Contribution from the Laboratory of Analytical Chemistry,
Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

Kinetic Studies on the Reaction of Hydrogen Peroxide with (5,10,15,20-Tetrakis(4-*N*-methylpyridiniumyl)porphine(2+))oxotitanium(IV) in Aqueous Solutions.[1] Evidence for an Associative-Interchange Mechanism

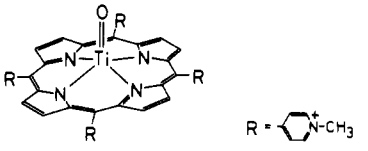
MASAHIKO INAMO, SHIGENOBU FUNAHASHI, and MOTOHARU TANAKA*

Received January 30, 1985

The kinetics of the reaction of hydrogen peroxide with (5,10,15,20-tetrakis(4-*N*-methylpyridiniumyl)porphine(2+))oxotitanium(IV) ($[Ti^{IV}O(tmpyp)]^{4+}$) leading to the corresponding 1:1 monoperoxo complex, $[Ti^{IV}(O_2)(tmpyp)]^{4+}$, has been investigated in aqueous solutions at $I = 1.00$ M at various temperatures and pressures. The peroxo complex formation is first order in the concentration of hydrogen peroxide and the titanium(IV) complex. Activation parameters for the proton-independent path (rate constant k) and the proton-catalyzed path (rate constant k_H) were obtained as follows: $k(25\ °C) = 2.86 \times 10^{-2}\ M^{-1}\ s^{-1}$, $\Delta H^{\ddagger} = 40.1 \pm 1.0$ kJ mol^{-1}, $\Delta S^{\ddagger} = -140 \pm 3$ J K^{-1} mol^{-1}, $\Delta V^{\ddagger} = -18.6 \pm 0.3$ cm^3 mol^{-1}; $k_H(25\ °C) = 5.17 \times 10\ M^{-2}\ s^{-1}$, $\Delta H_H^{\ddagger} = 38.5 \pm 1.0$ kJ mol^{-1}, $\Delta S_H^{\ddagger} = -83 \pm 3$ J K^{-1} mol^{-1}, $\Delta V_H^{\ddagger} = -3.9 \pm 0.2$ cm^3 mol^{-1}. The rate-determining step is considered to be the insertion of hydrogen peroxide into the inner coordination sphere of titanium(IV). An associative-interchange mechanism is most probably operative. This reaction is also catalyzed by acetic acid as well as by bases such as hydroxide, acetate, some morpholinoalkanesulfonates, etc. The catalytic effect is related to the strength of the relevant acids and bases.

Introduction

Current interest has been focused on dioxygen complexes of metalloporphyrins because of their relevance to the biological systems.[2] These dioxygen complexes are known to be produced through the reaction of the corresponding metalloporphyrins with dioxygen, superoxide ion, or hydrogen peroxide. Reaction mechanisms of the dioxygen uptake by some metalloporphyrins have been extensively investigated,[3] while little is known about the reactions with hydrogen peroxide. Most metalloporphyrins of early transition metals have strongly bound oxygen atoms as an axial ligand, and some of these are known to react with hydrogen peroxide to produce one type of dioxygen complex, i.e., a peroxo complex.[4,5] Thus diperoxomolybdenum(VI) porphyrins have been obtained from the corresponding molybdenum(V) porphyrins,[5a] while peroxotitanium(IV) porphyrins are formed through the oxo substitution by hydrogen peroxide.[4b] It has been demonstrated by labeling experiments that the oxo group in $Ti^{IV}O(por)$[6] was in fact substituted by the peroxo group.[4c] An associative mechanism has been proposed for this reaction.[4c] we are interested in the formation kinetics of peroxo complexes of early-transition-metal ions[1] and have previously reported the kinetics of the reaction of hydrogen peroxide with a water-soluble titanium(IV) porphyrin complex (Ti(IV)–TPyP[6]) in an acidic aqueous solutions.[4g] Here we report the kinetics of the similar reaction of a Ti(IV)–TMPyP[6] complex (**1**) over a wider pH range



$[Ti^{IV}O(tmpyp)]^{4+}$ (1)

extending to the basic aqueous solution. One of our purposes is to elucidate the mechanism of substitution of an oxo group in the metalloporphyrin by hydrogen peroxide in aqueous solutions.

Experimental Section

Reagents. Reagent grade sodium nitrate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was recrystallized twice from distilled water. Nitric acid of special purity (Wako) was used as obtained. About 60% hydrogen peroxide solution containing no stabilizing agent (donated from Mitsubishi Gas Chemical Co. Inc, Kanagawa, Japan) was purified by distillation under reduced pressure. The hydrogen peroxide concentration was determined with a standard solution of potassium permanganate. 2-Morpholinoethanesulfonic acid (MES or Hmes), 3-morpholinopropanesulfonic acid (MOPS or Hmops), and 3-((tris(hydroxymethyl)methyl)amino)propanesulfonic acid (TAPS or Htaps) (Wako) used in pH buffers were recrystallized twice from aqueous ethanol.

