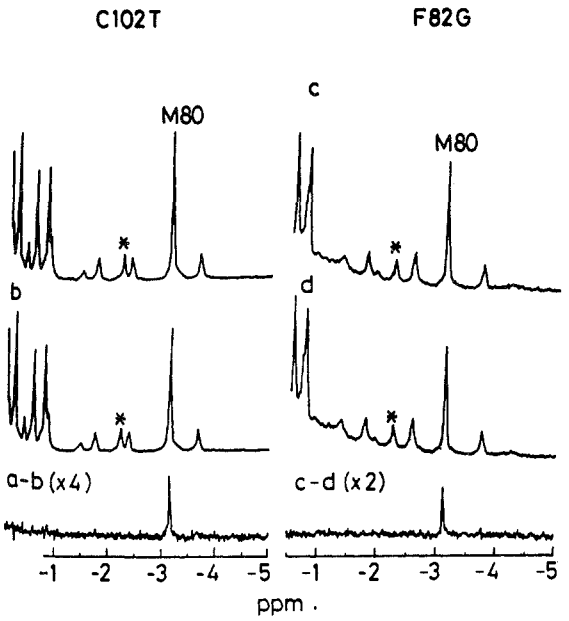

Figure 1. NMR spectra (600-MHz) showing saturation transfer from the Met-80 methyl group of ferricytochrome *c* to the corresponding group of ferrocytochrome *c* for two variants of yeast iso-1-cytochrome *c*. On- and off-resonance spectra are shown for the Cys-102-Thr, and Cys-102-Thr, Phe-82-Gly variants. The total protein concentration was 2 mM; all measurements were made in 50 mM phosphate, pH 6.3, and with a presaturation pulse of 2 s (temp 300 K). The * indicates the presence of peaks due to ferricytochrome *c*. On-resonance spectra were irradiated at the frequency of the ϵ-methyl of the Met-80 group in the oxidized protein.

of 50 000 Hz on 16K data points. A presaturation period of 1 s was employed in both on- and off-resonance spectra that were otherwise recorded under identical conditions. T_1 measurements were recorded using the standard nonselective inversion recovery pulse sequence ($D1-\pi-t-\pi/2$).

Results

Horse heart cytochrome *c* has been the subject of many electron self-exchange studies, and in this study we compare the exchange properties with those of the two variants of yeast iso-1-cytochrome *c*. The preparation and initial characterization of these mutants are described in earlier papers.[5] The replacement of Cys-102 was done to circumvent any potential problems that might arise from the formation of intermolecular disulfide bonds. For all three variants the spectra of ferrocytochrome *c* in the presence and absence of ferricytochrome *c* contained a distinctive, resolved methyl resonance at approximately 3.25 ppm. This resonance has been assigned to the ϵ-methyl of Met-80, one of the two axial ligands to the iron center, and its chemical shift in the spectra of the two yeast variants was characteristic of the reduced protein in its folded state. The presence of a paramagnetic center in ferricytochrome *c* shifts the resonance of the same group upfield by approximately 20 ppm.

The electron self-exchange rate of mitochondrial cytochrome *c* is modulated strongly by ionic strength and counterions, but in the absence of multivalent anions it is normally slow on the NMR timescale.[6] Concordant with this observation, the presence of ferricytochrome *c* in samples of ferrocytochrome *c* had no detectable effect on NMR line widths in any of the proteins, even in the presence of 50 mM potassium phosphate (spectra not shown). However, slow chemical exchange between ferro- and ferricytochrome *c* was evident from the measurement of saturation transfer. Preirradiation at the frequency of the Met-80 methyl resonance of ferricytochrome *c*, at a power level sufficient to abolish the resonance in the spectrum, caused the intensity of the corresponding resonance of ferrocytochrome *c* to decrease for all three protein variants (Figure 1).

Table I. Comparison of Self-Exchange Rates of Horse and Yeast Cytochromes *c*[a]

variant	T_1 (s)	k_{ex} (s^{-1})
horse	1.29 (0.04)	850 (30)
Cys-102-Thr	1.61 (0.07)	200 (20)
Cys-102-Thr, Phe-82-Gly	1.49 (0.06)	350 (25)

[a] T_1 values for the Met-80 methyl resonance of ferrocytochrome *c* were obtained by curve fitting a single exponential function to inversion recovery data points obtained at 600 MHz. Bimolecular rate constants, k_{ex}, were obtained from lifetimes, *t*, of ferrocytochrome *c* in the presence of ferricytochrome *c* as indicated in the text.

