only the first and second atoms (a peripheral steric effect). However, the *tert*-butyl group does encounter some interaction with the adamantane group, as there is NMR evidence for restricted rotation of this group. By comparison, the (2×10^2)-fold greater reduction in *tert*-butyl isocyanide binding to the anthracene–heme–6,6-cyclophane compared with the adamantane–heme–6,6-cyclophane perhaps is a result of the anthracene "roof" being unable to swing to one side of the ligation area. In this instance there would be steric hindrance directly above the iron atom (a central steric effect). The anthracene–heme–7,7-cyclophane is almost as effective in preventing *tert*-butyl isocyanide binding as is the adamantane–heme–6,6-cyclophane, even though the two complexes differ by about 8×10^2 with regard to CO binding. The most striking effect of steric differentiation is seen in the $K_B^{CO}/K_B^{t\text{-BuNC}}$ comparison. This ratio goes from 1.4×10^3 to 4×10^3 to 6 in the series anthracene–7,7-cyclophane, anthracene–6,6-cyclophane, adamantane–6,6-cyclophane. It is 2 for chelated protoheme. Hence the adamantane–cyclophane does not effectively differentiate *t*-BuNC from CO by a steric effect.

The size of the steric effect for a given ligand depends upon the particular cyclophane cap, the adamantane– and alkyl–cyclophanes providing side hindrance (peripheral effect) and the anthracene–cyclophanes providing a roof (central effect). Thus, as for the heme proteins, by proper design of the model system, for example the cyclophane–heme, large ligands can be specifically differentiated by steric effects on the basis of both their size and shape. This corroborates the general idea of steric differentiation of ligands by size and shape.[1,6,12,13,40]

Steric Effects on Dioxygen Binding. In the series anthracene–heme–7,7-cyclophane (**1b**), anthracene–heme–6,6-cyclophane (**1a**), and adamantane–heme–6,6-cyclophane (**5**), the dioxygen dissociation rate changes little, although all these hemes, like the strapped hemes (**2a,b**), have lower values of $k_B^{-O_2}$ than do simple hemes, such as chelated mesoheme. Therefore, the hindered hemes **1a** and **5** can be compared with the unhindered heme **1b** directly. In this series the $P_{O_2}{}^{1/2}$ values increase from 1.4 torr to 7×10^2 and 3.0×10^2 torr, respectively, while the M value, $K_B^{CO}/K_B^{O_2}$, changes from 1.5×10^3 to 4.1×10^3 to 5.3×10^2. By the criterion of the M value[18] there seems to be little, if any, steric discrimination between CO and O_2. With the association rates as measures of the distal steric effects,[1] there appears to be a small discrimination against CO in the adamantane–heme **5**. The ratios $k_B^{O_2}/k_B^{CO}$ are 10, 3, and 16 for **1b**, **1a**, and **5**, respectively. The effect is, however, very small. Therefore, neither the anthracene cap, which displays a central steric effect toward isocyanide, nor the adamantane cap, which displays a peripheral steric effect toward isocyanide, shows significant steric differentiation between CO and O_2 binding.

Conformational Effects. The crystal structure of the adamantane–porphyrin–cyclophane **5P** clearly indicates that the adamantane group must move to accommodate ligands. A similar situation exists in deoxyhemoglobin.[4] The adamantane group is in rapid conformational equilibrium in the deoxy form and only a fraction of the conformations are sufficiently open for ligand binding. Once the ligand is bound, some of the conformations are no longer available.

The geometry of the bound CO in **5** is not known. However, its dissociation rate is not accelerated relative to unstrained systems. We conclude that even at these high levels of distal-side steric hindrance the bound state is not particularly strained and the binding constant is controlled by association rates.

