Table I. Second-Order Rate Constants for Monocomplex Formation on $Mo(H_2O)_6^{3+}$ at 298 K

incoming group	k_f^{298}, M^{-1} s^{-1}	ref	incoming group	k_f^{298}, M^{-1} s^{-1}	ref
Cl^-	4.6×10^{-3} [a]	5	$Co(C_2O_4)_3^{3-}$	0.340[b]	7
NCS^-	0.268[a]	5	$MoO_2(H_2O)_5^{3+}$	42.0[b]	8
	0.317[a]	this work	O_2	180.0[b]	8
$HC_2O_4^-$	0.490[a]	6			

[a] μ = 1.00 M. [b] μ = 2.00 M. The presence of an accompanying redox reaction in these studies is noted.

Table II. Kinetic Parameters for NCS^- Anation on $Mo(H_2O)_6^{3+}$ [a]

	this work	ref 5
k_f^{298}, M^{-1} s^{-1}	0.317 ± 0.014	0.268 ± 0.013
$\Delta H^{\ddagger}$, kJ mol^{-1}	67.2 ± 2.7	68.1 ± 1.7
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	-29.2 ± 9.4	-26.7 ± 5.4
$\Delta V^{\ddagger}$, cm^3 mol^{-1}	-11.4 ± 0.5[b]	

[a] μ = 1.00 M. [b] At 285 K.

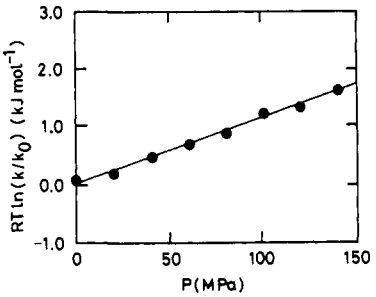

Figure 1. Pressure dependence of the rate constant for the formation of $Mo(H_2O)_5NCS^{2+}$ at 285 K in normalized logarithmic scale.

of most commercial spectrophotometers and was used in the same instrument as for the temperature work.

Plots of $\ln(A_\infty - A_t)$ vs. time, linear over more than four half-lives, yielded pseudo-first-order rate constants k_{obsd} at eight pressures (Figure 1). The k_f values obtained from $k_{obsd} = k_f[Mo^{3+}]$ were fitted as $\ln k_f = \ln k_0 - \Delta V_f^{\ddagger}P/RT$ with $\ln k_0$ and $\Delta V_f^{\ddagger}$ as adjustable parameters. The resulting volume of activation, $\Delta V_f^{\ddagger} = -11.4 \pm 0.5$ cm^3 mol^{-1}, is given in Table II with the other kinetic parameters for the reaction.

Comparison of this activation volume with the values reported for water exchange or substitution reactions with uncharged ligands on trivalent cations is not directly possible, since substitution of water by an anionic ligand like NCS^- induces a large reduction in solvent electrostriction due to charge neutralization. The overall reaction can be described by eq 1. It appears second

$$M(H_2O)_6^{n+} + L^{n-} \overset{K_{os}}{\rightleftharpoons} [M(H_2O)_6,L]^{(n-m)+} \xrightarrow{k_l} M(H_2O)_5L^{(n-m)+} + H_2O \quad (1)$$

