probe and appear to give peak manifolds corresponding to the borate ester, multiple hydroboration, and degradation. For example, *m/e* cutoffs of 262 and 176 are consistent with $^{34}S^{11}B_8H_5(^{12}C_2H_5{}^{16}O)_3$ and $^{34}S^{11}B_8H_7(^{12}C_2H_5{}^{16}O)$, respectively. Fragments such as *m/e* 217 might correspond to CH_3 loss from $^{34}S^{11}B_9H_9(^{12}C_2H_5O)_2$ upon electron impact; however, the same envelopes appear using chemical ionization conditions.

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Distances of Electron Transfer to and from Metalloprotein Redox Sites in Reactions with Inorganic Complexes

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Abstract: A modified form of Hopfield's equation relating rate constants to electron-transfer distances has been applied to a series of metalloprotein redox reactions. For proteins containing redox centers with minimal inner-sphere reorganization barriers, the relationship between one-half the intersite distance (R_p, Å) and the self-exchange rate constant at infinite ionic strength (k_{11}^{∞}) is estimated to be $R_p = 6.2 - 0.35 \ln (k_{11}^{\infty})$. Calculated R_p values based on redox reactions of heme *c*, blue copper, and iron-sulfur proteins with inorganic complexes support the conclusion that hydrophobic, π-conducting ligands are able to penetrate into protein interiors, thereby reducing the distance over which electron transfer occurs. The following estimates of metalloprotein redox site-to-surface distances (ΔR_p's) have been made based on $Fe(EDTA)^{2-}$ rate data: cytochrome *c*, 3.4; cytochrome c_{551}, 4.0; plastocyanin, 2.6; azurin, 5.5; HiPIP, 5.8 Å. These kinetically determined distances accord reasonably well with estimates of metalloprotein redox site-to-surface distances based on examination of molecular models. The electron-transfer distance in the ferricytochrome *c*-$Fe(CN)_6^{4-}$ complex has been estimated from kinetics data to be 10 Å, which accords closely with an estimate of 7–10 Å based on spectroscopic measurements.

Introduction

A problem that has received attention in recent years is the determination of the distance over which electron transfer occurs in metalloprotein redox reactions.[1-3] The basis for this attention is seated in the implications of the distance of transfer for our understanding of metalloprotein redox mechanisms and their specificity. Owing to our continuing interest in this subject, we have undertaken an investigation with the goal of developing a method that allows conventional rate data to be used in a systematic way to estimate the distances of electron transfer to and from redox sites in blue copper, heme *c*, and iron-sulfur proteins in reactions with inorganic complexes.

Methods

General Considerations. We assume first that the electron-transfer rate constant, k, is related to a tunneling matrix element, T_{ab}, as given in the equation

$$k = C_0 |T_{ab}|^2 \tag{1}$$

According to standard electron-transfer theory, the magnitude of T_{ab} is dependent on the extent of donor and acceptor electronic wave function overlap.[3-9] C_0 is a complicated function whose value is dependent on a number of properties of the donor and acceptor sites.[10] In general, the matrix element T_{ab} can be expressed as an exponential function of the intersite distance R (as originally derived by Gamow[11]), as in the equation

$$k = Ce^{-2aR} \tag{2}$$

Taking $a = 0.72$ Å^{-1}, as proposed by Hopfield,[3] we have

$$R = -0.694 \ln (k/C) \tag{3}$$

Previous calculations employing estimates for the parameters that comprise C in eq 3 have enjoyed reasonable success.[12-14] However, we prefer to reduce our assumptions by appealing to experiment to obtain an acceptable value for C without attempting to estimate values for its various components. This approach will work only if it is referenced to a system where we know the rate and have a good estimate for the distance of electron transfer. The system we have chosen for the analysis is discussed in the following section.

Analytical Procedure and Rationale. The reactions of interest in this analysis are those between a metalloprotein and a substitutionally inert transition metal complex. This selection is

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advantageous because of the large volume of data available for consideration[15-18] and because of the mechanistic insight provided by previous detailed examination of these reactions. Three experimental quantities are required for the calculations:[17] (1) the rate constant for the electron transfer cross-reaction between the protein and the complex (k_{12}) (the protein is reactant 1 and the complex is reactant 2); (2) the self-exchange electron transfer rate constant for the complex (k_{22}); and (3) the driving force for the cross-reaction (ΔE°_{12}).