Oxo(5,10,15,20-tetra-4-pyridylporphinato(2–))titanium(IV) (Ti(IV)–TPyp), $Ti^{IV}O(tpyp)$, was prepared as described previously.[4g] (5,10,15,20-Tetrakis(4-*N*-methylpyridiniumyl)porphine(2+))oxotitanium(IV) iodide, $[Ti^{IV}O(tmpyp)]I_4$, was obtained by the following procedure. A 70-mg portion of the Ti(IV)–TPyP complex and a large excess of methyl iodide (5 cm^3) were dissolved in 50 cm^3 of *N*,*N*-dimethylformamide. The mixture was kept at 150 °C for 1 h, then cooled to room temperature, and mixed with 100 cm^3 of ethyl ether. The precipitated product was collected on a funnel and washed with ethyl ether several times. Anal. Calcd for $C_{44}H_{36}N_8OTiI_4$: C, 42.33; H, 2.91; N, 8.98. Found: C, 42.12; H, 3.28; N, 8.84. $[Ti^{IV}O(tmpyp)]I_4$ displays a sharp and intense band at 944 cm^{-1} in the IR spectra obtained for the solid in a KBr disk on a JASCO IRA-3 infrared spectrometer. This is assigned as due to the stretching of the Ti=O bond. This complex is soluble in aqueous solutions over the entire pH range. Visible absorption spectra were recorded on a highly sensitive spectrophotometer (type SM401, Union Giken, Osaka, Japan). Vis (λ_{max}/nm (log (ϵ/M^{-1} cm^{-1}))): 435 (5.47), 565 (4.31), 605 (3.65) (M = mol dm^{-3}, molar units).

Measurements. The temperature of reaction solutions was controlled to within ±0.1 °C. The ionic strength was maintained at 1.00 M with sodium nitrate and nitric acid. The hydrogen ion concentration at pH >2.0 was measured by an Orion Research pH meter (Model 701A) with a glass electrode (Metrohm, Type EA109) and a reference electrode (Metrohm, Type EA404). A 1.000×10^{-2} M aqueous solution of nitric

(1) Reactions of Hydrogen Peroxide with Metal Complexes. 9. Part 8: Inamo, M.; Funahashi, S.; Ito, Y.; Hamada, Y.; Tanaka, M. *Inorg. Chem.*, preceding paper in this issue.
(2) (a) Basolo, F.; Hoffman, B. M.; Ibers, J. A. *Acc. Chem. Res.* **1975**, *8*, 384. (b) Collman, J. P. *Acc. Chem. Res.* **1977**, *10*, 265. (c) Fuhrhop, J.-H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 648. (d) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, *79*, 139. (e) Ullrich, V.; Ahr, H. J.; Castle, L.; Kuthan, H.; Nastainczyk, W.; Ruf, H. H. In "The Biological Chemistry of Iron"; Dunford, H. B., Dolphin, D., Raymond, K. N., Sieker, L., Eds. D. Reidel: Dordrecht, Holland, 1982; p 413.
(3) James, B. R. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press; New York, 1978; Vol. V, p 205.
(4) (a) Guilard, R.; Fontesse, M.; Fournari, P.; Lecomte, C.; Protas, J. *J. Chem. Soc., Chem. Commun.* **1976**, 161. (b) Guilard, R.; Latour, J.-M.; Lecomte, C.; Marchon, J.-C.; Protas, J.; Ripoll, D. *Inorg. Chem.* **1978**, *17*, 1228. (c) Latour, J.-M.; Galland, B.; Marchon, J.-C. *J. Chem. Soc., Chem. Commun.* **1979**, 570. (d) Latour, J.-M.; Marchon, J.-C.; Nakajima, M. *J. Am. Chem. Soc.* **1979**, *101*, 3974. (e) Rhomer, M. M.; Barry, M.; Dedieu, A.; Veillard, A. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1977**, *4*, 337. (f) Boreham, C. J.; Latour, J.-M.; Marchon, J.-C.; Boisselier-Cocolios, B.; Guilaid, R. *Inorg. Chim. Acta* **1980**, *45*, L69. (g) Inamo, M.; Funahashi, S.; Tanaka, M. *Inorg. Chem.* **1983**, *22*, 3734.
(5) (a) Chevrier, B.; Diebold, Th.; Weiss, R. *Inorg. Chim. Acta* **1976**, *19*, L57. (b) Ledon, H.; Bonnet, M.; Lallemand, J.-Y. *J. Chem. Soc., Chem. Commun.* **1979**, 702. (c) Kadish, K. M.; Chang, D.; Malinski, T.; Ledon, H. *Inorg. Chem.* **1983**, *22*, 3490.
(6) Ligand abbreviations: por, porphyrin dianion; H_2tpyp (TPyP), 5,10,15,20-tetra-4-pyridylporphine; H_2tmpyp (TMPyP), 5,10,15,20-tetrakis(4-*N*-methylpyridiniumyl)porphine; H_2oep (OEP), 2,3,7,8,12,13,17,18-octaethylporphine; H_2tpps, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine.

0020-1669/85/1324-2475$01.50/0

Table I. Rate Constants k and k_H at Various Temperatures[a]

	T/°C[b]	10^2k/M^{-1} s^{-1}	$10^{-1}k_H$/M^{-2} s^{-1}
	15.0 (20)	1.61 ± 0.03[c]	2.87 ± 0.06
	25.0 (24)	2.86 ± 0.06	5.17 ± 0.10
	35.0 (20)	5.10 ± 0.08	8.72 ± 0.13
$\Delta H^{\ddagger}$/kJ mol^{-1}		40.1 ± 1.0	38.5 ± 1.0
$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}		−140 ± 3	−83 ± 3

[a] At 0.1 MPa. [b] The number of data points at each temperature is given in parentheses. [c] Error limits are 1 standard deviation.

acid at I = 1.00 M was used as a pH standard solution. In all kinetic measurements reactions were followed spectrophotometrically under pseudo-first-order conditons. An SM401 spectrophotometer and a sample mixing device (Type MX7, Union Giken) were used to obtain the kinetic data. The progress of fast reactions under pressures up to 120 MPa was measured by a high-pressure stopped-flow apparatus exploited in our laboratory,[7] while a high-pressure static cell connected to a spectrophotometer was used for slower reactions.