order with rate constant $k_f = K_{os}k_l$. The volume of activation is accordingly given by $\Delta V_f^{\ddagger} = \Delta V_{os}^0 + \Delta V_l$. The formation constant K_{os} and volume ΔV_{os}^0 of the outer-sphere reaction can be estimated from purely electrostatic models.[14,15] For uncharged ligands, ΔV_{os}^0 is assumed to be zero. For the anation of a monovalent anion with a trivalent cation, a positive contribution of about +5.3 cm^3 mol^{-1} is expected. The volume of activation for the interchange step in the formation of $Mo(H_2O)_5NCS^{2+}$, $\Delta V_l^{\ddagger}$, is therefore likely to be about -17 cm^3 mol^{-1}. This is far more negative than the values reported for the volumes of activation for water exchange $\Delta V_{ex}^{\ddagger}$ on the early trivalent elements of the first-row transition series, -12.1 cm^3 mol^{-1} for Ti^{3+}, -8.9 cm^3 mol^{-1} for V^{3+}, and -9.6 cm^3 mol^{-1} for Cr^{3+}.[3] It is also much larger than the extreme value of -13.5 cm^3 mol^{-1} proposed by Swaddle[16] for a limiting associative mechanism in the case of the simple, perfectly symmetrical, solvent exchange reaction. The overall volume change involved in this asymmetrical substitution process is not known. Nevertheless, large positive volumes of reaction have been obtained for NCS^- anation on V^{2+} (ΔV^0 = +9.4 cm^3 mol^{-1})[17] and Fe^{3+} (ΔV^0 = +7.5, +8.3, +8.9, and +17.5 cm^3 mol^{-1}),[4] and although an exact figure cannot be extrapolated, one can safely assume that the volume of reaction will also be positive for Mo^{3+}. It means that the volume of activation, $\Delta V_r^{\ddagger}$, for the reverse aquation reaction (not measurable) must be even more negative than $\Delta V_f^{\ddagger}$. The character of the reaction under study is thus undoubtedly associative. For such an associative mode of activation, the $\Delta V_l^{\ddagger}$ value is not expected to match the value of the activation volume for the corresponding water exchange, $\Delta V_{ex}^{\ddagger}$, in the same way that k_l values are not supposed to be equal to k_{ex}, but the $\Delta V_{ex}^{\ddagger}$ can be used as a reference value to define the degree of associativeness of the reaction. Following this idea, we can conclude that, in comparison with the $\Delta V_{ex}^{\ddagger}$ values mentioned above, the value of $\Delta V_l^{\ddagger} \simeq -17$ cm^3 mol^{-1} is characteristic of a very strong associative interchange mechanism, if not even of a limiting A mechanism.

Acknowledgment. Y.D. and A.E.M. thank the Swiss National Science Foundation (Grant 2.854-0.85) and D.T.R. the Convention Intercantonale Romande pour l'Enseignement du 3eme Cycle en Chimie for financial support.

(12) It consists of a beryllium-copper pressure-tight cylinder (80 mm in length and 45-mm o.d.) with a sapphire window inbedded at one end and a closing beryllium-copper assembly at the other end, in which a second sapphire window is enclosed. The sample is contained in a 2-cm "pill box" quartz cell,[13] immersed in the pressure vessel. A 100-Ω platinum resistance is introduced sideways along the internal face of the cylinder to measure the temperature in the vicinity of the sample. A capillary connector transmits the applied pressure through the side wall. The cell is thermostated by circulation of a fluid in a helical groove carved in the outer surface of the cylinder, covered by a beryllium-copper envelope and thermally insulated from the outside by a cylindrical vacuumed quartz Dewar.

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(15) Hemmes, P. *J. Phys. Chem.* **1972**, *76*, 895.
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(17) Nichols, P. J.; Ducommun, Y.; Merbach, A. E. *Inorg. Chem.* **1983**, *22*, 3993.

Synthesis and Electron-Transfer Rates of Coplanar Diporphyrins: Models for (Heme) Protein-Protein Electron-Transfer Reactions

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Received September 25, 1986

Despite the crucial biological role of protein electron-transfer reactions, the factors which control this reactivity remain poorly understood.[1,2] One simple approach to developing such understanding is to develop "small" molecule model systems to mimic intramolecular electron transfer in the protein systems. Appropriate models require that the donor and acceptor be held at a fixed distance and orientation which correspond to those in the protein-protein complex. Current information[3] suggests that in the reactive heme protein-protein complexes, the heme centers are constrained at relatively long distances. For example in the cyt c/cyt b_5 complex, the Fe-Fe distance is ca. 16 Å, and in the cyt c/cyt c peroxidase complex, the Fe-Fe distance is ca. 24 Å. It is believed that the mean heme planes are held parallel in these complexes. In the models described herein, however, the protein

(1) Reviews include: (a) *Tunneling in Biological Systems*; Chance, B., ed.; Academic Press: Philadelphia, 1979. (b) Guarr, T. Mclendon, G. *Coord. Chem. Rev.* **1985**, *68*, 1-52. (c) Marcus, R.; Sutin, N. *Biochim. Biophys Acta* **1985**, *811*, 265-322.
(2) For recent work in this area, see: (a) Winkler, J.; Nocera, D.; Mauk, A. G.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798-5800. (b) McLendon, G.; Miller, J. *J. Am. Chem. Soc.* **1985**, *107*, 7811-7816. (c) McGourty, J.; Blough, N.; Hoffman, B. *J. Am. Chem. Soc.* **1983**, *105*, 4470-4472. (d) Cave, R.; Klippenstein, S.; Marcus, R. J. *Chem. Phys.* **1986**, *84*, 3089-3098.
(3) Review of Protein complexes include: Poulos, T.; Finzel, B. *Pept. Protein Rev.* **1985**, *5*, 1-56.