We first correct for nonspecific electrostatic effects by using Debye-Hückel theory to adjust the rate constants (k_{12} and k_{22}) to infinite ionic strength:[17]

$$k^\infty_{12} = \exp\left[\ln(k) + 3.576 \times \left(\frac{\exp(-\kappa R_1)}{1+\kappa R_2} + \frac{\exp(-\kappa R_2)}{1+\kappa R_1}\right)\left(\frac{Z_1 Z_2}{R_1 + R_2}\right)\right] \quad (4)$$

At 25 °C

$$\kappa = 0.329\mu^{1/2}\ \text{Å}^{-1}$$

In eq 4, Z_1, Z_2, R_1, and R_2 are the charges and radii of the reactants.

Next, correction for the thermodynamic driving force[19] and the reactivity properties of the small molecule is accomplished through the use of the Marcus relationships[20] for adiabatic (or uniformly nonadiabatic)[21] electron transfer reactions:

$$k_{12} = (k_{11}k_{22}fK_{12})^{1/2} \quad (5)$$

$$\log(f) = [\log(K_{12})]^2/[4\log(k_{11}k_{22}/Z^2)] \quad (6)$$

where Z is the collision frequency (taken to be 10^{11} M^{-1} s^{-1}). The procedure is to calculate the self-exchange rate constant for the protein at infinite ionic strength (k^∞_{11}) from the k^∞_{12} and k^∞_{22} values obtained from eq 4.[22] If a protein obeys Marcus theory, then the values of k^∞_{11} calculated from its reactions with a variety of complexes will be the same (within an order of magnitude, the limit of experimental and theoretical error). If a protein does not obey Marcus theory, then k^∞_{11} may vary over several orders of magnitude, indicating that the nonadiabatic character of the electron-transfer process depends strongly on the nature of the protein's reaction partner. In the treatment that follows we shall assume that a decrease in k^∞_{11} from a set upper limit reflects an increase in the nonadiabatic character of the reaction in question, which in turn can be related to an increase in the intersite electron transfer distance from a fixed reference point.

The intersite distance, R, for electron transfer between a protein and an inorganic complex is defined as the sum of a contribution from the protein (R_p) and from the reagent (R_r). Thus the distance calculated from the protein self-exchange rate constant using eq 3 is $2R_p$. The reference protein reactions that we have selected for the determination of C in eq 3 are those of the blue copper protein stellacyanin. This protein was chosen because it is well behaved from the standpoint of Marcus theory;[15] that is, the copper redox site apparently is relatively accessible to solvent and there are no unusual protein-reagent interactions. Moreover, the stellacyanin k^∞_{11} values are generally at the upper limit of those calculated for protein-small molecule reactions, indicating that electron transfer is adiabatic or nearly so. Finally, stellacyanin is one of the few proteins that is able to exchange electrons with electrodes reasonably well without the aid of mediators, further suggesting an accessible redox site.[23] Thus we shall assume that the k^∞_{11} for stellacyanin is associated with a "closest contact" electron transfer distance for a blue copper site, that distance being roughly 3.7 Å ($=2R_p$), or twice the van der Waals radius of an aromatic carbon atom located on the periphery of an imidazole ring of a histidine ligand.[24] Any increase in the redox partner contact distance from the reference limit should lead to nonadiabatic electron transfer, and the "nonadiabatic distance" will be calculated from eq 3.[25]

For the reference rate constant at $2R_p = 3.7$ Å, we shall take the average of the k^∞_{11} values calculated for stellacyanin in its reactions with $Fe(EDTA)^{2-}$ ($k^\infty_{11} = 2.3\ (10^5)\ M^{-1}\ s^{-1}$), $Co(phen)_3^{3+}$ ($k^\infty_{11} = 3.3\ (10^5)\ M^{-1}\ s^{-1}$), and $Ru(NH_3)_5py^{3+}$ ($k^\infty_{11} = 1.6\ (10^5)\ M^{-1}\ s^{-1}$).[15,18] This average, $2.4\ (10^5)\ M^{-1}\ s^{-1}$, determines C in eq 3, yielding the following expression:[26]