Determination of Acid Dissociation Constants of MES, MOPS, and TAPS. The acid dissociation constants of MES, MOPS, and TAPS were determined potentiometrically at 25.0 °C and I = 1.00 M ($NaNO_3$): $pK_a(HB) = -\log([H^+][B^-][HB]^{-1}/M)$, where HB and B^- represent an acid and its conjugate base, respectively (All data are given in Figure S1.).[8] The pK_a values obtained are as follows: pK_a(MES) = 6.166 ± 0.003, pK_a(MOPS) = 7.188 ± 0.010, and pK_a(TAPS) = 8.365 ± 0.003.

Results and Discussion

Formation of the Peroxo Complex. $[Ti^{IV}O(tmpyp)]I_4$ was soluble over the pH range 1–12, and no substantial spectral change was observed with pH variation. It has been reported that, in the crystalline state of the Ti(IV)–OEP[6] complex, $Ti^{IV}O(oep)$, the titanium atom lies 60.3 pm from the mean plane of the porphyrin skeleton.[4b] Therefore, we conclude that in water the Ti(IV)–TMPyP complex exists as $[Ti^{IV}O(tmpyp)]^{4+}$ in which the titanium(IV) atom is pentacoordinated by the four nitrogen atoms and the oxygen atom; i.e., no water coordinates to the central metal and the oxo group is not protonated even at very low pH.

The reaction of hydrogen peroxide with oxotitanium(IV) porphyrins, $Ti^{IV}O(por)$, affords the corresponding peroxotitanium(IV) porphyrin, $Ti^{IV}(O_2)(por)$.[4b] The reaction of the Ti(IV)–TPyP complex with hydrogen peroxide at pH 1.0–2.1 produces a similar peroxo complex, $Ti^{IV}(O_2)(tpypH_4)^{4+}$.[4g] Therefore, in the present system the reaction of the Ti(IV)–TMPyP complex **1** with hydrogen peroxide is expressed as

$$\mathbf{1} + H_2O_2 \rightleftharpoons \underset{\mathbf{2}}{[Ti^{IV}(O_2)(tmpyp)]^{4+}} + H_2O \qquad (1)$$

The visible absorption spectrum of **2** also does not change with pH as in the case of **1**. The spectra of **1** and **2** are shown in Figure S2.[8] The kinetics of reaction 1 was studied with a large excess of hydrogen peroxide over the Ti(IV)–TMPyP complex. Equilibrium 1 is much favored to the right under the present experimental conditions, so the reverse reaction is negligible in the kinetic studies. First-order plots were linear at least for 3 half-lives. First-order rate constants were proportional to the concentration of hydrogen peroxide over the range from 10^{-3} to 10^{-1} M at constant acidity. Therefore the rate law is described as

$$-d[\mathbf{1}]/dt = d[\mathbf{2}]/dt = k_{0(H)}[\mathbf{1}][H_2O_2] \qquad (2)$$

where $k_{0(H)}$ represents the conditional second-order rate constant. In Figure 1 is given the dependence of $k_{0(H)}$ on $-\log([H^+]/M)$ under conditions with no added pH buffer reagent. The slope of the plot tends to be −1 at higher acidity and 0 at lower acidity. This is well described by eq 3, where k and k_H are rate constants

$$-d[\mathbf{1}]/dt = (k + k_H[H^+])[\mathbf{1}][H_2O_2] \qquad (3)$$

for the proton-independent path and for the proton-assisted path, respectively. Judging from the values of the proton dissociation

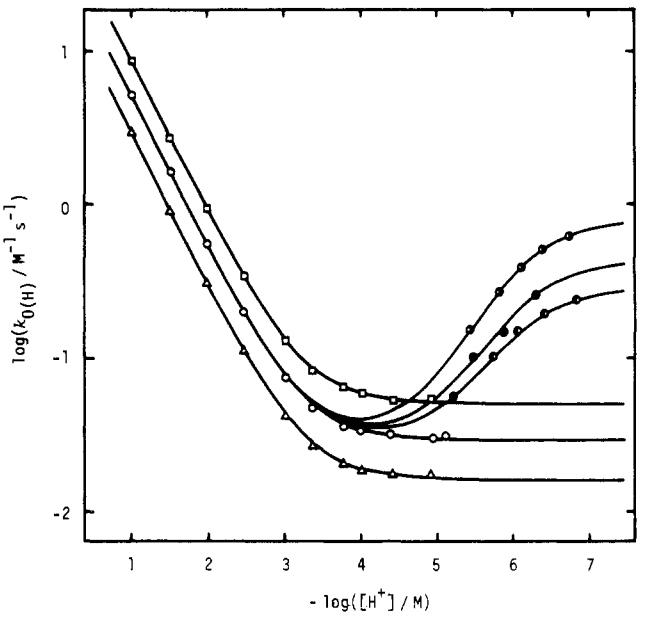


Figure 1. Hydrogen ion concentration dependence of the second-order rate constant $k_{0(H)}$ in the absence of any pH buffer agents at 15.0 °C (Δ), 25.0 °C (○), and 35.0 °C (□) and in the presence of MES at 25.0 °C and C_{MES} = 3.23 × 10^{-3} M (◐), 5.00 × 10^{-3} M (●), and 9.68 × 10^{-3} M (◑). Each point in Figures 1–3 is the average value of several determinations.

constants for $H_3O_2^+$ and H_2O_2,[9] $[H^+][H_2O][H_3O_2^+]^{-1} \simeq 10^3$ M and $[H^+][HO_2^-][H_2O_2]^{-1} = 10^{-11.27}$ M, the contribution of $H_3O_2^+$ and HO_2^- can be precluded under the present experimental conditions. In the k_H path the reaction may be assisted by the preequilibrium protonation to the oxo group of **1**.[4g] Values of k and k_H and the corresponding activation parameters are tabulated in Table I.

