

A New Catalytic System for Activation of Molecular Oxygen within the Cavity of a Dinuclear Macrocyclic Complex. Redox Reaction of the (μ -Mesoxalato)(μ -peroxo)dnicobalt-BISDIEN Complex

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Potentiometric p[H] studies on the coordination of the mesoxalate anion to the dinuclear cobalt(II) complex of 1,4,10,13,16,22-hexaaza-7,19-dioxacyclotetrasane (BISDIEN) in aqueous solution are reported. Potentiometric p[H] measurements are also described for related equilibria involving the coordination of cobalt(II) with mesoxalate and the coordination of mesoxalate with the protonated (metal ion free) forms of BISDIEN and with the protonated and unprotonated mononuclear Co(II)-BISDIEN complexes. Equilibrium constants are reported for all complex species formed in solution in measurable concentrations. The aqueous BISDIEN-cobalt(II)-mesoxalate system has also been investigated potentiometrically under dioxygen as a function of pH; a unique triply bridged dinuclear cobalt(III)-BISDIEN complex has been detected in which mesoxalate, peroxo, and hydroxo groups are coordinated in a bridging fashion to both metal centers, and the equilibrium constant for its formation is reported. Distribution curves are presented, showing the concentrations of all dioxygen complex species, including the tribridged mesoxalato dinuclear complex, as a function of p[H]. At moderately elevated temperatures the dinuclear dicobalt-dioxygen-mesoxalate complex undergoes an intramolecular redox reaction in which mesoxalate is completely oxidized to CO₂ and dioxygen is reduced to water. An ¹⁸O tracer experiment shows that dioxygen is converted to reaction products with the isotopic label equilibrated between water and CO₂ (as bicarbonate). The redox reaction is catalytic, with the number of turnovers seemingly limited only by the amount of mesoxalate supplied to the reaction mixture.

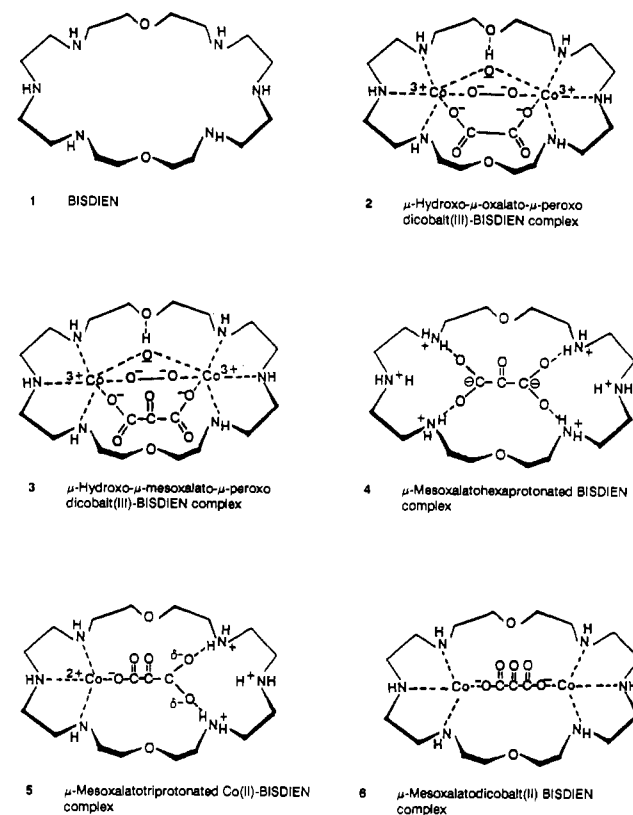
Introduction

Recently, an equilibrium study of the interaction of Co(II), BISDIEN (1) (see Chart I), oxalic acid, and dioxygen in dilute aqueous solution was reported.^{1,2} It was found that, in the pH range in which a significant concentration of the μ -oxalato- μ -peroxo complex 2 is present, a facile intramolecular redox reaction occurs when the solution is gently warmed to 45.0 °C. The μ -peroxo group (coordinated dioxygen) was found to take up two electrons to produce water, while the coordinated oxalate lost two electrons to form carbon dioxide. In this process the coordinated cobalt(II) ions undergo metal-centered oxidation to substitution-inert cobalt(III). The electron transfer from oxalate to dioxygen was interpreted as flowing through the metal centers.