$$R_p = 6.2 - 0.35\ln(k^\infty_{11}) \quad (7)$$

The discussion leading to eq 7 has tacitly assumed that kinetically detectable precursor complexes are not involved in the reactions under consideration. However, straightforward modification of our procedures allows us to estimate electron-transfer distances in reactions of the following type:[27]

$$A^{ox} + B^{red} \overset{K}{\rightleftharpoons} [A^{ox}|B^{red}]$$

$$[A^{ox}|B^{red}] \xrightarrow{k_{et}} [A^{red}|B^{ox}]$$

$$[A^{red}|B^{ox}] \longrightarrow A^{red} + B^{ox}$$

In this scheme, precursor complex formation is assumed to be rapid and the electron-transfer step represented by k_{et} is taken to be rate limiting. Straightforward application of transition-state theory assuming spherically symmetric reactants allows us to estimate a "synthetic" second-order rate constant ($k^{est}_{12} \approx (\nu/Z)k_{et} \approx 10^{-2}\ M^{-1}\ k_{et}$). This second-order rate constant is then treated as described above to estimate an electron-transfer distance.

Reliability of Calculated Distances. Before drawing conclusions from the present calculations, we emphasize that distances estimated for proteins with blue copper redox centers should be more reliable than those estimated for *c*-type cytochromes or iron-sulfur proteins. The potential source of error in applying eq 7 to the latter systems arises from differences in site characteristics that contribute to the value of C.[10] The error should be small in the cases included in the present analysis because the inner-sphere reorganization barriers predicted for iron-sulfur proteins, *c*-type cytochromes, and blue copper proteins are minimal.[15] In such cases, it may reasonably be argued that $Fe(phen)_3^{2+/3+}$ is an appropriate reference redox system for the calculations. *We emphasize, however, that eq 7 should not be applied to systems in which there are substantial inner-sphere reorganization barriers.*

The precision of our R_p calculations is determined by the uncertainty of the Marcus treatment. Rate constants calculated from Marcus theory are generally regarded as being reliable within an order of magnitude. From eq 7 it can be seen that an error of this size translates into an uncertainty of ±0.8 Å. This is the minimum error, therefore, that must be considered when comparing R_p values obtained from reactions of a given protein with a series of complexes or when comparing R_p values from reactions of a given complex with several proteins of a given structural type.

Results and Discussion

The results for the electron-transfer reactions of several inorganic complexes with *c*-type cytochromes, blue copper proteins, and an iron-sulfur protein are set out in Table I. It is apparent that complexes having hydrophilic ligand surfaces (e.g., $Fe(EDTA)^{2-}$) are associated with R_p values that are larger than those calculated from reactions with complexes having hydrophobic, π-conducting ligand surfaces (e.g., $Co(phen)_3^{3+}$, $Ru(NH_3)_5py^{3+}$). This result is consistent with the conclusion that complexes of the latter type are able to interact