Peroxo Complex Formation in the Presence of Buffers. Results described above are obtained in the absence of any pH buffer agents. In the presence of agents such as MES, MOPS, TAPS, and acetate, the second-order rate constant $k_{0(H)}$ is dependent not only on the hydrogen ion concentration but also on the concentration of buffers. Data collected in the MES buffer are shown in Figure 1. Values of $k_{0(H)}$ increase with increase both in pH and in the total concentration of MES, while $k_{0(H)}$ approaches the value observed in the absence of buffers when the pH becomes lower. This indicates the general-base catalysis of the conjugate base of MES (mes^-) in the peroxo complex formation. Therefore, in the presence of MES the rate constant $k_{0(H)}$ is written as

$$k_{0(H)} = k + k_H[H^+] + k_{mes}[mes^-] \qquad (4)$$

where k_{mes} is the rate constant for the base-catalyzed path. The value of k_{mes} was determined to be (7.61 ± 0.14) × 10 M^{-2} s^{-1} at 25.0 °C.

A similar general-base catalysis was observed in both the TAPS and MOPS buffers, but enhancement in rates in these buffers cannot entirely be attributable to the general-base catalysis by $taps^-$ or $mops^-$. In these systems the pH of solutions is higher than that for the MES system: the range of $-\log([H^+]/M)$ is 5.44–6.84 for the MES system, 6.45–7.93 for the MOPS system, and 7.48–8.69 for the TAPS system. Thus in the last two systems the specific base catalysis by hydroxide was also observed. The plot of $\{k_{0(H)} - (k + k_H[H^+])\}$ vs. $[taps^-]$ deviates systematically from a straight line (Figure S3).[8] This deviation is attributable to the catalysis by OH^-. The same situation prevails for the MOPS system. Therefore, $k_{0(H)}$ is generally expressed as eq 5, where B

$$k_{0(H)} = k + k_H[H^+] + k_B[B] + k_{OH}[OH^-] \qquad (5)$$

refers to the conjugate base of an acid HB (charges on B and HB

(7) (a) Funahashi, S.; Inamo, M.; Ishihara, K.; Tanaka, M. *Inorg. Chem.* **1982**, *21*, 447. (b) Ishihara, K.; Funahashi S.; Tanaka, M. *Rev. Sci. Instrum.* **1982**, *53*, 1231.
(8) Supplementary material.
(9) Evans, M. G.; Uri, N. *Trans. Faraday Soc.* **1949**, *45*, 224.

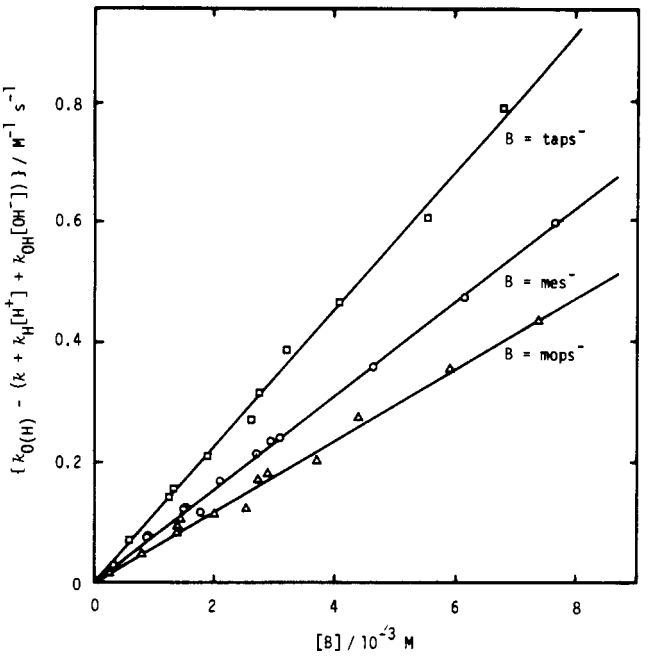


Figure 2. General-base catalysis of the peroxo complex formation at 25.0 °C. [B] is the concentration of the basic form of buffer agents.

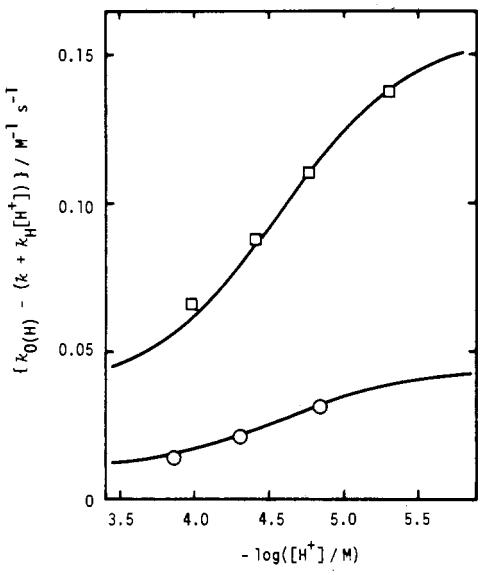


Figure 3. Catalysis by the acetate buffer at 25.0 °C and C_{HOAc} = 4.03 × 10^{-3} M (○) and 1.45 × 10^{-2} M (□). Solid lines are computer fits of the data to eq 6.