According to the classical theory of magnetization transfer, the ratio of the reduced (I) to normal (I_0) intensities when measured at equilibrium is given by

$$I/I_0 = t/(t + T_1) \quad (1)$$

where *t* is the oxidation state lifetime, a function of the exchange rate between two states, of ferrocytochrome *c*, and T_1 is the normal longitudinal relaxation time of the Met-80 ϵ-methyl group measured in the absence of ferricytochrome *c*. Thus, a knowledge of the longitudinal relaxation times (T_1's) of the Met-80 methyl group of each reduced protein measured from conventional inversion recovery pulse sequences[7] enabled the oxidation state lifetime, *t*, to be estimated from corresponding intensity decreases. From an integration of NMR peaks and spectrophotometric measurements, the concentration of ferro- and ferricytochrome *c* was known and used to calculate bimolecular self-exchange rates, k_{ex}, using the equation

$$k_{ex} = 1/(t[Fe^{III}Cyt\ c]) \quad (2)$$

The T_1 values, measured at 600 MHz, and exchange rates derived from oxidation state lifetimes are given in Table I, which reveals that the electron self-exchange reactivities of all three protein, when measured under the same experimental conditions, are between 200 and <1000 $M^{-1}\ s^{-1}$, with the yeast iso-1-cytochromes *c* showing consistently slower bimolecular exchange rates.

Electron "Cross-Exchange" Measurements. The use of NMR to determine the oxidation-state lifetimes of redox proteins in solution is not confined to electron-exchange measurements. Cytochrome *c* can be cycled between its two oxidation states by virtue of electron exchange with a second, different, redox species present in the same solution. Electron exchange with the ferri-/ferrocyanide redox couple, for example, has been detected by NMR methods[8] as has exchange between cytochrome *c* and the nonphysiological redox partner plastocyanin.[8b] The main constraint on such measurements is that the cross-electron-exchange rate should be high enough to render chemical exchange between ferro- and ferricytochrome *c* amenable to detection by NMR line broadening or saturation transfer, but not so high that the system enters the fast-exchange region. Using the Met-80 methyl resonance of ferrocytochrome *c* as an indicator of exchange means that any decrease of the oxidation-state lifetime of the reduced protein in the presence of a second redox species must be comparable to the longitudinal relaxation time (T_1) of the Met-80 methyl resonance if it is to be detectable by saturation transfer. With these considerations in mind, we used NMR to determine the effects of the tryptic fragment of bovine microsomal ferricytochrome b_5 on the apparent rate of chemical exchange between ferro- and ferricytochrome *c*, initially for the horse heart protein and then for the two yeast iso-1-mutants.

At low ionic strengths, cytochrome *c* and cytochrome b_5, when present in equimolar concentrations in solution, form a tight ($K_a \sim 10^5\ M^{-1}$) 1:1 complex[9b] with an estimated difference in their

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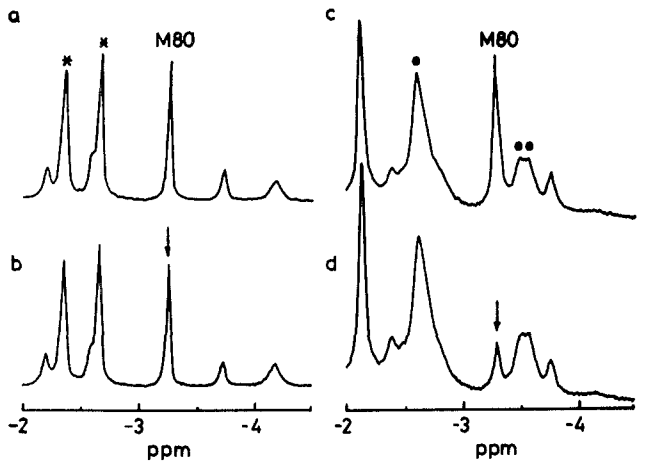