Table I. Calculations[a] of k_{11}^{∞} and R_p

protein	reagent	k_{12}, $M^{-1}\,s^{-1}$	ΔE_{12}°, V	μ, M	R_1, Å/ R_2, Å	Z_1/Z_1'	Z_2/Z_2'	k_{12}^{∞}, $M^{-1}\,s^{-1}$	k_{22}^{∞}, $M^{-1}\,s^{-1}$	ΔE_{12}^{∞}, V	k_{11}^{∞}, $M^{-1}\,s^{-1}$	R_p, Å
cytochrome *c*	$Fe(EDTA)^{2-}$	$2.57(10^4)$	0.140	0.10	16.6/4	7.5/6.5	−2/−1	$9.87(10^3)$	$6.9(10^4)$	0.126	$1.2(10^1)$	5.3
(horse heart)	$Ru(NH_3)_6^{2+}$	$3.8(10^4)$	0.209	0.10	16.6/3	7.5/6.5	2/3	$1.15(10^5)$	$2.5(10^5)$	0.200	$3.0(10^1)$	5.0
	$Ru(NH_3)_5py^{3+}$	$9.26(10^3)$	−0.007	0.10	16.6/3.5	6.5/7.5	3/2	$3.52(10^4)$	$1.7(10^4)$	0.001	$7.1(10^4)$	2.3
	$Co(phen)_3^{3+}$	$1.5(10^3)$	0.110	0.10	16.6/7	6.5/7.5	3/2	$3.43(10^3)$	$9.8(10^1)$	0.115	$1.5(10^3)$	3.6
cytochrome c_{551}	$Fe(EDTA)^{2-}$	$4.2(10^3)$	0.140	0.10	14.4/4	−2/−3	−2/−1	$5.83(10^3)$	$6.9(10^4)$	0.142	$2.3(10^0)$	5.9
(*Pseudomonas aeruginosa*)	$Co(phen)_3^{3+}$	$5.3(10^4)$	0.110	0.10	14.4/7	−3/−2	3/2	$3.26(10^4)$	$9.8(10^1)$	0.103	$2.2(10^5)$	1.9
stellacyanin	$Fe(EDTA)^{2-}$	$4.3(10^5)$	0.064	0.50	19.5/4	0/0	−2/−1	$4.3(10^5)$	$6.9(10^4)$	0.064	$2.3(10^5)$	1.9
(*Rhus vernicifera*)	$Ru(NH_3)_5py^{3+}$	$1.94(10^5)$	0.069	0.10	19.5/3.5	0/0	3/2	$1.94(10^5)$	$1.7(10^4)$	0.069	$1.6(10^5)$	2.0
	$Co(phen)_3^{3+}$	$1.8(10^5)$	0.186	0.10	19.5/7	0/0	3/2	$1.8(10^5)$	$9.8(10^1)$	0.186	$3.3(10^5)$	1.8
plastocyanin	$Fe(EDTA)^{2-}$	$8.2(10^4)$	0.227	0.20	15.8/4	−9/−10	−2/−1	$1.72(10^5)$	$6.9(10^4)$	0.235	$7.3(10^1)$	4.7
(*Phaseolus vulgaris*)	$Ru(NH_3)_5py^{3+}$	$7.1(10^3)$	−0.094	0.50	15.8/3.5	−10/−9	3/2	$3.88(10^3)$	$1.7(10^4)$	−0.100	$4.9(10^4)$	2.4
	$Co(phen)_3^{3+}$	$4.9(10^3)$	0.023	0.10	15.8/7	−10/−9	3/2	$1.2(10^3)$	$9.8(10^1)$	0.009	$1.1(10^4)$	2.9
azurin	$Fe(EDTA)^{2-}$	$1.3(10^3)$	0.184	0.20	17.2/4	−1/−2	−2/−1	$1.39(10^3)$	$6.9(10^4)$	0.184	$2.8(10^{-2})$	7.4
(*Pseudomonas aeruginosa*)	$Ru(NH_3)_5py^{3+}$	$2.0(10^3)$	−0.051	0.10	17.2/3.5	−2/−1	3/2	$1.36(10^3)$	$1.7(10^4)$	−0.058	$1.1(10^3)$	3.8
	$Co(phen)_3^{3+}$	$3.2(10^3)$	0.066	0.20	17.2/7	−2/−1	3/2	$2.82(10^3)$	$9.8(10^1)$	0.064	$7.0(10^3)$	3.1
HiPIP	$Fe(EDTA)^{2-}$	$1.7(10^3)$	0.230	0.10	15.5/4	−2.5/−3.5	−2/−1	$2.44(10^3)$	$6.9(10^4)$	0.233	$1.5(10^{-2})$	7.7
(*Chromatium vinosum*)	$Ru(NH_3)_6^{2+}$	$3.1(10^5)$	0.299	0.10	15.5/3	−2.5/−3.5	2/3	$2.04(10^5)$	$2.5(10^5)$	0.311	$2.0(10^0)$	6.0
	$Ru(NH_3)_5py^{3+}$	$1.1(10^3)$	−0.097	0.50	15.5/3.5	−3.5/−2.5	3/2	$8.83(10^2)$	$1.7(10^4)$	−0.100	$2.5(10^3)$	3.5
	$Co(phen)_3^{3+}$	$2.8(10^3)$	0.020	0.10	15.5/7	−3.5/−2.5	3/2	$1.70(10^3)$	$9.8(10^1)$	0.013	$1.8(10^4)$	2.8

[a] Input parameters are from ref 15, 18, and 23.

effectively with hydrophobic regions of proteins to shorten the intersite distance of electron transfer, whereas complexes with hydrophilic ligand surfaces are not capable of such penetration.[15-18] It is further apparent that, of all the proteins examined, the range in distances of transfer is relatively large for the proteins azurin and HiPIP. As these proteins are thought to have relatively inaccessible metal sites,[18] the influence of reagent structure on the electron-transfer distance is expected to be greater.