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Registry No. 1a, 78505-28-9; **1b**, 78505-29-0; **2a**, 90552-84-4; **2b**, 90531-33-2; **5**, 90531-22-9; **5P**, 90531-20-7; **5P**·$2Cl_2CH_2$, 90531-21-8; **5**-DCIm, 90531-23-0; **5**-DCImCO, 90531-24-1; **5**-MeImCO, 90531-25-2; **5**-Me_2Im, 90531-26-3; **5**-Me_2ImCO, 90531-27-4; **5**-*t*-BuNC, 90531-28-5; **5**-(*t*-BuNC)$_2$, 90531-29-6; **5**-(*t*-BNC)(MeIm), 90531-30-9; **5**-*n*-BuNC, 90531-31-0; **5**-(*n*-BuNC)$_2$, 90531-32-1; O_2, 7782-44-7; CO, 630-08-0; TMIC, 36635-61-7; 8,18-bis(2-carbobenzoxyethyl)-3,13-bis(3-aminopropyl)-2,7,12,17-tetramethylporphyrin, 90552-83-3; chelated mesoheme, 53814-06-5; *tert*-butyl isocyanide, 7188-38-7; 1,3-adamantanediacetyl chloride, 31898-14-3.

Supplementary Material Available: Table III, thermal parameters for the non-hydrogen atoms of 1,3-adamantane–3,13-porphyrin–6,6-cyclophane; Table IV, positional and thermal parameters for the hydrogen atoms of 1,3-adamantane–3,13-porphyrin–6,6-cyclophane; Table V, structure amplitudes; Table VIII, least-squares planes for 1,3-adamantane–3,13-porphyrin–6,6-cyclophane; Table IX, torsion angles for 1,3-adamantane–3,13-porphyrin–6,6-cyclophane (34 pages). Ordering information is given on any current masthead page.

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Generation of Binuclear ($d^8 \cdot d^8$)pσ Platinum and Rhodium Complexes by Pulse Radiolysis

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Abstract: Two ($d^8 \cdot d^8$)pσ binuclear complexes, $Pt_2(pop)_4{}^{5-}$ (pop = $P_2O_5H_2{}^{2-}$) and $Rh_2(TMB)_4{}^+$ (TMB = 2,5-dimethyl-2,5-diisocyanohexane), have been generated by pulse radiolysis of aqueous and acetonitrile solutions, respectively, of the corresponding $d^8 \cdot d^8$ species. An intense absorption system attributable to the allowed $d\sigma^* \rightarrow p\sigma$ transition is observed in the spectrum of each of the transients (420 nm, $Pt_2(pop)_4{}^{5-}$; 570 nm, $Rh_2(TMB)_4{}^+$).

In 1980 we reported that $Rh_2{}^+$ species could be produced by reductive quenching of the $^3d\sigma^*p\sigma$ states of $Rh_2(TMB)_4{}^{2+}$ and $Rh_2b_4{}^{2+}$ (TMB = 2,5-dimethyl-2,5-diisocyanohexane; b = 1,3-diisocyanopropane).[2] At the time the transient absorption spectra of these species could not be established unambiguously, because of the absorptions of the oxidized quenchers. We have now found that $Rh_2(TMB)_4{}^+$ and a binuclear platinum analogue, $Pt_2(pop)_4{}^{5-}$ (pop = $P_2O_5H_2{}^{2-}$), can be generated by pulse radiolysis of solutions containing their $d^8 \cdot d^8$ parents. The absorption spectra of these

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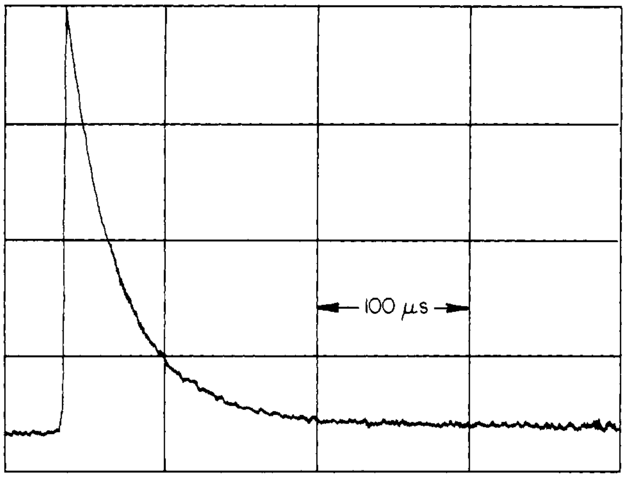

Figure 1. The 420-nm absorbance vs. time plot for the decay of $Pt_2(pop)_4^{5-}$ following a 100-ns pulse of a 5×10^{-5} M aqueous solution of $Pt_2(pop)_4^{4-}$.

reduced species are consistent with an electronic structural model in which the extra electron occupies a bonding metal–metal $p\sigma$ orbital.