Figure 2. The effect of ferricytochrome b_5 on the relative decrease in the magnitude of the intensity of the Met-80 ϵ-methyl resonance of ferrocytochrome c observed upon presaturation of the corresponding resonance in ferricytochrome c. (a,b) off-resonance spectra in the absence of ferricytochrome b_5; (c,d) on-resonance spectra observed in the presence of equimolar ferricytochrome b_5. All spectra were recorded at 600 MHz and 303 K. Samples a and b contain 2 mM ferri-/ferrocytochrome c in 20 mM phosphate, pH 7.0, while samples c and d contain 1 mM ferro-/ferricytochrome b_5 in the presence of 10 mM phosphate, pH 7.0. * and ● indicates the positions of peaks in the spectra of ferricytochrome c and ferricytochrome b_5, respectively.

midpoint potentials of approximately 200 mV.[10] This difference in midpoint potentials is such that the equilibrium shown below lies firmly to the left:

ferrocytochrome c:ferricytochrome b_5 ⇄
ferricytochrome c:ferrocytochrome b_5

Slow electron exchange against the thermodynamic barrier from ferrocytochrome c to ferricytochrome b_5 followed by rapid reverse electron transfer could therefore affect the oxidation-state lifetime of ferrocytochrome c without generating more than a trace amount of the oxidized protein.

"Cross" Exchange between Horse Cytochrome c and Cytochrome b_5. Initially, we performed saturation transfer experiments on mixtures of horse heart ferro- and ferricytochrome c in both the absence of a protein partner and in the presence of equimolar ferricytochrome b_5. In the latter case, the solution conditions (2 mM protein, 10 mM phosphate, pH 7.1) were conductive to the formation of a binary complex. In both cases preirradiation at the frequency of the Met-80 methyl resonance of ferricytochrome c (−24.2 ppm) resulted in decreases in the intensities of the corresponding resonance of ferrocytochrome c, indicative of slow chemical exchange. However, the presence of ferricytochrome b_5 had a dramatic effect on the size of the intensity reduction (Figure 2). From the intensity decreases we determined t/T_1, the ratio of the oxidation-state lifetime of ferrocytochrome c to the intrinsic longitudinal relaxation time of the Met-80 methyl group using the standard equation (1). The t/T_1 ratio was determined to be 9 in the absence of a protein partner and approximately 0.4 in the presence of equimolar ferricytochrome b_5. As the T_1 of the Met-80 methyl resonance increases only from 1.29 s to 1.78 s in the presence of ferrocytochrome b_5, we conclude from these data that ferricytochrome b_5 is effective in shortening the oxidation state lifetime of ferrocytochrome c above that caused by ferricytochrome c alone.

In the case of bimolecular electron self-exchange, the lifetime shortening of ferrocytochrome c is inversely proportional to the total concentration of ferricytochrome c. It is significant therefore that the results of further saturation transfer experiments revealed that the intensity reduction of the Met-80 methyl resonance of ferrocytochrome c determined in the presence of ferricytochrome

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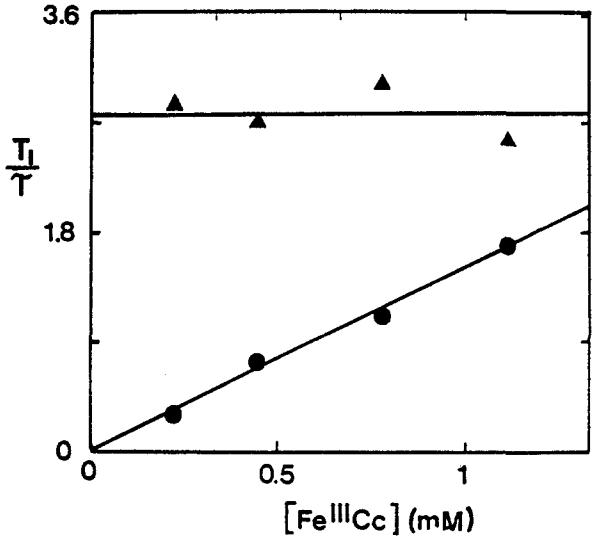

Figure 3. The magnetization transfer ratio $(I_0 - I)/I$ as a function of the ferri-/ferrocytochrome c mole ratio in the absence of a protein partner and in the presence of equimolar ferricytochrome b_5. (●) absence of ferricytochrome b_5, (▲) presence of equimolar ferricytochrome b_5. Conditions were as for Figure 2.