The R_p calculated for an electron-transfer reaction with a nonpenetrating reactant such as $Fe(EDTA)^{2-}$ minus the van der Waals contact (1.85 Å) should approximate the shortest distance from the active site to the surface of the protein. For the proteins we have analyzed, these ΔR_p values based on $Fe(EDTA)^{2-}$ are as follows: cytochrome *c*, 3.4; cytochrome c_{551}, 4.0; plastocyanin, 2.6; azurin, 5.5; HiPIP, 5.8 Å. As protein crystallographic data generally are not presented in suitable form to obtain a "structural ΔR_p", it is necessary to rely on careful examination of molecular models to estimate this aspect of protein structure. Our estimates of ΔR_p values for cytochrome *c* (3.0 ± 1.0 Å), cytochrome c_{551} (3.0 ± 1.0 Å), and HiPIP (4.5 ± 1.0 Å) accord remarkably well with the kinetically determined ones, giving us confidence that distances based on eq 7 are reliable. We also note that similar ΔR_p values for cytochrome *c* (3.2 Å) and HiPIP (4.1 Å) may be estimated from the $Ru(NH_3)_6^{2+}$ data given in Table I.

We now take up the matter of estimating electron-transfer distances in reactions involving kinetically detectable precursor complexes. In doing so, we point out that observing rate saturation at high concentrations of reagent is not sufficient to establish precursor complex involvement; other mechanisms may also account for such behavior,[28] and these are difficult to rule out.

The most carefully documented example of precursor complex formation is the ferricytochrome *c*-$Fe(CN)_6^{4-}$ reaction. Miller and Cusanovich first detected rate saturation for this reaction that is consistent with a stability constant of ca. $3.0(10^2)$ M^{-1} (μ = 0.1 M, pH 7.0).[29] Independent demonstration of $Fe(CN)_6^{4-}$ binding to cytochrome *c* was provided by the equilibrium dialysis study of Stellwagen and Cass in which an association constant was obtained that was comparable to that determined from the kinetics analysis.[30]

Parameters for the ferricytochrome *c*-$Fe(CN)_6^{4-}$ calculation are as follows: $k_{et} \simeq 1.3(10^2)\ s^{-1}$ ($k_{12}^{est} = 1.3\ M^{-1}\,s^{-1}$); $k_{22}^{\infty} = 4.6(10^5)\ M^{-1}\,s^{-1}$; $\Delta E_{12}^{\circ} = -0.16$ V. Based on $k_{11}^{\infty} = 2.3(10^{-3})\ M^{-1}\,s^{-1}$, we obtain $R_p = 8.3$ Å from eq 7. The total intersite electron transfer distance is predicted to be slightly greater than 8.3 Å (~10 Å), as R_r for $Fe(CN)_6^{4-}$ is about 2 Å. Our estimated electron-transfer distance of 10 Å falls within the 7-10-Å range obtained by Potasek and Hopfield[31] from an experiment in which excitation modulation spectroscopy was employed to detect a charge-transfer band in the iron hexacyanide/cytochrome *c* complex. It is encouraging that electron-transfer distances derived from two very different experiments accord so closely.

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(10) These properties have been discussed by several authors[3-7] but have been presented most recently by Hopfield as follows:

$$C_0 = P\left(\frac{2\pi}{\hbar}\right)(2\pi\sigma^2)^{-1/2}\exp\left[\frac{-(E_a - E_b - \Delta)^2}{2\sigma^2}\right]$$

$$\sigma^2 = \Delta k_B T_a \coth(T_a/2T)$$

Δ is the vibronic coupling parameter and reflects the shape of the potential-energy surface for a reaction center. One major contribution to Δ is the Franck-Condon activation barrier for the site. T_a is the characteristic temperature, and $T_a/2$ is the temperature at which electron transfer becomes thermally activated (k_B is Boltzmann's constant). $E_a - E_b$ is the potential difference between the sites, T is the temperature at which the rate is to be determined, and P is a collision frequency factor for bimolecular reactions.
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$$\Delta E_{12}^{\circ\circ} = \Delta E_{12}^{\circ} + 0.09182\left(\frac{\exp(-\kappa R_2)}{1+\kappa R_1} + \frac{\exp(-\kappa R_1)}{1+\kappa R_2}\right) \times \left(\frac{Z_1Z_2 - Z'_1Z'_2}{R_1+R_2}\right)$$