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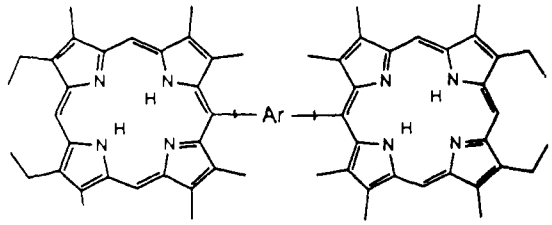
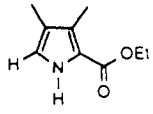
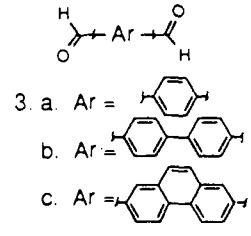
matrix is replaced by a simple synthetic spacer. We now wish to report a general approach to the synthesis of such models, of general structure **1**. In addition, initial data are reported for the simplest members of the series, in which the spacer is a phenyl group.



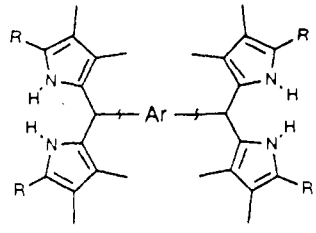
1. a. Ar =
b. Ar =
c. Ar =
d. Ar =



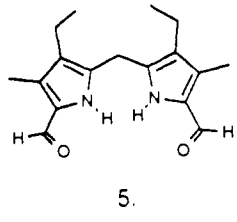
2.



3. a. Ar =
b. Ar =
c. Ar =



4. a. R = CO_2Et



5.

The synthetic route to these diporphyrins is analogous to previously published syntheses of meso-bridged cofacial diporphyrins.[4] 3,4-dimethyl-5-ethoxycarbonylpyrrole (**2**), prepared by the method of Kleinspehn,[5] was condensed with appropriate aryl dialdehydes **3a–d** to form a bis(dipyrromethane) tetraester **4a**[6] which includes the spacer. The tetraester was then hydrolyzed and decarboxylated to the tetra-5*H* bis(dipyrromethane) **4b**. The diporphyrin is then obtained by the acid-catalyzed, Zn-mediated coupling[7] of the bis(dipyrromethane) **4b** with 3,3′-diethyl-4,4′-dimethyl-5,5′-diformyl-2,2′-dipyrromethane (**5**)[8] in methylene chloride followed by air oxidation.[9] Methylene chloride was chosen as solvent in order to assure mutual solubility of the reactants. In this manner, we were able to produce the first examples of coplanar porphyrins which afford models for heme stereochemistry of biological heme protein complexes.[10]

The alkyl substituents are believed to be crucial in the construction, to ensure that the aryl bridge remains perpendicular to the mean porphyrin plane, thereby minimizing direct π interaction between the porphyrin and the bridge.

By changing the identity of the spacer, a wide variety of reaction distances, (**1a** vs. **1c**), geometries (of **1c** vs. **1d**), and coupling energies (**1a** vs. **1b**) can be accessed.[11]

With such molecules available and characterized, the key questions become, (1) what are the rates of electron transfer in the models and (2) how do these rates compare with other small molecule "bichromophoric" systems[12] and with protein–protein complexes?

Several initial approaches have been taken to answer this question.[13] The first method is based on NMR measurements of electron exchange in the mixed-valence $Fe^{II}P$–spacer–$Fe^{III}P$ system[14] (spacer = phenyl). Although the results are complicated by competing unimolecular and bimolecular pathways, the key finding is that under conditions where bimolecular electron transfer is minimal, the mixed-valence system undergoes fast electron exchange ($k_{et} > 10^4\ s^{-1}$). This rate includes not only the electronic coupling as affected by reactant separation and stereochemistry but also contains the reorganization (activation) energy for the charge transfer which is expected to be substantial when $\Delta G = 0$. In order to minimize the activation energy, we have measured the rate of the excited state reaction in the "half-filled" dimer H_2P–phenyl–$Fe^{III}P(Im)_2$. In this molecule, the fluorescence lifetime, measured using picosecond excitation and a jitter-free streak camera,[13b] is 2×10^{-11} s. This directly measured rate is in good agreement with rate estimates from static emission intensity quenching in comparison to the metal-free material. This corresponds to a fluorescence quenching rate of $5 \times 10^{10}\ s^{-1}$ (MeOH solution, 25 °C). We assign this quenching process to electron transfer. It is >100-fold faster than the rate expected for energy transfer[15] but well within the range observed for other electron transfer in donor acceptor assemblies.[12] Assuming for this latter reaction that $\Delta G \cong \lambda$, we can estimate the electronic nonadiabaticity as $k_{obsd} \cong 10^{10} \cong [(\pi/h^2)\lambda_s kT]^{1/2}(V(r))^2(FCF)$. We assume the Franck–Condon factor $\cong 1$ when $\Delta G = \lambda \cong 1$ V. $V(r)$ depends primarily on the coupling of the reactant and product wave functions (including both through-space and through-bond coupling).