Experimental Section

$K_4Pt_2(pop)_4 \cdot 2H_2O$[3-5] and $Rh_2(TMB)_4(PF_6)_2$[2] were prepared by standard procedures. The experimental setup for pulse radiolysis has been described previously.[6] Electron pulses of 100-ns duration were delivered to samples contained in a quartz cell having a 2.4-cm optical path length. Absorptions of transient species produced by the pulse were measured by using a conventional xenon lamp, monochromator, and photomultiplier tube assembly, and the signals were digitized by a Biomation 8100 transient recorder. Analysis of the signals was accomplished using an on-line PDP11/70 minicomputer.

The absorption of radiation by water produces the primary radicals e^-_{aq}, OH, and H, with G values of 2.7, 2.7, and 0.55, respectively.[7] Addition of *tert*-butyl alcohol to the system removes both OH and H radicals in reactions that produce the relatively stable *tert*-butyl alcohol radical. Thus reduction of an added solute by e^-_{aq} may be observed without complications arising from other, possibly absorbing, species formed from OH or H radical attack.

Rate constants for electron addition to the binuclear complexes were obtained by measuring the rate of decay of hydrated electron absorption at 550 nm as a function of the concentration of added complex. The observed decay rate, k_{obsd}, at a concentration C of complex is given simply by $k_{obsd} = k_1 + k_2C$, where k_1 is the rate of decay of e^-_{aq} in the absence of the added complex and k_2 is the second-order rate constant for electron addition to the complex.

Extinction coefficients for each reduced complex were calculated from the initial absorption of the hydrated electron, the maximum absorbance of the reduced complex, and a knowledge of the extinction coefficient of the electron (taken to be 9.3×10^3 M^{-1} cm^{-1}).[8] The calculated extinctions were then corrected for the fraction of electrons that decay by means other than reaction with the complex. In general the 100-ns pulse produced ca. 3×10^{-6} M e^-_{aq}, as measured by using the extinction coefficient given above, and the concentration of metal complex was always such that 90% of the e^-_{aq} reacted with the complex. Thus corrections to the calculated extinction coefficients were very small.

Transient absorption spectra were generated from successive signals at different wavelengths by the on-line computer.

Results and Dicussion

Solutions of $Pt_2(pop)_4^{4-}$ (10^{-4}–10^{-5} M) in 2 mM phosphate buffer (pH ~6.8 with 1% *tert*-butyl alcohol) were saturated with nitrogen. The rate constant for the reaction between $Pt_2(pop)_4^{4-}$ and hydrated electrons was measured over the concentration range (1.4–0.2) $\times 10^{-4}$ M. The second-order rate constant for the reaction was found to be 1.80 (7) $\times 10^{10}$ M^{-1} s^{-1}. Figure 1 shows the absorbance vs. time plot monitored at 420 nm for the decay of the reduced complex after a 100-ns electron pulse. The decay was found to be first order[8] with a rate constant of 2.9×10^4 s^{-1}. The observed reaction is attributable to the reoxidation of $Pt_2(pop)_4^{5-}$.

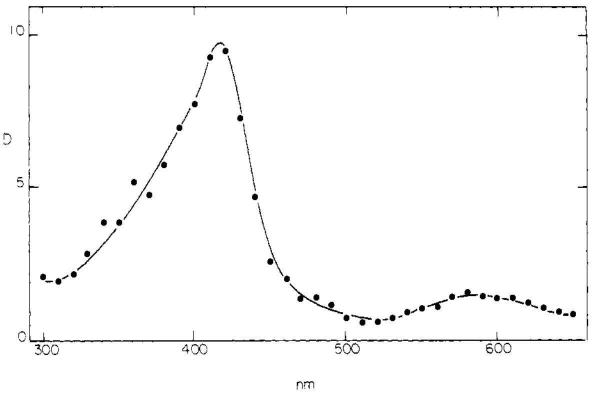

Figure 2. The transient absorption spectrum (absorbance vs. wavelength plot) recorded 8 μs after pulse radiolysis of ~5×10^{-5} M $Pt_2(pop)_4^{4-}$ in degassed 2 mM phosphate buffer containing 1% *tert*-butyl alcohol (pH ~6.8).