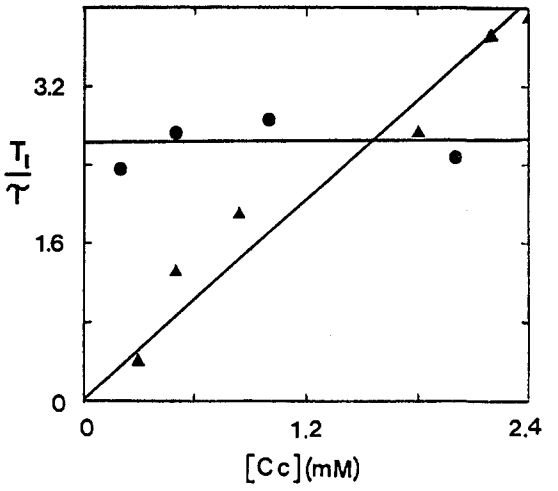

Figure 4. The dependence of the magnetization transfer ratio $(I_0 - I)/I$ on the total protein concentration in the presence and absence of equimolar ferricytochrome b_5: (▲) absence of ferricytochrome b_5, (●) presence of 2 mM ferricytochrome b_5. Intensity measurements were made from saturation transfer spectra recorded at 600 MHz and 303 K. In the absence of ferricytochrome b_5, measurements were made in 50 mM phosphate, pH 7.0, whereas in the presence of ferricytochrome b_5 the solution conditions were 10 mM sodium phosphate, pH 7.1.

b_5 is independent of the bulk concentration of ferricytochrome c. Neither uniform dilution of ferrocytochrome c, ferricytochrome c, and ferricytochrome b_5 in the concentration range 0.2–2.0 mM nor alteration of the ferri-/ferrocytochrome c ratio in the presence of a constant concentration of ferricytochrome b_5 led to significant changes in the degree of saturation transfer (Figures 3 and 4). In marked contrast, the intensity reduction measured in the absence of ferricytochrome b_5 was always dependent on protein concentration. Additionally, increasing the ionic strength of the solution causes a decrease in ferricytochrome b_5 catalyzed saturation transfer that reflects the decline in the formation of protein complexes. This is also apparent from an observed shortening of the T_1's of the Met-80 ϵ-methyl of ferrocytochrome c upon elevating the ionic strength of a solution containing equimolar ferrocytochrome c and b_5 (results not shown).

Taken together these results indicate that the lifetime of ferrocytochrome c in the presence of ferricytochrome b_5 is not governed by bimolecular electron self-exchange, since this would require a dependence of saturation transfer on the concentration

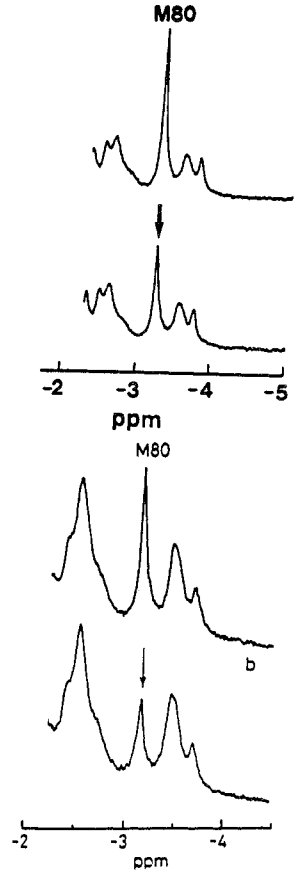

Figure 5. Saturation transfer spectra showing the effect of preirradiating at the frequency of the Met-80 methyl resonance of ferricytochrome *c* on the intensity of the corresponding resonance of ferrocytochrome *c* for the Cys-102-Thr and Thr-102-Cys, Phe-82-Gly variants of yeast iso-1-cytochrome *c* in the presence of equimolar ferricytochrome b_5: (a) Cys-102-Thr, (b) = Cys-102-Thr, Phe-82-Gly. In each case the total protein concentration was 2 mM (10 mM phosphate, pH 7.2), temp 303 K.