are omitted hereafter), i.e., taps⁻, mops⁻, or mes⁻, and k_B and k_{OH} are the rate constants for the reactions catalyzed by B and OH^-, respectively. Values of rate constants k_{taps}, k_{mops}, k_{mes}, and k_{OH} were calculated by applying eq 5 to all data obtained in the TAPS, MOPS, and MES systems.[10] The following values were obtained: k_{taps} = (1.14 ± 0.04) × 10^2 M^{-2} s^{-1}, k_{mops} = (5.91 ± 0.16) × 10 M^{-2} s^{-1}, k_{mes} = (7.61 ± 0.14) × 10 M^{-2} s^{-1}, and k_{OH} = (5.13 ± 0.35) × 10^4 M^{-2} s^{-1} at 25.0 °C. For the MES system the k_{OH}[OH^-] term is missing in eq 4 because of relatively low pH. The general-base catalysis is illustrated in Figure 2 as a plot of $\{k_{0(H)} - (k + k_H[H^+] + k_{OH}[OH^-])\}$ vs. [B].

Now we shall describe the results obtained in the acetate buffer over the pH range 3.9–5.3, where the contribution of the term

Table II. Rate Constants k and k_H at Various Pressures[a]

	P/MPa	10^2k/ mol^{-1} kg s^{-1}	$10^{-1}k_H$/ mol^{-2} kg^2 s^{-1}
	0.1	2.69 ± 0.08[b]	4.69 ± 0.07
	19.6	3.10 ± 0.02	4.88 ± 0.07
	39.2	3.65 ± 0.03	5.04 ± 0.13
	58.8	4.33 ± 0.10	5.09 ± 0.12
	78.5	4.81 ± 0.03	5.34 ± 0.08
	98.1	5.62 ± 0.06	5.51 ± 0.10
	117.7	6.48 ± 0.13	5.66 ± 0.07
ΔV^*/cm^3 mol^{-1}		−18.6 ± 0.3	−3.9 ± 0.2

[a] At 25.0 °C. [b] Each value is the average of two to seven determinations.

k_{OH}[OH^-] is practically negligible. In this system there is an appreciable effect of acid used in the buffer in addition to the general-base catalysis by acetate ion. The catalytic effect of the acetate buffer is shown in Figure 3 and is quantitatively accounted for by eq 6, where k_{HOAc} and k_{OAc} are the rate constants of reaction

$$k_{0(H)} = k + k_H[H^+] + k_{HOAc}[HOAc] + k_{OAc}[OAc^-]$$

$$= k + k_H[H^+] + \frac{k_{HOAc} + k_{OAc}(K_a(HOAc))[H^+]^{-1}}{1 + (K_a(HOAc))[H^+]^{-1}} C_{HOAc} \quad (6)$$

paths catalyzed by acetic acid and acetate, respectively, K_a(HOAc) is the acid dissociation constant of acetic acid,[11] and C_{HOAc} is the total concentration of acetate buffer. The values of rate constants of k_{HOAc} and k_{OAc} were estimated to be 2.59 ± 0.15 and (1.10 ± 0.03) × 10 M^{-2} s^{-1}, respectively, at 25.0 °C.

Peroxo Complex Formation under High Pressures. Rate constants k and k_H, obtained under pressures up to 120 MPa, are summarized in Table II and are plotted as a function of pressure (MPa) in Figure S4.[8] According to the transition-state theory the pressure effect on the rate constant k is related to the activation volume ΔV^* by $(\partial \ln k/\partial P)_T = -\Delta V^*/RT$. Since the logarithmic values of rate constants are linearly related to pressure, activation volumes are presumed to be pressure independent under the present experimental conditions. We obtained ΔV^* = −18.6 ± 0.3 cm^3 mol^{-1} for k and ΔV_H^* = −3.9 ± 0.2 cm^3 mol^{-1} for k_H at 25.0 °C.

The measured activation volume is the sum of an intrinsic part, ΔV_{intr}^*, and an electrostrictive part, ΔV_{el}^*.[12] ΔV_{intr}^* is the change in partial molar volume caused by bond breaking or bond making in the transition state, and ΔV_{el}^* is the change in volume with variation of solvation. Since hydrogen peroxide is noncharged, there seems to be little or no contribution of solvation change in the activation process.

A large negative activation volume for the proton-independent path (k) points to an associative mode of activation with the transition state involving hydrogen peroxide bonded as a monodentate. A hydrogen peroxide molecule would approach a face of the square pyramid of $[Ti^{IV}O(tmpyp)]^{4+}$. This mechanism is also consistent with a large negative value of activation entropy of k.

On the other hand, the k_H path involves the protonation of the oxo group in **1** as a preequilibrium. Thus, the activation parameter for the k_H path corresponds to the sum of the thermodynamic parameter of the protonation of the oxo group in **1** and the activation parameter of the reaction of the protonated species of **1** with hydrogen peroxide. The effect of protonation must involve a small positive contribution to ΔV^*, judging from the small negative partial molar volume of proton[13] and a small electrostriction on the oxo group, as expected from its very weak basicity.