More recently, the study of this reaction system involving the macrocyclic BISDIEN- μ -peroxo-dinuclear cobalt complex was extended to the use of catechol and TIRON as bifunctional bridging reducing substrates.³ Significantly, it was found that hydroquinone, which does not have the appropriate dimensions and geometry for bridging the metal centers, was not oxidized in the macrocyclic cavity, while 1,2-dihydroxybenzenes were converted to the corresponding quinones but at higher temperatures. The nature of the thermal stabilization of the bridging substrates was considered due to the stabilities of the geometric arrangements of the bifunctional bridging substrates and dioxygen within the cavity of the macrocyclic dinuclear complex.

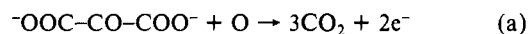
In order that the dinuclear complex containing bridging dioxygen and a bridging reducing substrate constitute a catalytic system, it is necessary to match the number of electrons available from the substrate with the number of electrons required by the dioxygen. Therefore, a four-electron-reducing and bridging substrate would be a candidate for this type of system, since the oxidation state of cobalt would remain unchanged after the redox reaction between the guest molecules within the macrocyclic cavity. Initially, a dioxygen molecule would pick up two electrons (one from each Co(II)), forming a μ -peroxo complex. The bifunctional donor substrate (i.e., the four-electron reductant) could then conceivably occupy a bridging position between the two partially oxidized metal centers in a manner analogous to that indicated in 2. Providing the required activation energy by warming the system would then result in the four-electron oxidation of the substrate and reconversion of the metal ions to Co(II), which would

Chart I



then be capable of coordinating additional dioxygen and substrate.

A bifunctional reducing coordinating ligand that appears to meet these requirements is ketomalonic acid (KMA, mesoxalic acid). Because mesoxalic acid is highly hydrated in aqueous solution,^{4,5} it is also known as "dihydroxymalonic acid". Four-electron oxidation of this ligand would produce 3 mol of carbon dioxide/mol of reductant, which conceivably would take place by the loss of two electrons with concomitant uptake of an oxygen atom (oxygen insertion) in accordance with the following reaction:



(1) Martell, A. E.; Motekaitis, R. J. *J. Chem. Soc., Chem. Commun.* **1988**, 915.

(2) Martell, A. E.; Motekaitis, R. J. *J. Am. Chem. Soc.* **1988**, *110*, 8059.

(3) Szponganicz, B.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1990**, *29*, 1467.

(4) Strelow, H. Z. *Elektrochem.* **1962**, *66*, 393.

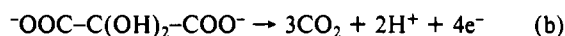
(5) Ahren, M. L. *Ber. Bunsen-Ges. Phys. Chem.* **1968**, *72*, 691.

Table I. Potentiometric p[H] Equilibrium Determinations and Equilibrium Constants Obtained

composn of soln	information obtained
mesoxalic acid (KMA)	protonation constants and "molecular weight"
KMA + Co(II), 1:1	metal chelate stability constant
BISDIEN-6HBr (BD)	protonation constants and "molecular weight"
BD + Co(II), 1:1/Ar	stability constant and protonation constants of 1:1 complex ^a
BD + Co(II), 1:2/Ar	stability constant and deprotonation constants of dinuclear complex ^a
BD + Co(II), 1:2/O ₂	oxygenation constant and hydroxy forms ^a
KMA + BD, 1:1	affinity constants of KMA for protonated forms of BD
Co(II) + BD + KMA, 1:1:1/Ar	1:1:1 mixed-ligand stability constants
Co(II) + BD + KMA, 2:1:1/Ar	2:1:1/KMA-bridged mixed-ligand stability constants
Co(II) + BD + KMA, 2:1:1/O ₂	oxygenation constant of dicobalt KMA-bridged BD complex

^a Previously reported for KNO₃ ionic medium ($\mu = 0.100$), $t = 25.0$ °C.