Allowing for uncertainty in the FCF, we estimate a coupling strength of 3.5 cm^{-1} (corresponding to a "nonadiabaticity" of ca. 10^{-3}), reflecting separation of the donor and acceptor by the phenyl spacer. This magnitude can be compared with studies of other bichromophoric systems. A simple through-space interaction[1] would suggest a nonadiabaticity of $\kappa \cong \exp^{-1.2R}$ where R is the donor acceptor distance (Å) ($R \cong 6$ Å for the phenyl bridge, $\kappa = 7 \times 10^{-4}$). For aliphatic cyclohexane spacers, Closs and Miller found coupling strengths of ca. 1–8 cm^{-1}, in interesting comparison with our result. Both comparisons suggest that any interaction

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(9) Analytical data for **1a**: $(M + H)^+$ calcd 975.580 167 g/mol; obsd 975.580 165 g/mol. Vis ϵ 404.5 (1.3×10^5), 505.0 (8800), 535.0 (5600), 569.5 (5200), 623.5 nm (2200); ^1H NMR ($CDCl_3$) δ 10.13 (4 H, s, meso), 9.93 (2 H, s, meso), 8.22 (4 H, s, phenyl), 4.12 (8 H, quar, J = 7.2 Hz, CH_2CH_3), 3.60 (12 H, s, Me), 3.47 (12 H, s, Me), 2.38 (12 H, s, Me), 1.83 (12 H, t, J = 7.2 Hz, CH_2CH_3), −3.22 (2 H, br s, NH), −3.34 (2 H, br s, NH).

(10) We note that analogous approaches are being pursued elsewhere; e.g.: Collman, J.; Kim, personal communication. Wasielewski, M., personal communication.

(11) The compounds **1a–c** have all been synthesized and characterized and are currently under investigation.

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(15) For dipolar energy transfer, with R = 14 Å, $k \cong 10^8\ s^{-1}$, for the H_2–porphyrin/Fe^{III}–poprhyrin pair.

with the phenyl spacer in these molecules does not lead to anomalously strong D–A coupling. However, a detailed evaluation of donor–spacer–acceptor coupling must await more detailed studies. We note that analogous studies by Beyerle[12d] of phenyl linked D–A compounds suggest coupling occurs primarily through the σ framework. However, the compounds studied by Beyerle are significantly more flexible than those presented here.

Finally, we note that the diporphyrin model reacts *many* times faster (ca. 10^4) than a similar protein–protein system at similar ΔG (e.g., Zn cyt c/Fe^{III} cyt b_5 R $\cong$ 8 Å, $k_{max} \cong 10^6$ s^{-1}).[16] This result minimally suggests that the protein matrix does not accelerate the electron-transfer rate.

Acknowledgment. This work was supported by grants from the National Science Foundation (CHE8303896 and PCM83-02601) and in part by the sponsors of the Laser Fusion Feasibility Project at the University of Rochester. We also acknowledge the experimental assistance of Professor Tom Nordlund and helpful discussions with Drs. Mike Wasielewski, James Collman, and C. K. Chang.

(16) Roughly 1 order of magnitude of this difference can easily be attributed to distance (8 vs. 6 Å) k(6 Å)/k(8 Å) $\cong \exp^{-1.2(2)}$.

Flavohemoglobin: A Semisynthetic Hydroxylase Acting in the Absence of Reductase

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Received August 15, 1986

Heme proteins involved in redox reactions, such as cytochrome P-450,[3] function only by single electron transfers in each step and require an electron transport system to work with the usual biological electron donor, e.g., NADPH, which acts as a two-electron donor. From the viewpoint of practical applications alteration of the heme proteins, allowing them to react directly with two-electron donors without the intervention of the electron-transport system, would be a most challenging target in the protein engineering field.

Hemoglobin is known to catalyze a wide variety of monooxygenase reactions[4] in a typical P-450-like electron-transport system consisting of NADPH, O_2, and NADPH-cytochrome P-450 reductase (EC 1.6.2.4), a major flavoprotein in the microsomal electron-transport system.[5] Therefore, we started with hemoglobin as a representative heme protein and undertook to replace the reductase in the electron-transport system by an isoalloxazine (flavin) moiety covalently attached to hemoglobin in the vicinity of the heme. We report here that the resulting molecular conjugate, flavohemoglobin (Fl-Hb^{3+}), served as a hydroxylase for aniline without requiring the P-450 reductase.