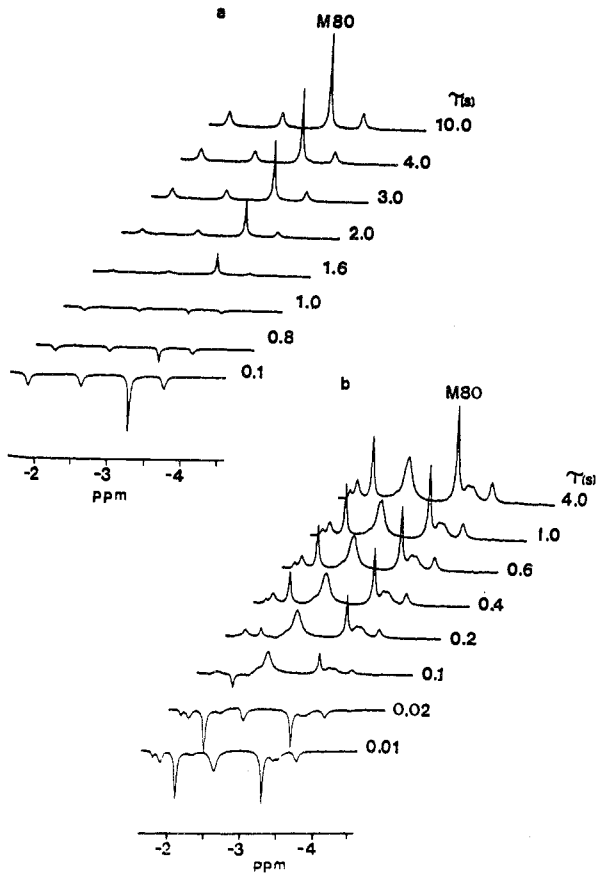

Figure 6. Representative spectra of inversion recovery for horse heart and Cys-102-Thr cytochromes *c* in the presence and absence of ferricytochrome b_5. Spectra show the Met-80 ϵ-methyl resonance of horse heart cytochrome *c* in the presence of ferrocytochrome b_5 (a) and ferricytochrome b_5 (b). Spectra were recorded at 600 MHz and 300 K. Protein concentration was 1 mM ferrocytochrome *c* and 1 mM ferro-/ferricytochrome b_5 in 10 mM phosphate, pH 7.1.

of ferricytochrome *c*, but by a unimolecular redox process. An obvious candidate for such a process is slow electron transfer from ferrocytochrome *c* to ferricytochrome b_5 within a 1:1 complex of the two proteins. Rapid electron transfer in the reverse direction ensures that only a trace amount of ferricytochrome *c* is ever involved in the chemical-exchange process.

Cross Exchange between Yeast Iso-1 Variants and Cytochrome b_5. Similar saturation transfer experiments performed with the yeast iso-1-cytochrome *c* variants in the presence and absence of ferricytochrome b_5 revealed an analogous effect of the protein partner on intensity reductions of the Met-80 methyl group of ferrocytochrome *c* (Figure 5). In the case of both the Thr-102-Cys and the Thr-102-Cys, Phe-82-Gly mutants, preirradiation at the frequency of the Met-80 methyl resonance of ferricytochrome *c* caused a negligible intensity decrease for the corresponding resonance of ferrocytochrome *c* when the relative amount of ferricytochrome *c* was no more than 10%. In the presence of equimolar ferricytochrome b_5, however, the intensity decreases for comparable mixtures were approximately 44% and 53% for the Thr-102-Cys and Thr-102-Cys, Phe-82-Gly mutants, respectively. The T_1 values of the Met-80 ϵ-methyl group of both variants of yeast iso-1-ferrocytochrome *c* were not altered significantly by the addition of equimolar ferrocytochrome b_5 (data not shown), indicating that the intensity reductions were caused by a shortening of the oxidation-state lifetime of ferrocytochrome *c* by ferricytochrome b_5. Moreover, for both yeast variants the effects of ferricytochrome b_5 on saturation transfer were independent of the total protein concentration.