Because the reductant exists in aqueous solution mainly as the hydrate, one may also write



On the other hand, an alternate redox pathway would involve two-electron reduction:



There remains the additional question of whether the four-electron reduction of coordinated dioxygen would occur by oxygen atom transfer to mesoxalate or simply by deprotonation of its hydrate, with electrons flowing through the coordinated metal ions.

In this work, the thermodynamics of mesoxalic acid, Co(II), BISDIEN, and dioxygen interaction are reported and the kinetics of the redox reaction between the encapsulated dioxygen and substrate are determined.

Experimental Section

Materials. The hexaaza macrocyclic ligand BISDIEN hexahydrobromide dihydrate was synthesized and characterized as described previously.^{2,3} Mesoxalic acid was obtained from Aldrich Chemical Co., and its "molecular weight", which reflected the presence of a trace of water, was determined by potentiometric titration. Potassium chloride (supporting electrolyte), cobalt(II) nitrate, sulfuric acid, and other compounds employed were all of reagent grade quality and were used without further purification. Co(II) stock solutions were prepared by weight, and the exact concentration was determined by titration with standard EDTA solution, as described by Schwarzenbach.⁶ For potentiometric titrations, 0.100 M KOH was prepared from carbonate-free "Dilut-it" ampules and was standardized against primary standard grade potassium acid phthalate purchased from American Drug and Chemical Co. For quantitative preparations of other solutions, 1.000 M standard KOH was purchased from Fisher Scientific Co. and dispensed as needed from a microburet.

Potentiometric Equilibrium Measurements. The potentiometric procedures followed here are described in detail by Motekaitis and Martell.⁷ The potentiometric equilibrium determinations performed in order to compute all of the equilibrium constants defining the BISDIEN-mesoxalate-cobalt(II)-dioxygen system are shown in Table I.

The binary metal-free ligand system was characterized by measurement of slow equilibria in the pH range 6–8, for which about 45 min was required for establishment of each equilibrium point. Similar behavior was observed for the ternary systems with molar ratios of ligand to cobalt(II) of 1:1 or 1:2, except that up to 90 min was required for

Table II. Protonation Constants of Mesoxalic Acid and Related Di- and Monocarboxylic Acids^a

compd	formula	log K_1^{H}	log K_2^{H}
ketomalonic acid (mesoxalic acid)	$\begin{array}{c} \text{OH} \\ \\ \text{HOOC}-\text{C}-\text{COOH} \\ \\ \text{OH} \end{array}$	3.52	1.82
hydroxymalonic acid ⁸ (tartronic acid)	$\begin{array}{c} \text{OH} \\ \\ \text{HOOC}-\text{C}-\text{COOH} \\ \\ \text{H} \end{array}$	4.24	2.02
malonic acid ⁸	$\begin{array}{c} \text{H} \\ \\ \text{HOOC}-\text{C}-\text{COOH} \\ \\ \text{H} \end{array}$	5.28	2.65
propanoic acid ⁸	$\begin{array}{c} \text{H} \\ \\ \text{Me}-\text{C}-\text{COOH} \\ \\ \text{H} \end{array}$	4.69	
2-hydroxypropanoic acid ⁸	$\begin{array}{c} \text{OH} \\ \\ \text{Me}-\text{C}-\text{COOH} \\ \\ \text{H} \end{array}$	3.66	
2-ketopropanoic acid ⁸ (pyruvic acid)	$\begin{array}{c} \text{O} \\ \\ \text{Me}-\text{C}-\text{COOH} \end{array}$	2.26	

^a $t = 25.0$ °C; $\mu = 0.100$.

equilibrium. The ternary systems under dioxygen were modified to include larger increments of base added and longer intervals of time allowed for obtaining equilibrium. These time intervals were of 3–4 h duration.

