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Pressure Effects on the Rates of Intramolecular Electron Transfer in Ruthenium-Modified Cytochrome *c*. Role of the Intervening Medium in Tuning Distant Fe^{2+}:Ru^{3+} Electronic Couplings

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The rates of electron-transfer (ET) processes exhibit characteristic pressure dependences that provide information about the transition states for these reactions.[1-12] Recent studies on ruthenium-modified cytochrome *c* (cyt *c*) using high-pressure pulse radiolysis and stopped-flow techniques[11,12] have revealed negative activation volumes for the following reactions: intramolecular ET in horse heart $(NH_3)_5Ru^{2+}(His33)Fe^{3+}$-cyt *c* and *Candida krusei* $(NH_3)_5Ru^{2+}(His39)Fe^{3+}$-cyt *c* and intermolecular ET between horse heart Fe^{3+}-cyt *c* and $Ru(NH_3)_6^{2+}$ ($\Delta V^{\ddagger} = -17.7 \pm 0.9$, -18.3 ± 0.7, and -15.6 ± 0.6 $cm^3\ mol^{-1}$, respectively).[11] The intermolecular reduction of horse heart Fe^{3+}-cyt *c* by $Ru(NH_3)_5isn^{2+}$ (isn = isonicotinamide) has $\Delta V^{\ddagger} = -17.2 \pm 1.5$ $cm^3\ mol^{-1}$, whereas the reverse process exhibits a positive $\Delta V^{\ddagger}$ (16.0 ± 1.5 $cm^3\ mol^{-1}$).[12] The change in sign in the latter case, as well as the similar magnitude of all cyt *c* $\Delta V^{\ddagger}$ values, suggests that the observed activation volumes are mainly associated with solvent electrostriction around the ruthenium ammine centers. In fact, the overall reaction volume change for the one-electron oxidation of $Ru(NH_3)_5isn^{2+}$, calculated to be -33 ± 3 $cm^3\ mol^{-1}$ from the difference in $\Delta V^{\ddagger}$ values for the forward and reverse reactions (and measured to be -31 ± 1 $cm^3\ mol^{-1}$ from the pressure dependence of the overall equilibrium constant for the redox reaction with cytochrome *c*),[12] is very close to the value of -28.7 ± 0.8 $cm^3\ mol^{-1}$ determined from the pressure dependence of the redox couple $Ru(NH_3)_6^{2+/3+}$ after correction for the volume change associated with the Ag/Ag^+ reference electrode.[13-15] It follows that all the available data indicate that the observed volume changes associated with the redox reactions between cytochrome *c* and ruthenium ammine complexes can be interpreted in terms of volume changes associated with the ruthenium center.[16-18]

The ET reactions of $(NH_3)_5Ru(His33)Zn$-cyt *c* have been investigated at ambient pressure using a laser flash kinetic technique.[19-21] Intramolecular ET is initiated in this system by photoexcitation of the Zn-porphyrin (ZnP) to its strongly reducing triplet excited state (ZnP*). This triplet state decays by ET to the histidine-bound $Ru(NH_3)_5^{3+}$ complex (ET*) to produce the metastable $Ru^{2+}ZnP^{\bullet +}$ species, which relaxes via a thermal ET process (ET^b) to reform the ground-state $Ru^{3+}ZnP$ complex.[19-21] Both ET processes (ET* and ET^b) have been studied as a function of pressure up to 150 MPa.[22-26] The $ZnP^* \rightarrow (NH_3)_5Ru^{3+}(His33)$ rate constant exhibits no significant dependence on pressure, whereas the ET^b rate $[(NH_3)_5Ru^{2+}(His33) \rightarrow ZnP^{\bullet +}]$ increases markedly with increasing pressure ($\Delta V^{\ddagger} = -12 \pm 1$ $cm^3\ mol^{-1}$; Figure 1). The higher driving force for the Zn-cyt *c* reaction[20] may account for the smaller pressure effect compared to the Fe-cyt *c* case. The observation of both a negative activation entropy[21] and a negative activation volume indicates that the transition state for the ET^b process is highly ordered. The absence of any significant pressure dependence for the ET* process can be interpreted in terms of roughly equal and opposite volume changes associated with the donor and acceptor redox complexes. Reduction of Ru^{3+} to Ru^{2+} should be accompanied by a volume increase due to a decrease in solvent electrostriction.[11,12] However, ET* occurs from ZnP*, which is expected to have a higher partial molar volume than $ZnP^{\bullet +}$ (possibly by as much as 10–15 $cm^3\ mol^{-1}$).[1,27-29] The ET* reaction therefore involves a ZnP* to $ZnP^{\bullet +}$ volume collapse and a similar Ru^{3+} to Ru^{2+} volume increase.