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(21) For nonadiabatic electron transfer reactions the Marcus relationship (neglecting f) is

$$\frac{k_{12}}{p_{12}} = \left(\frac{k_{11}}{p_{11}}\frac{k_{22}}{p_{22}}K_{12}\right)^{1/2}$$

This general expression reduces to eq 5 if the reactions in question are "uniformly nonadiabatic" ($p_{12}^2 = p_{11}p_{22}$) (Sutin, N. *Acc. Chem. Res.* **1968**, *1*, 225).

(22) In terms of the Hopfield formalism, use of the protein self-exchange rate constant fulfills the assumption of equivalent sites. Furthermore, because this stratagem removes dependence of the rate on the kinetic properties of the inorganic complex, it eliminates the uncertainty in the values of the parameters[10] that describe the small molecule site characteristics.

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(24) The selection of 3.7 Å as the "closest contact" distance assumes that stellacyanin employs a self-exchange mechanism involving electron transfer through an imidazole ring of a histidine ligand. Alternatively, the protein may employ a mechanism involving a sulfur ligand (as is likely the case for the Fe–S proteins considered). In either case, the "closest contact" distance would be the same, since the van der Waals radius for S is also 1.85 Å. Thus, the calibration based on a 3.7-Å "closest contact" distance should be adequate for all reactions in which redox site contact occurs between aromatic carbon atoms (imidazole edges, heme edges) or sulfur atoms. The relevant structural data on cytochrome c[15,17] and HiPIP[15,18] have been discussed previously. The available structural information on the blue copper centers in plastocyanin, azurin, and stellacyanin has been reviewed recently (Gray, H. B. *Adv. Inorg. Biochem.* **1979**, *2*, 1).

(25) Put another way, we assume that the rate constants depend on the "nonadiabatic distance" of electron transfer as follows:

$$k_{12} = k_{12}^{\circ}[\exp(-2aR_1)][\exp(-2aR_2)]$$

where k_{12}° is the rate constant that would be observed if the reaction were adiabatic (R_1, $R_2 = 0$) and R_1 and R_2 are the "nonadiabatic distances" for reactants **1** and **2**, respectively. In our analysis $2R_p = 3.7$ Å for $R_1 = 0$.

(26) Even though the apoprotein does not impose any protein-reagent interactions on the kinetics of the blue copper center in stellacyanin, it does limit the surface area of the copper center that is available for reaction. This statistical factor is a major determinant of the absolute magnitude of k_{11}^{∞}. Sutin has treated this effect in detail for cytochrome c by showing that the self-exchange rate constant for the protein ($\sim 10^3$ M^{-1} s^{-1}) compared to that for $Fe(phen)_3^{3+}$ ($\sim 10^7$ M^{-1} s^{-1}) is consistent with crystallographic data showing that about 3% of the porphyrin surface is available for electron transfer (N. Sutin, "Bioinorganic Chemistry-II", *Adv. Chem. Ser.* **1977**, *No. 162*, 156). A similar comparison of the self-exchange rate constant for stellacyanin ($\sim 10^5$ M^{-1} s^{-1}) with that for $Fe(phen)_3^{3+}$ ($\sim 10^7$ M^{-1} s^{-1}) would suggest that roughly 10% of the copper center "surface" is available for electron transfer in this protein. The use of a single value of C for all calculations based on eq 3 implies that the same statistical factor holds for all the proteins considered. This assumption should be nearly valid for cases involving hydrophilic (nonpenetrating) reagents. However, the potential ability for penetrating (hydrophobic) reagents to sample a larger relative "surface area" of a "buried" site may contribute to a larger uncertainty in the R values calculated for proteins reacting with these reagents. The penetration of such reagents may also contribute to changes in the Franck–Condon factors (which are implicitly included in the constant C) and this would also introduce error in the calculated R values.