Gas Chromatography. Carbon dioxide formation was quantitatively followed with a Perkin-Elmer Model 8410 gas chromatograph (TC/He) fitted with a $1/4$ in. \times 6 ft SS column packed with Carboxphere 80/100 mesh maintained at 80 °C. Carbon dioxide eluted at 2.4 min as an isolated sharp peak. In order to follow carbonate formation, typically a 1.00-mL aliquot of a test solution was placed into a 6.2-mL septum-capped vessel, to which 0.30 mL of 1.8 M sulfuric acid was then added. The carbon dioxide in the gas phase was equilibrated with the solution by vigorous agitation for 15 min prior to the injection of 1.00-mL head space gas into the chromatograph with the aid of a Dynatech Model PS A-2 gas syringe equipped with a gas valve. The small background carbon dioxide detected in the calibration was subtracted from each chromatogram reading.

¹⁸O₂ Tracer Studies. An ¹⁸O₂ tracer experiment was run on a concentrated solution of the μ -mesoxalato- μ -peroxo-binuclear Co(III)-BISDIEN complex. The initial reaction mixture contained 1.25 mmol of BISDIEN, 2.51 mmol of mesoxalic acid, and 2.50 mM Co(II) nitrate, dissolved in 20.0 mL of H₂O. Then 1.45 mL of 10.0 M KOH was added to bring the pH up to 9.00. The solution was made up in a small reaction cell under argon, which was flushed with argon for 30 min, and the argon supply was then turned off. The solution was then exposed to the ¹⁸O₂ supply (100 mL of 99% purity gas from Cambridge Isotopes), which was thoroughly mixed with the small volume of argon in the reaction cell by repeated transfer to and from a secondary gas container. The redox reaction was run by heating the reaction mixture to 45.0 °C and holding it at that temperature for 15 h, with continued transfer of ¹⁸O₂ over the surface of the rapidly stirred reaction mixture. The reaction mixture obtained after completion of the redox reaction between mesoxalate and coordinated dioxygen was sampled for ¹⁸O by acidification with phosphoric acid followed by stripping of CO₂ in vacuo at room temperature. The ¹⁸O content of the carbon dioxide evolved was determined with a Finnigan MAT 251 isotope ratio mass spectrometer in the laboratory of Professor Ethan Grossman of the Department of Geology, Texas A&M University.

Computations. All computations involving equilibria were performed as indicated in Table I by the use of Program BEST.^{7–9} For pure ligands the molecular weights were adjusted slightly to reflect the presence of trace amounts of inert impurities such as water, thus giving rise to much

(6) Schwarzenbach, G.; Flaschka, M. *Complexometric Titrations*; Methuen: London, 1957.

(7) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*; VCH Publishers: New York, 1988.

(8) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 2403.

(9) Program and procedural details available by E-Mail at Bitnet address MARTELL@TAMCHEM.

Table III. Overall Protonation Constants (β Values) of BISDIEN (L) in the Absence and Presence of Mesoxalic Acid (H_2A) and Equilibrium Constants for the Binding of KMA to Protonated Forms of BISDIEN^a (K Values)

n	$\log \beta_{H_n L}^H$ ^b	$\log \beta_{H_n L}^H$ ^c	$\log K_{H_n L}^A$ ^d
6	46.30	41.15	5.15
5	42.72	38.20	4.52
4	38.09	34.44	3.65
3	30.98	26.82	4.16
2	22.37	18.54	3.83
1	13.01	9.62	3.39

^a $t = 25.0$ °C; $\mu = 0.100$ M (KCl). ^b $[H_n L] / [H^+]^n [L] [A]$.
^c $[H_n L] / [H^+]^n [L]$. ^d $[H_n L] / [H_n L] [A]$.

closer fits near sharp inflections. This is particularly true of mesoxalic acid, which is hygroscopic.

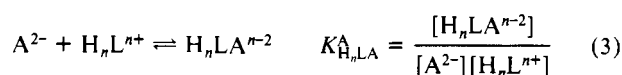
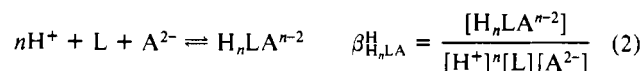
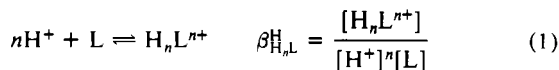
Results

Mesoxalic Acid (KMA). The log protonation constants of mesoxalic acid were computed from its potentiometric titration curve at 25.0 °C ($\mu = 0.100$ M, adjusted with KCl) and were found to be 3.52 and 1.82. The overall standard deviation is 0.003 log units. Table II contains a comparison of this result with the protonation constants of related di- and monobasic acids,¹⁰ which help to demonstrate the molecular nature of KMA in aqueous solution.