Effect of Ferricytochrome b_5 on Longitudinal Relaxation. The physical basis of saturation transfer lies in the effect of chemical exchange on longitudinal relaxation.[6b] In the case of the Met-80 methyl group of cytochrome *c*, electron exchange results in a mixing of the longitudinal relaxation properties of the group in ferrocytochrome *c* with those of the group in ferricytochrome *c*. In the slow-exchange limit, the classical theory of magnetization transfer predicts that such mixing should produce biexponential relaxation. To verify our interpretation of the saturation transfer data, we therefore determined the effect of ferricytochrome b_5 on the longitudinal relaxation properties of the Met-80 methyl group of ferrocytochrome *c* using the standard inversion recovery pulse sequence. We measured the dependence of peak height on the delay period for all three proteins initially in the presence of ferrocytochrome b_5 and then in the presence of equimolar ferricytochrome b_5. Examples of the relevant spectra and curve fitting are shown in Figures 6 and 7, respectively. For all three proteins, relaxation was monoexponential in the presence of ferrocytochrome b_5 (Figure 7, i and ii, top) but distinctly biexponential in the presence of ferricytochrome b_5 (Figure 7, i and ii, bottom). These data strengthen the view that saturation transfer in the presence of oxidized cytochrome b_5 is a result of intracomplex cross exchange.

Comparison of Cross-Exchange Rates. From the results above, we conclude that for yeast and horse cytochromes *c* there exists at equilibrium a small but finite amount of the complex $[Fe^{III}C_c{:}Fe^{II}C_{b_5}]$ in exchange with the predominant complex $[Fe^{II}C_c{:}Fe^{III}C_{b_5}]$. The exchange process is therefore

$$[Fe^{III}C_c{:}Fe^{II}_{b_5}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Fe^{II}C_c{:}Fe^{III}_{b_5}]$$

where k_1 and k_{-1} are the rapid, driving force assisted, and slow "reverse" rates of electron transfer, respectively. In the saturation-transfer experiment, magnetization arriving at the second site, corresponding to the Met-80 methyl group of ferricytochrome *c*,

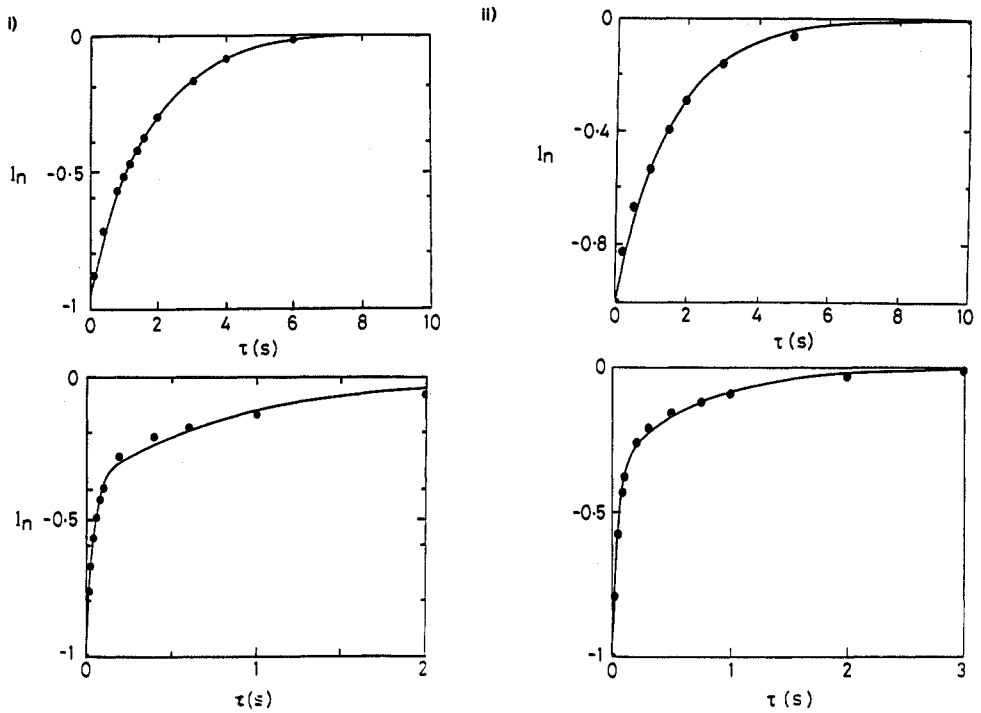

Figure 7. Curve-fitting data for horse heart and Cys 102 Thr cytochrome *c* in the presence and absence of ferricytochrome b_5. (i) Normalized intensity of the Met-80 methyl resonance as a function of the variable delay in the presence of equimolar ferrocytochrome b_5 (top) and ferricytochrome b_5 (bottom). (ii) normalized intensity of the Met-80 methyl resonance of Cys-102-Thr iso-1-cytochrome *c* as a function of the variable delay in the presence of equimolar ferrocytochrome b_5 (top) and ferricytochrome b_5 (bottom).