(27) Sutin, N. "Inorganic Biochemistry", Eichhorn, G. I., Ed.; Elsevier: Amsterdam, 1973; p 611.

(28) The possible mechanisms that give rise to saturation behavior have been enumerated (Yoneda, G. S.; Holwerda, R. A. *Bioinorg. Chem.* **1978**, *8*, 139): (a) precursor complex formation, (b) rate-limiting formation of an activated form of the protein, and (c) dead-end complex formation.

(29) This value of the formation constant was calculated by extrapolation (linear in $\mu^{1/2}$) of the ionic strength dependent values given by Miller and Cusanovich (Miller, W. G.; Cusanovich, M. A. *Biophys. Struct. Mech.* **1975**, *1*, 97).

(30) Stellwagen, E.; Cass, R. D. *J. Biol. Chem.* **1975**, *250*, 2095.

(31) Potasek, M. J.; Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3817.

Cobalt Metallacycles. 8.[1] Preparative and Kinetic Studies on the Reactivity of Cobaltacyclopentadienes with Phosphites

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Abstract: (η^5-Cyclopentadienyl)(triphenylphosphine)cobaltacyclopentadiene complexes, ($\overline{CoCR^1{=}CR^2CR^3{=}CR^4}$)($\eta^5$-$C_5H_5$)($PPh_3$) (**1**), react with phosphites, $P(OR^5)_3$ (**2**), yielding in successive steps (η^5-cyclopentadienyl)(phosphite)cobaltacyclopentadiene complexes, ($\overline{CoCR^1{=}CR^2CR^3{=}CR^4}$)($\eta^5$-$C_5H_5$)[$P(OR^5)_3$] (**3**) and isomeric 1-alkoxyphosphole oxide complexes (**4**). The structure of one isomer of the 1-methoxyphosphole oxide complex, **4ca-2**, was determined by an X-ray crystallographic structural analysis. The complexes **4**, when oxidized by Ce^{4+} ions, give 1-alkoxyphosphole oxides. Kinetic studies on the formation of **3** and of the conversion of **3** to **4** have been carried out and a first-order reaction has been verified in the complex concentration for both steps. Activation parameters for the reaction of **1c** (R^1, R^2, R^3, R^4 = Ph) with **2a** ($OR^5 = OCH_3$) are determined as $\Delta H^{\ddagger} = 31$ kcal mol^{-1} and $\Delta S^{\ddagger} = 4$ eu for the substitution, and as $\Delta H^{\ddagger} = 22$ kcal mol^{-1} and $\Delta S^{\ddagger} = -4$ eu for the conversion step, respectively. The preparative and kinetic investigations show that (1) both steric and electronic factors of the substituents of the cobalt metallacycles govern the substitution step, and (2) the electronic factor chiefly governs the conversion step. The steric effect of the alkyl groups of **2** is very distinctive in the conversion step.

I. Introduction

Cobaltacyclopentadienes have been considered to be key intermediates in the cobalt-catalyzed reactions of acetylenes.[2-4] Studies on the reactions of the stable (η^5-cyclopentadienyl)(triphenylphosphine)cobaltacyclopentadiene complexes, ($\overline{CoCR^1{=}CR^2CR^3{=}CR^4}$)($\eta^5$-$C_5H_5$)($PPh_3$) (**1**), with various unsaturated organic reagents L such as acetylenes,[5,6] olefins,[7] or compounds having a hetero multiple bond[8-10] have contributed to the understanding of the cobalt catalysis. In the catalytic process, it is believed that the L should first ligate on the cobalt by displacing the PPh_3 ligand to give an intermediate **3** (Scheme 1). Then the intermediate **3** may be converted to a final product via insertion of L into the cobalt metallacycle. However, when L is an acetylene, another path involving a coordinated Diels–Alder type addition reaction cannot be excluded.

The production of **3** as an intermediate has been postulated for most of these systems and recently the first step of the reaction, substitution of PPh_3 with L, has been proven to be dissociation controlled in the reaction of **1** (R^1, R^2, R^3, R^4 = Me) with 2-butyne.[11] However, little is known about the reactivities of the cobalt metallacycles toward the reagent L or about the factors governing the reactions, particularly with

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