(10) (a) The concentration of hydroxide ion was estimated by using the pK_w value of 13.75 at I = 1.00 M and 25.0 °C (Nakasuka, N.; Matsumura, K.; Kunimatsu, M.; Tanaka, M. *Inorg. Chem.* **1985**, *24*, 10). (b) Nakagawa, T.; Oyanagi, Y. "SALS (Statistical Analysis with Least-Squares Fitting)"; Program Library, Nagoya University Computation Center: Nagoya, Japan, 1979.

(11) K_a(HOAc) = [H^+][OAc^-][HOAc]$^{-1}$ = $10^{-4.609\pm0.005}$ M at I = 1.00 M and 25 °C (Portanova, R.; Di Bernardo, P.; Cassol, A.; Tondello, E.; Magon, L. *Inorg. Chim. Acta* **1974**, *8*, 233).

(12) Swaddle, T. W. *Coord. Chem. Rev.* **1974**, *14*, 217.

(13) $\bar{V}(H^+)$ = −4.5 cm^3 mol^{-1} (Palmer, D. A.; Kelm, H. *Inorg. Chem.* **1977**, *16*, 3139. Millero, F. J. *Chem. Rev.* **1971**, *71*, 147).

Table III. Activation Parameters for Substitution at the Axial Coordination Site of Metalloporphyrins

no.	reacn syst	$\Delta H^{\ddagger}$/ kJ mol^{-1}	$\Delta S^{\ddagger}$/ J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$/ cm^3 mol^{-1}	mechanism	remarks and ref
1	$[Co^{III}(tmpyp)(OH_2)_2]^{5+}$ + SCN^-	107 ± 3	129 ± 8	15 ± 4	D	*a*
2	$[Co^{III}(tpps)(OH_2)_2]^{3-}$ + SCN^-	153 ± 9	120 ± 31	15.4 ± 0.6	D	*b, c*
3	$[Rh^{III}(tpps)(OH_2)_2]^{3-}$ + SCN^-	137 ± 2	−86 ± 5	8.8 ± 0.4	I_d	*c, d*
4	$[Cr^{III}(tpps)(OH_2)_2]^{3-}$ + SCN^-	140 ± 3	−106 ± 12	7.4 ± 0.1	I_d	*c, e*
5	$[Mo^{V}O(tmpyp)OH_2]^{5+}$ + H_2O_2	37 ± 1	−63 ± 3	−0.2 ± 0.3	I	*f*
6	$[Ti^{IV}O(tpypH_4)]^{4+}$ + H_2O_2 + H^+	34.9 ± 0.5	−95 ± 1	−3.3 ± 0.2	I_a	*g*
7	$[Ti^{IV}O(tmpyp)]^{4+}$ + H_2O_2	40.1 ± 0.8	−140 ± 3	−18.6 ± 0.3	I_a	*h*
8	$[Ti^{IV}O(tmpyp)]^{4+}$ + H_2O_2 + H^+	38.5 ± 0.6	−83 ± 2	−3.9 ± 0.2	I_a	*h*

[a] Activation parameters are those for loss of a coordinated water molecule to produce a five-coordinated intermediate.[7a,14] [b] Reference 15. [c] Reference 16. [d] Reference 17. [e] Reference 18. [f] Reference 1. [g] Reference 4g. [h] This work (see text).

Table IV. Rate Constants[a] for the Acid- and Base-Catalyzed Formation of $[Ti^{IV}(O_2)(tmpyp)]^{4+}$

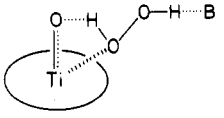
HB	pK_a(HB)	k_{HB}	k_B
H_3O^+	−1.73	(5.17 ± 0.10) × 10	(5.33 ± 0.11) × 10^{-4}
HOAc	4.609	2.59 ± 0.15	(1.10 ± 0.03) × 10
Hmes	6.166 ± 0.003	4.2 × 10^{-1} [b]	(7.61 ± 0.14) × 10
Hmops	7.188 ± 0.010	2.1 × 10^{-1} [b]	(5.91 ± 0.16) × 10
Htaps	8.365 ± 0.003	9.3 × 10^{-2} [b]	(1.14 ± 0.04) × 10^2
H_2O	15.48	(5.33 ± 0.11) × 10^{-4}	(5.13 ± 0.35) × 10^4

[a] In units of M^{-2} s^{-1}, at 25.0 °C. [b] Estimated by assuming a linear relationship in Figure 4.

Therefore, the activation volume for the rate-determining step of the k_H path is expected to be more negative than the obtained value of −3.9 cm^3 mol^{-1}. Thus the k_H path should be also associatively activated.

The values of activation volume for the axial substitution reaction of metalloporphyrins studied so far are summarized in Table III together with $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. The oxo substitution by hydrogen peroxide is associatively activated, while the axial water substitution reaction is known to be dissociative in character.[7a,14-18]

General-Acid and -Base Catalyses. Rate constants for the peroxo complex formation catalyzed by acids (HB) and bases (B) are summarized together with pK_a values of HB in Table IV. In Figure 4 is given the Brønsted plot: the relationship between the rate constant of reaction catalyzed by bases B (k_B) and the pK_a values of HB. The plot includes also the rate constant for the water-catalyzed path (k_{H_2O}): $k_{H_2O} = k[H_2O]^{-1} = 5.33 \times 10^{-4}$ M^{-2} s^{-1}. The slope of the straight line in Figure 4, the so-called Brønsted coefficient α, was estimated to be 0.33 ± 0.03 by a linear least-squares treatment if the point for H_2O is excluded (vide infra). The transition state of the base-catalyzed path should involve such species as **1**, H_2O_2, and B (B = CH_3COO^-, mes^-, $mops^-$, $taps^-$, OH^-). At the transition state (**3**) the base (B)