One free cysteine residue titratable with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) exists in the β-chain of hemoglobin, i.e., two exposed cysteine residues in the whole hemoglobin molecule.[6] The cysteine (β-93) is located adjacent to the histidine (β-92) which is coordinated to the heme iron. A flavin reagent designed to modify this thiol residue under mild conditions was derived from the parent 7-cyanoisoalloxazine (Scheme I), which is more reactive toward NADPH than flavin coenzymes.[7]

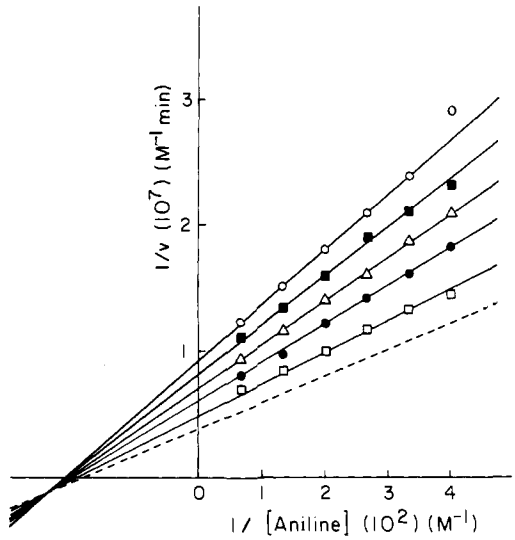

Figure 1. Double-reciprocal plots of the rate of para-hydroxylation against aniline concentration at various concentrations of NADPH. The reaction mixtures (1 mL) consisted of 1 μM Fl-Hb^{3+} (4 μM with respect to heme), 20 mM potassium phosphate, pH 7.5, aniline, and NADPH at 0.15 (○), 0.188 (■), 0.25 (△), 0.375 (●), and 0.75 mM (□). The reactions were allowed to proceed for 15 min at 37 °C and then terminated by addition of ice-cold 20% trichloroacetic acid. Product *p*-aminophenol was determined according to the phenol–indophenol method of Mieyal et al.[4a] Since substrate inhibition was significant at higher concentrations of aniline (>20 mM),[4d] the kinetic measurements were carried out at concentrations less than 3 times the value of K_m. The dashed line in the figure represents the predicted dependence of the rate at an infinite concentration of NADPH.

Hemoglobin A was prepared from normal human erythrocytes[8] and converted to carbon monoxyhemoglobin ($COHb^{2+}$). $COHb^{2+}$ (0.05 mM) was modified by treatment with 0.5 mM of **5** in 0.01 M potassium phosphate, pH 7.0 at 25 °C for 30 min. The major product, Fl-$COHb^{2+}$, was isolated by ion-exchange chromatography on CM-Sepharose.[9] Fl-$COHb^{2+}$ was then subjected to hydrophobic interaction HPLC analysis[10] using a TSK Phenyl-5PW column,[11] and the homogeneity (>96% purity) of the modified protein was confirmed. The absorption difference at 280 nm between Fl-$COHb^{2+}$ and $COHb^{2+}$ ($\Delta\epsilon_{280}$ = 95 000) corresponded to the absorbance of 1.9 equiv of the flavin, based on ϵ_{280} = 51 000 of *N*-3-alkyl-7-cyanoisoalloxazine. Together with the observed decrease in the free thiol content from 2.1 equiv in $COHb^{2+}$ to 0.1 equiv in Fl-$COHb^{2+}$, these data are consistent with the conclusion that two flavin moieties were introduced into the $COHb^{2+}$ molecule. Ferric flavohemoglobin (Fl-Hb^{3+}) was subsequently prepared by the oxidation of 0.1 mM Fl-$COHb^{2+}$ with 4 mM potassium ferricyanide at 25 °C for 1 h and isolated by gel filtration on Sephadex G-25.[4e]

(1) Laboratory of Bioorganic Chemistry and Biochemistry.
(2) Laboratory of Metabolism and Pharmacology.
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(11) Toyo Soda Ltd., Japan. Fl-$COHb^{2+}$ and $COHb^{2+}$ were well resolved on a reverse linear gradient of 1–0 M $(NH_4)_2SO_4$.

0002-7863/87/1509-0606$01.50/0