The values themselves are such that at $p[H]$ 2 nearly 40% of KMA is in the form of the monoanion, while above $p[H]$ 5 virtually all of the KMA is in the form of the dianion. The importance of this finding is that (vide supra), over the entire $p[H]$ range employed in the study, KMA is completely dissociated.

Although it is seen to be a weak base, the KMA dianion is expected to coordinate to cobalt(II) and its encapsulated forms (i.e., with its macrocyclic complexes). Potentiometric measurements showed that 1:1 complexes of Co(II) with KMA do indeed form, albeit weakly, with no apparent tendency to form a 2:1 ligand to metal complex. The log stability constant of the 1:1 complex was found to be 2.42, and because the formation constant is low, a 1.0 mM solution forms only about 35% of the neutral complex species, CoKMA.

BISDIEN (BD)-KMA Interactions. The protonation and complex formation reactions of BISDIEN and the oxygenation constants of its dicobalt(II) complex have been reported.^{1,2,11} However, in order to first determine the details of the binding of the KMA anion with the protonated macrocycle, the protonation constants of the hexahydrobromide of BD were remeasured under the experimental conditions employed in this investigation and were found to be (log values) 9.61, 8.92, 8.28, 7.62, 3.75, and 2.95. These values are similar to (but not identical with) those obtained originally for a 0.100 M KNO_3 medium. The presence of mesoxalate in a solution of BISDIEN was found to increase considerably the $p[H]$ values of the potentiometric equilibrium curve (i.e., hydrogen ions are more strongly bound to BISDIEN in the presence of KMA). The differences provide an indication of extensive interactions between mesoxalate and all the protonated forms of BISDIEN. In the absence of KMA a set of six overall protonation constants defined by eq 1 were computed, while from



H_2A = mesoxalic acid; L = BISDIEN; H^+ = hydrogen ion

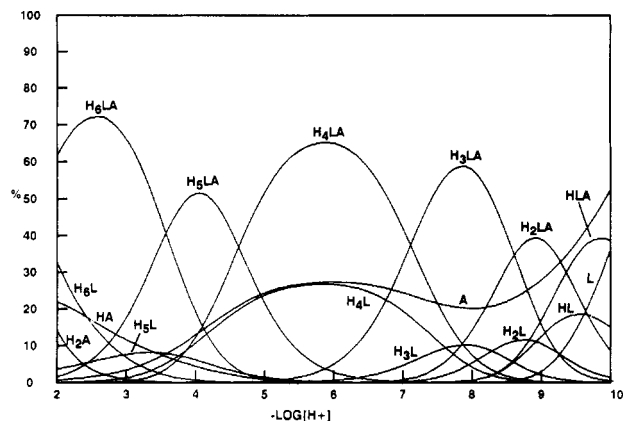


Figure 1. Distribution of species present as a function of $p[H]$ in a solution containing a 1:1 molar ratio of BISDIEN (L) and mesoxalic acid (H_2A) at 25.0 °C and $\mu = 0.100$ (KCl). $T_A = T_L = 2.00 \times 10^{-3}$ M.

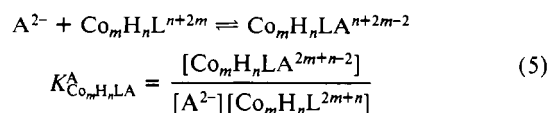
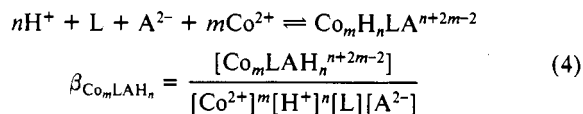
Table IV. Overall Stability Constants (β values) for the Binding of KMA to the Co(II) Complexes of BISDIEN and Specific Interaction Constants (K values)^a

species	$\log \beta$	$\log K$
(KMA)(BD)(Co)	15.01	5.28
(KMA)(BD)(Co)(H)	23.76	6.45
(KMA)(BD)(Co)(H) ₂	29.79	6.51
(KMA)(BD)(Co)(H) ₃	35.08	
(KMA)(BD)(Co)(H) ₋₁	4.74	
(KMA)(BD)(Co) ₂	20.03	7.60
(KMA)(BD)(Co) ₂ (H) ₋₁	8.9 ^b	