The transient absorption spectrum of $Pt_2(pop)_4^{5-}$ recorded 8 μs after the electron pulse (at which time all electron decay is complete) exhibits peaks at ~420 (ϵ ~1.3×10^4) and ~600 nm (ϵ ~1.7×10^3) (Figure 2). Analogous experiments with $Rh_2(TMB)_4^{2+}$ were performed in degassed CH_3CN containing 1% *tert*-butyl alcohol. Importantly, we found that the spectrum of $Rh_2(TMB)_4^+$ (~740, ~570 nm) accords well with the transient difference spectrum obtained 50 μs after conventional flash photolysis of $Rh_2(TMB)_4^{2+}$ and TMPD in methanol.[2]

Extensive spectroscopic studies of binuclear $d^8 \cdot d^8$ complexes containing rhodium[9-11] and platinum[4,12-14] have shown that their lowest excited states are derived from the $d\sigma^*p\sigma$ electronic configuration. It is reasonable, therefore, that one-electron reduction of such $d^8 \cdot d^8$ species would produce a $(d^8 \cdot d^8)p\sigma$ complex, which features a formal one-half $p\sigma$ bond. Alternatively, reduction might yield a $d^8 \cdot d^9$ ground state, with the extra electron in an orbital derived from $d\sigma^*(d_{x^2-y^2})$. Our spectroscopic measurements are consistent with the unusual "$p\sigma$-bonded" ($^2A_{1g}$) ground state for $Pt_2(pop)_4^{5-}$ and $Rh_2(TMB)_4^+$. With the assumption that the metal–metal bond contraction upon population of the $p\sigma$ orbital is less than that observed for the $^3d\sigma^*p\sigma$ state,[14] the $^2A_{2u}$ energy should fall between the energies of the $^1d\sigma^*p\sigma$ and $^3d\sigma^*p\sigma$ excited states of the parent $d^8 \cdot d^8$ complex. Since both $Pt_2(pop)_4^{5-}$ (420 nm) and $Rh_2(TMB)_4^+$ (570 nm) exhibit intense bands at slightly lower energies than the $d\sigma^* \rightarrow p\sigma$ singlets in their $d^8 \cdot d^8$ parents,[9,13] it is logical to assign these bands to the $^2A_{1g} \rightarrow {}^2A_{2u}$ ($d\sigma^* \rightarrow p\sigma$) transitions.[15] Although such close correspondence to the parent spectra would not be expected if the reduced species adopted a

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[15] The spectrum we have assigned to $Pt_2(pop)_4^{5-}$ is quite different from that reported[5] for the product of chromous reduction of $Pt_2(pop)_4^{4-}$. It was proposed[5] that a simple two-electron reduction product, $Pt_2(pop)_4^{6-}$, was formed in the latter reaction. This proposal may require revision in view of our finding that $Pt_2(pop)_4^{5-}$ is very rapidly oxidized in aqueous solution; for example, it is possible that some rearrangement of the bridging ligand structure accompanies the chromous reduction.

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d^8-d^9 configuration, it is not possible with the limited information in hand to rule out entirely the latter ground state.

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Kinetics of Intramolecular Electron Transfer from Ru^{II} to Fe^{III} in Ruthenium-Modified Cytochrome *c*