We have examined the effects of pressure on distant electronic coupling in $Ru(bpy)_2(im)$-modified (bpy = 2,2′-bipyridine; im = imidazole) His33 and His72 cytochrome *c* derivatives, whose $Fe^{2+} \rightarrow Ru^{3+}$ reactions are activationless ($-\Delta G^\circ \sim \lambda$).[24] Photoexcitation of $(bpy)_2(im)Ru^{2+}(His)Fe^{2+}$-cyt *c* in the presence of a quencher results in the rapid formation of $(bpy)_2(im)Ru^{3+}(His)Fe^{2+}$-cyt *c*, which undergoes $Fe^{2+} \rightarrow Ru^{3+}$ ET to produce $(bpy)_2(im)Ru^{2+}(His)Fe^{3+}$-cyt *c*. In the case of the His33-modified system, the observed $Fe^{2+} \rightarrow Ru^{3+}$ ET rate constant exhibits no dependence on pressure within experimental error limits. However, the rate constant for the His72-modified protein increases significantly with increasing pressure (Figure 1), corresponding to an *apparent* negative activation volume of -6 ± 2 $cm^3\ mol^{-1}$.

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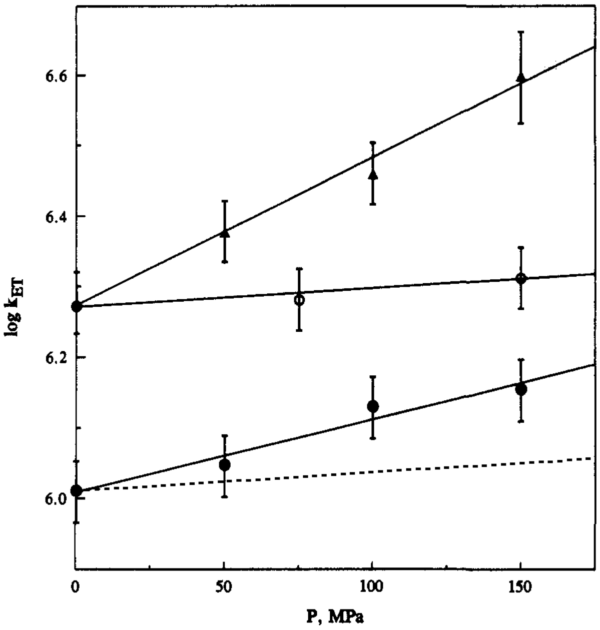

Figure 1. Pressure dependences of intramolecular ET rates in Ru-modified cytochromes *c*. Solid lines are linear least-squares fits. (▲) $Ru^{2+} \rightarrow ZnP^{\bullet+}$ ET in $(NH_3)_5Ru(His33)Zn$-cyt *c*. (○) $Fe^{2+} \rightarrow Ru^{3+}$ ET in $(bpy)_2(im)Ru(His33)Fe$-cyt *c*. (●) $Fe^{2+} \rightarrow Ru^{3+}$ ET in $(bpy)_2(im)Ru(His72)$-Fe-cyt *c*. Dashed line indicates the expected pressure dependence based on the His33 data.

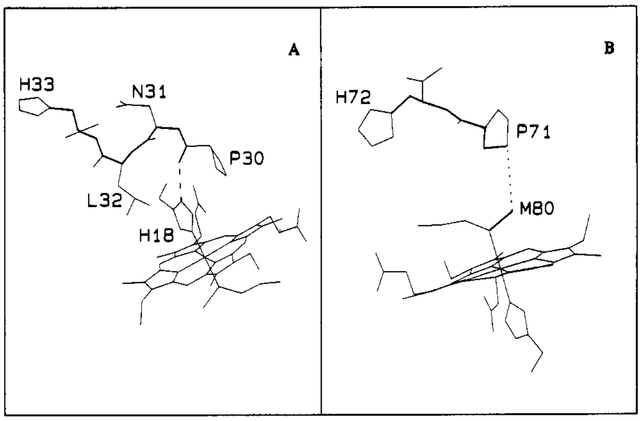

Figure 2. Dominant σ-tunneling pathways for intramolecular ET in $(bpy)_2(im)Ru(HisX)Fe$-cyt *c*: (A) X = 33; (B) X = 72.[24,31,33]

Since a negative ΔV^* is exactly opposite that expected on the basis of a decrease in electrostriction during reduction of the $Ru(bpy)_2(im)(His)^{3+}$ moiety, we conclude that the increase in the ET rate constant is a result of an increase in electronic coupling at elevated pressure.[30]

We suggest that the application of moderate pressures causes a slight compression of cyt *c* that in turn shrinks the through-space gaps that are key units in the calculated electron-tunneling pathways between the heme and His72.[24,31,32] In the most direct σ-tunneling pathway,[24] the electronic coupling depends strongly on the distance between Pro71 and Met80 (Figure 2). Any decrease in the Pro71–Met80 distance will increase the Fe^{2+}:Ru^{3+} electronic coupling in the His72-modified protein and accelerate the ET process. A more quantitative analysis of the observed effect can be obtained from the correlation reported between maximum ET rate constants and σ-tunneling lengths.[24,31,33] Accordingly, the increase in ET rate constant from 1.0×10^6 to 1.4×10^6 s^{-1} on increasing the pressure by 150 MPa corresponds to a decrease of 0.46 Å in the tunneling path length. This in turn indicates that there is an average decrease of 0.1 Å in Pro71–Met80 and other space jumps in the heme-to-His72 tunneling pathways.[24,31-33] The absence of an effect on the $Fe^{2+} \rightarrow Ru^{3+}$ ET rate in the His33-modified species suggests that electronic coupling through covalent bonds and hydrogen bonds is less sensitive to pressure than is coupling *via* van der Waals gaps.

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