3

interacts with hydrogen peroxide in the inner sphere, thus playing an important role in proton abstraction. Since water is also a base, it is likely that it contributes to the rate in a similar manner. However, the hydrogen peroxide in the transition state may make hydrogen bonds not only with a water molecule through the lone-paired electrons of the latter but also with the hydrogen atom of another water molecule via the lone-paired electrons of hydrogen peroxide. Thus it is reasonable that the general-base catalysis

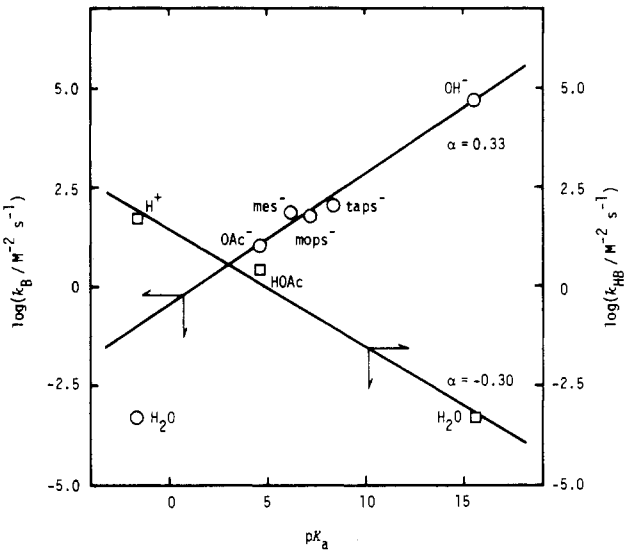


Figure 4. Brønsted plot of peroxo complex formation rate constants for acid catalysis (□) and base catalysis (○).

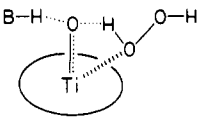
of water is less effective than expected from plots in Figure 4.

As described earlier, the hydrogen ion assists the peroxo complex formation by the protonation of the oxo group of **1**. Acetic acid may act as a general-acid catalyst in the same way. The k path is regarded as the reaction catalyzed by water through hydrogen bonding to the oxo group of the metalloporphyrin. As shown in Figure 4, the Brønsted plot for the acid catalysis (log (k_{HB}/M^{-2} s^{-1}) vs. pK_a) gives a linear relationship with a slope of −0.30 ± 0.04. If the relationship is also applicable to the MES, MOPS, and TAPS systems, the contribution of the acid (HB) in these buffers to the rate through general-acid catalysis is expected to be less than 2% of the observed rate in the MES system and to be much less in the MOPS and TAPS systems under the present experimental conditions. Therefore, it is reasonable that such a general-acid catalysis was not observed in these buffer systems.

It is very difficult for an oxo group to dissociate from the central metal ion.[4c,19] So it is reasonable that the oxo substitution by hydrogen peroxide occurs by an associative-interchange (I_a) mechanism via a transition state having both the entering and the leaving ligands adjacent to each other on the same side of the porphyrin.[4c] Leaving of the oxo group is accelerated by its interaction with acids, while entering of a peroxo group is assisted by the proton abstraction from hydrogen peroxide in the inner sphere by bases. A proposed transition state for the oxo substitution by hydrogen peroxide is presented as **3** for the base catalysis and **4** for the acid catalysis. The substitution of the oxo group by peroxide would be assisted to some extent by the proton

(14) Ashley, K. R.; Berggren, M.; Cheng, M. *J. Am. Chem. Soc.* **1975**, *97*, 1422.
(15) Ashley, K. R.; Au-Young, S. *Inorg. Chem.* **1976**, *15*, 1937.
(16) Leipoldt, J. G.; van Eldik, R.; Kelm, H. *Inorg. Chem.* **1983**, *22*, 4146.
(17) Ashley, K. R.; Shyu, S.-B.; Leipoldt, J. G. *Inorg. Chem.* **1980**, *19*, 1613.
(18) Ashley, K. R.; Leipoldt, J. G.; Joshi, V. K. *Inorg. Chem.* **1980**, *19*, 1608.

(19) A mixture of the chloroform solution of the Ti(IV)–TPyP complex and the ^{17}O-enriched water was refluxed at 60 °C for 1 week. The extent of the oxygen exchange between the oxo group of the complex and the bulk water was examined by monitoring the ^{17}O NMR spectra of the complex, but only less than 10% of the oxo group was proved to be displaced under the present experimental conditions (Inamo, M.; Funahashi, S.; Tanaka, M., unpublished results).



4

transfer from hydrogen peroxide to the oxo group in the transition state.

Acknowledgment. S.F. gratefully acknowledges financial support by a grant from the Kurata Foundation. Financial support from the Ministry of Education, Science, and Culture through a Grand-in-Aid for Scientific Research (No. 59430010) is gratefully acknowledged.

Registry No. $[Ti^{IV}O(tmpyp)]$, 96503-16-1; $[Ti^{IV}O(tmpyp)]I_4$, 96503-17-2; MES, 4432-31-9; MOPS, 1132-61-2; TAPS, 29915-38-6; H_2O_2, 7722-84-1; HOAc, 64-19-7.

Supplementary Material Available: Figure S1, data for the determination of pK_a values of MES, MOS, and TAPS, Figure S2, visible absorption spectra of **1** and **2**, Figure S3, dependence of $\{k_{0(H)} - (k + k_H[H^+])\}$ on the concentration of taps$^-$, and Figure S4, pressure dependence of rate constants k and k_H (4 pages). Ordering information is given on any current masthead page.