is immediately quenched. As a consequence, the reduction in the intensity of the Met-80 methyl resonance of ferrocytochrome *c* is related directly to the lifetime of ferrocytochrome *c* bound to ferricytochrome b_5, and hence k_{-1}. On analyzing the intensity reductions with eq 1 and a modified form of eq 2 representing a first-order exchange process ($k_{ex} = 1/t$), we obtained values for k_{-1} of 1.4 (0.2) s^{-1}, 0.5 (0.2) s^{-1}, and 0.7 (0.2) s^{-1}, respectively, for horse heart, yeast iso-1-Thr-102-Cys and yeast iso-1-Thr-102-Cys, Phe-82-Gly cytochromes *c* (300 K, 10 mM phosphate, pH 7.1). Significantly, these values are of similar magnitude, which suggests that the rate of electron transfer from ferrocytochrome *c* to ferricytochrome b_5 is not affected markedly by substitutional differences between the variants and, in particular, by the substitution of Phe-82 by Gly.

Discussion and Conclusions

Effect of Phe-82 on Bimolecular Electron Self-Exchange. In assessing the role of Phe-82 in electron transfer, there are at least two major factors to consider. First, the bulk of the side chain is such that the residue presumably fills an important volume within the protein structure and thereby contributes to the stability of the heme crevice. When the Phe side chain is removed, X-ray crystallographic studies of the Gly-82 variant suggest that the protein's conformation changes although the heme edge exposure to solvent remains constant.[2e] However, the same mutations also lead to an increase in the susceptibility of the heme ligands to nucleophilic substitution[11] as well as a disruption of the internal H-bond network centered around an internal water molecule (WAT-112). Perturbation of the polypeptide fold is borne out by the change in the redox potential that accompanies the Phe-82-Gly mutation.[11b] All other things being equal, the removal of the Phe ring might be expected to facilitate electron transfer by reducing the average separation distance of heme centers. Second, it is thought that the delocalized electron density of the aromatic ring could enhance electronic coupling by forming a conduction path between the donor and acceptor metal centers. Support for the involvement of aromatics in electron transfer has come from the detection of Tyr and Trp radicals in high-energy intermediates in the catalytic cycle of biological systems,[12] although the involvement of an aromatic ring in conduction may only occur when the driving force for the reaction is relatively high (0.5–1.0 V).[12f]

For electron self-exchange reactions the thermodynamic driving force is zero, and it is clear from the results presented in this study that Phe-82 of cytochrome *c* does not contribute directly to electron transfer with cytochrome b_5 under these conditions. It should be noted that in many physiological redox reactions the driving forces are frequently less than 100 mV, for example, the reduction and oxidation of cytochrome *c* by cytochrome c_1 of complex III and the Cu_a center of cytochrome oxidase.[13]

Effect of Phe-82 on Cross Exchange with Cytochrome b_5. The saturation transfer data indicate that the lifetime of ferrocytochrome *c* in the presence of ferricytochrome b_5 is significantly shortened owing to first-order electron transfer from ferrocytochrome *c* to ferricytochrome b_5 within the preformed binary complex. For horse heart cytochrome *c* the rate of this reaction, which occurs against a thermodynamic driving force of approximately 200 mV, is 1.4 s^{-1} at 303 K. In the absence of potentially complicating factors such as conformational changes or electron "gating", the thermodynamically favorable reaction can be described according to the equation $k_f = k_b K_{eq}$ and is predicted to be 3×10^3 s^{-1}. Using a pulse radiolysis technique, McLendon and Miller[14a] determined the rate of the forward electron-transfer reaction to be 1.6×10^3 s^{-1} at low ionic strength (1 mM phosphate) and room temperature. In view of the known variation of the rate