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Abstract: The kinetics of intramolecular electron transfer in $Ru(NH_3)_5(histidine\text{-}33)^{2+}$–ferricytochrome *c* ($PFe^{III}$–$Ru^{II}$) and intermolecular electron transfer from $Ru(NH_3)_5L^{2+}$ (L = histidine, imidazole, NH_3) to ferricytochrome *c* (PFe^{III}) have been studied by transient absorption and stopped-flow spectroscopic techniques. Electron transfer from electronically excited $Ru(bpy)_3{}^{2+}$ (bpy = 2,2′-bipyridine) to PFe^{III}–Ru^{III} produces PFe^{III}–Ru^{II} in fivefold excess to PFe^{II}–Ru^{III}, and in the presence of EDTA (which rapidly reduces $Ru(bpy)_3{}^{3+}$) the PFe^{III}–Ru^{II} decays mainly by intramolecular electron transfer to PFe^{II}–Ru^{III}. At pH 7 (μ = 0.1 M) the rate constant (30 (3) s^{-1}, 23 °C) does not vary substantially over the temperature range 0–80 °C, thereby allowing an upper limit of 1.5 kcal mol^{-1} to be placed on $\Delta H^{\ddagger}$. Above 80 °C, intramolecular $Ru^{II} \rightarrow Fe^{III}$ electron transfer is not observed, owing to the displacement of methionine-80 from the iron coordination sphere. Combining the activation enthalpy of the intramolecular electron-transfer reaction with the redox thermodynamic parameters for the $Ru(NH_3)_5L^{2+}/PFe^{III}$ system allows an upper limit of 8 kcal mol^{-1} to be placed on the $PFe^{III/II}$ reorganizational enthalpy. The very small activation enthalpies for both the intramolecular and intermolecular electron-transfer reactions demonstrate that the enthalpic contribution to the free energy of formation of the $Ru(NH_3)_5L^{2+}/PFe^{III}$ precursor complex is almost zero. The activation entropy for the intramolecular process (−48 (2) eu) is more negative than that for the $Ru(NH_3)_5His^{2+}/PFe^{III}$ reaction (−36 (1) eu), which suggests that the electron-transfer distance is shorter than 11.8 Å in the intermolecular precursor complex.

Introduction

Several research groups have begun to report the results of experiments that bear directly on the role of redox-site separation distance on metalloprotein electron-transfer rates.[2–6] An approach that we have found attractive is to measure the electron-transfer kinetics in semisynthetic systems in which redox-active inorganic reagents are bound covalently to specific residues on the polypeptide chain of a structurally well-characterized electron-transfer metalloprotein. In these semisynthetic systems the electron-transfer distances are fixed and known.

It has been shown that horse heart ferricytochrome *c* (PFe^{III}) reacts with excess $Ru(NH_3)_5OH_2{}^{2+}$ to produce pentaammine(histidine-33)ruthenium(III)–ferricytochrome *c* (PFe^{III}–Ru^{III}) (Figure 1).[2,3,7] Preliminary experiments with the modified protein demonstrated that intramolecular electron transfer from Ru^{II} to PFe^{III} proceeds at a significant rate.[2,3a] We now have completed a thorough study of the intramolecular electron-transfer kinetics in this semisynthetic system. The interpretation of the results has been aided by an investigation of the thermodynamics as well as the kinetics of the intermolecular electron-transfer reaction between $Ru(NH_3)_5His^{2+}$ and PFe^{III}.

Experimental Section

Materials. All protein and buffer solutions were prepared by using distilled water that was purified by passage through a Barnstead Nanopure water purification system. Phosphate buffers were prepared from analytical grade reagents, and HEPES buffers were prepared from the sodium salt and acid forms of HEPES (Calbiochem). Horse heart cytochrome *c* (type VI; Sigma Chemical Co.) was purified on a CM-cellulose (Watman CM52) column prior to use in order to remove deamidated forms of the protein.[8]

The chloride salt of hexaammineruthenium(II), prepared by the method of Lever and Powell,[9] was recrystallized according to the following procedure. Two grams of crude product was dissolved in 20 mL of boiling aqueous ammonia (15%). To ensure complete reduction of the ruthenium, zinc powder was added to the ammonia solution, which was then filtered while hot. The filtrate was placed under an argon counterflow, and NH_4Cl was added to the solution. After the solution was cooled to 0 °C, the yellow-orange precipitate was collected, washed with cold aqueous ammonia and cold acetone, and dried under vacuum. $[Ru(NH_3)_5Cl]Cl_2$ was prepared from $[Ru(NH_3)_6]Cl_2$ (Matthey-Bishop)[10] and recrystallized from 0.1 M HCl.[11] $[Ru(NH_3)_5His]Cl_3{\cdot}H_2O$ was obtained from the reaction of L-histidine (Sigma) with $[Ru(NH_3)_5Cl]Cl_2$[12] over zinc amalgam and was purified by ion-exchange chromatog-

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