Contribution from the Departments of Chemistry, Emory University, Atlanta, Georgia 30322, and Georgia State University, Atlanta, Georgia 30303

Interactions of a Copper Antitumor Drug with DNA: [1-(α-Pyridylmethylene)-2-(α-pyridyl)hydrazine]copper(II) Chloride Does Not Intercalate

DEBRA L. BANVILLE,† W. DAVID WILSON,*‡ and LUIGI G. MARZILLI*†

Received January 9, 1985

The interaction of the copper metallodrug [1-(α-pyridylmethylene)-2-(α-pyridyl)hydrazine]copper(II) chloride with DNA (calf thymus, salmon sperm, Col E_1 plasmid) was selectively examined by UV–vis spectroscopy (binding constants, spectral changes, and flow dichroism), viscometry, and NMR spectroscopy (^{31}P NMR and Redfield ^1H NMR of the imino H). None of the physical measurements were consistent with an intercalation binding mode. The data were most consistent with an outside binding mode involving interaction of the drug with the phosphodiester group. The drug proved to be remarkably effective at broadening the ^{31}P NMR signal of DNA. In contrast, Cu^{2+} ion selectively broadened the GN(1)–H signal of DNA, consistent with proposals in the literature that Cu^{2+} binds to GC base pairs in DNA. Regardless of binding mode, the bound form of the drug is protonated at the noncoordinated hydrazine nitrogen. Although informative, this study did not provide a rationale for the very potent cytotoxicity of the drug toward certain tumor cell lines.

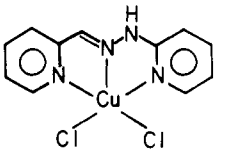
Introduction

The interactions of metal complexes with DNA are important both for their medicinal effects and as probes of DNA interactions and dynamics. A variety of drugs of such varied structure as *cis*-dichlorodiammineplatinum(II) (cisplatin),[1] bleomycin,[2] and metallocenes[3,4] of titanium and vanadium have shown wide spectrum antineoplastic action with DNA as the proposed biological target. In addition, other antitumor drugs such as the anthracyclines, which are thought to act at least partially at the DNA level in vivo, form metal complexes that have a pronounced effect on their DNA interactions.[5]

Metal complexes with planar aromatic bases have been used extensively as probes of DNA structure. Copper complexes of 1,10-phenanthroline, for example, cause DNA degradation in the presence of oxygen and a reducing agent, and the rate of degradation is dependent on the helical conformation (A, B, or Z).[6] Barton et al. have shown that Δ- and Λ-[tris(phenanthroline)-ruthenium(II)](2+) cations exhibit enantiomeric selectivity in intercalative binding to left- and right-handed helical forms of DNA.[7] Dervan and co-workers have synthetically coupled an EDTA derivative to the intercalator methidium cation.[8] This new agent, when complexed with iron, can also degrade DNA and has been extensively used in "footprinting" experiments to establish binding-site specificity for a variety of compounds that interact with DNA.

Porphyrin compounds that have no or easily removed axial ligands can also bind to DNA, presumably by intercalation, and are useful probes of DNA dynamics.[9-11] Lippard and co-workers have conducted an extensive study of metallointercalators containing Pt(II) and have carefully defined the structural requirements for intercalation to occur.[12]

It has recently been shown that the copper complex of the tridentate ligand 1-(α-pyridylmethylene)-2-(α-pyridyl)hydrazine (PCPH) inhibits DNA synthesis in neoplastic cells in tissue culture



CuPCPH

at concentrations similar to or less than those normally used for cisplatin and bleomycin.[13,14] The copper complex also causes dramatic inhibition of tumor growth in vivo in mice and, at the dosages used, produces no observable toxic side effects in the animals. CuPCPH, for example, gives 50% inhibition of DNA synthesis in cultured human melanoma cells at a concentration of only 0.05 ng/mL or several thousand times less than the

† Emory University.
‡ Georgia State University.

(1) Roberts, J. J. *Adv. Inorg. Biochem.* **1981**, *3*, 273.
(2) Dabrowiak, J. C. *Adv. Inorg. Biochem.* **1982**, *4*, 69.
(3) Kopf-Maier, P.; Krahl, D. *Chem. Biol. Interact.* **1983**, *44*, 317.
(4) Keller, H. J.; Keppler, B.; Schmahl, D. *J. Cancer Res. Clin. Oncol.* **1983**, *105*, 109.
(5) Dabrowiak, J. C. "Metal Ions in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1980; Vol. 11, p 305.
(6) Pope, L. E.; Sigman, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3.
(7) Barton, J. K. *J. Biomol. Struct. Dyn.* **1983**, *1*, 621.
(8) Schultz, P. G.; Dervan, P. B. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1133.
(9) Carvlin, M. J.; Fiel, R. J. *Nucleic Acids Res.* **1983**, *11*, 6121.
(10) Pasternack, R. F.; Antebi, A.; Ehrlich, B.; Sidney, D.; Gibbs, E. J.; Bassner, S. L.; Depoy, L. M. *J. Mol. Catal.* **1984**, *23*, 235.
(11) Banville, D. L.; Marzilli, L. G.; Wilson, W. D. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 148.
(12) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211.
(13) Pickart, L.; Goodwin, W. H.; Burgua, W.; Murphy, T. B.; Johnson, D. K. *Biochem. Pharmacol.* **1983**, *32*, 3868.
(14) Pickart, L. *Lymphokines (N.Y.)* **1983**, *8*, 425.

0020-1669/85/1324-2479$01.50/0