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of this reaction with temperature and ionic strength, the numbers are generally within experimental error. The reaction rates observed here are also comparable with those measured for the rate of unimolecular electron self-exchange within oligomers of cytochrome c.[14b] It is perhaps useful to compare the estimated first-order electron-transfer rates with the bimolecular rate for electron transfer from ferrocytochrome b_5 to ferricytochrome c.[14c,15] At 298 K, this rate is $\sim 3 \times 10^7$ M^{-1} s^{-1} (100 mM phosphate, pH 7.0), which, assuming a 200-mV driving force, leads to a hypothetical reverse electron-transfer rate of approximately 10^3 M^{-1} s^{-1}. If the concentration of ferricytochrome b_5 is 1 mM, this would give a notional "lifetime" for ferrocytochrome c of 1 s under these solution conditions. The notional lifetime is similar to the actual lifetime measured at low ionic strength, implying that complex formation may not promote electron transfer. One explanation for this would be that, in the ground state of the binary complex formed at low ionic strength, cytochrome c and cytochrome b_5 are locked in a geometry that requires a form of configurational rearrangement prior to electron transfer.[14c] The replacement of Phe-82 with Gly in yeast iso-1-ferrocytochrome c has no effect on the lifetime of the protein in the presence of ferricytochrome b_5, indicating that the presence of an aromatic group at position 82 is not essential for electron transfer from ferrocytochrome c to ferricytochrome b_5. Taken together with the electron self-exchange data, the results argue strongly against the direct involvement of the aromatic group in physiological electron-transfer reactions whose driving forces are relatively low. In conclusion, we have measured the reverse rate of electron transfer within a preformed binary complex of cytochrome c and cytochrome b_5 by NMR methods. This rate is not accessible to measurement using many prevailing methods and has given insight into the possible role of phenylalanine-82 of cytochrome c in biological electron transfer.

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Reactions of Co_{1-4}^+ and $Co_4(CO)_n^+$ with Cyclohexane: C-H Activation as a Function of Cluster Size and Ligand Substitution

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Abstract: Rate constants and product distributions for the bimolecular gas-phase reaction of cyclohexane with Co^+, Co_2^+, Co_3^+, $Co_4(CO)_n^+$ (n = 0-12), Ir_2^+, and Ir_3^+ are reported. All of the ions except Co_2^+ and $Co_4(CO)_n^+$ (n = 2-4, 8-12) react with observable rates. In most cases the predominant product is C_6H_6 bound to the metal cluster. Observed products of the Co^+ reaction and the failure of Co_2^+ to react agree with previously reported results. The results for $Co_4(CO)_n^+$ are compared with previously reported results for $Ir_4(CO)_n^+$ and $Re_4(CO)_n^+$. Like the third-row clusters, the reactivity of the Co tetramer diminishes and disappears as ligands are added to increase the number of cluster valence electrons to more than 48. $Co_4(CO)_7^+$ reacts slowly and the $Co_4(CO)_{8-12}^+$ do not react at an observable rate. This can be rationalized on the basis of frontier orbital theory. It is found on examining the cluster molecular orbitals with extended Hückel calculations that the failure of Co_2^+ and $Co_4(CO)_{2-4}^+$ to react can also be rationalized on the basis of frontier orbital theory. The contrast between the failure of $Co_4(CO)_4^+$ to react and the reactivity of $Co_4(CO)_5^+$ is attributed to a change from a high-spin electronic configuration of the former to a low-spin configuration for the latter.

Introduction

The activation of C-H bonds by gas-phase metal clusters has been the subject of a number of recent studies[1-6] which raise a variety of interesting questions. The Mn_2^+ and Co_2^+ dimer ions are unreactive with alkanes,[1a] but $Co_2(CO)^+$ reacts with butane to form $Co_2(CO)C_4H_8^+$.[1b] $Re_3(CO)_n^+$,[1c] $Re_4(CO)_n^+$,[1c] and $Ir_4(CO)_n^+$[1d] all dehydrogenate cyclohexane provided n is not too large. The critical value of n for each case can be rationalized in terms of frontier orbital theory.[1d] Several MFe^+ species (M = Co,[2b] V,[2c] Cu[2d]) do not react with alkanes, but $LaFe^+$ and Co_2Fe^+ do.[2e,a] Neutral Pt_n (n = 2-8) clusters dehydrogenate small alkanes in a fast flow reactor.[3a] In this case both the extent of dehydrogenation and the number of molecules absorbed increase with cluster size. Neutral Nb_n (n = 4-13) clusters dehydrogenate benzene.[3b,6] The reactivity of the Nb clusters varies with cluster size in the same way that ionization potential varies with cluster

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