^a $t = 25.0$ °C; $\mu = 0.100$ M (KCl). ^b Minor species.

the KMA-containing system a set of overall formation constants defined by eq 2 were computed. The ratios of the corresponding degrees of protonation were employed to calculate the specific interaction constants defined by eq 3 for hydrogen-bonded complexes between the mesoxalate dianion and each protonated form of BISDIEN. The results are shown in Table III. The corresponding species distribution curves in Figure 1 show that the KMA complexes predominate in concentration over the corresponding protonated forms of BISDIEN and that the protonation constants (crossover points between species) are higher than those of the BISDIEN species in the absence of KMA.

BISDIEN-KMA-Cobalt(II) Interactions. It was found that the dianion of KMA has strong affinity for all of the Co(II) complexes with BISDIEN. The complexes of Co(II) formed are $Co(BD)(KMA)$, $Co(BD)H(KMA)$, $Co(BD)H_2(KMA)$, $Co(BD)H_3(KMA)$, $Co(BD)OH(KMA)$, $Co_2(BD)(KMA)$, and $Co_2(BD)(OH)(KMA)$. Table IV lists the log overall formation constants for these species, defined by eq 4, as well as specific formation constants for combination of the preformed chelates of Co(II) expressed as indicated by eq 5.



The distributions of metal complex species formed as a function of $p[H]$ are given in Figure 2. The formation of three of the species in Table IV with mesoxalate bridging groups cannot be expressed in terms of reaction of mesoxalate with the preformed BISDIEN complex, because such complexes do not form in the absence of mesoxalic acid. The triprotonated species CoH_3L^{5+} does not form, and therefore its stability constant cannot be determined. The hydroxo species $CoL(OH)^+$ cannot form, since the amino nitrogens of BISDIEN coordinate all of the coordination sites of the metal ion at high pH. The olated $Co_2(OH)L^{3+}$ species

(10) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York, 1977, 1982, 1989; Vols. 3, 5, 6.

(11) Motekaitis, R. J.; Martell, A. E.; Lecomte, J. P.; Lehn, J. M. *Inorg. Chem.* 1983, 22, 609.

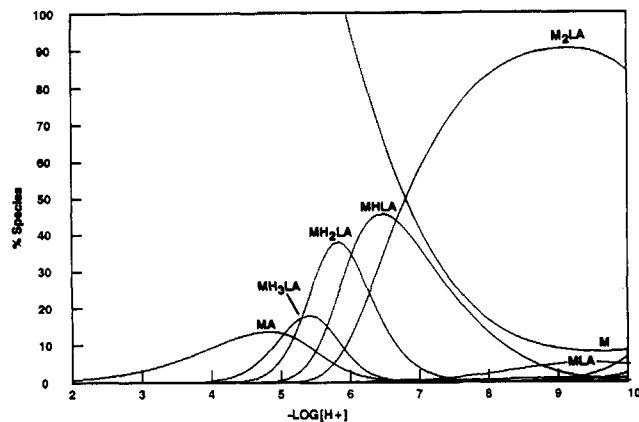


Figure 2. Distribution of cobalt-containing species present as a function of $p[H]$ in a solution containing a 1:1:2 molar ratio of BISDIEN (L), mesoxalic acid (H_2A), and cobalt(II), respectively, at 25.0 °C and $\mu = 0.100$ (KCl). $T_L = T_A = \frac{1}{2}T_{Co(II)} = 2.00 \times 10^{-3}$ M. The region above pH 9 is supersaturated with respect to $Co(OH)_2$.

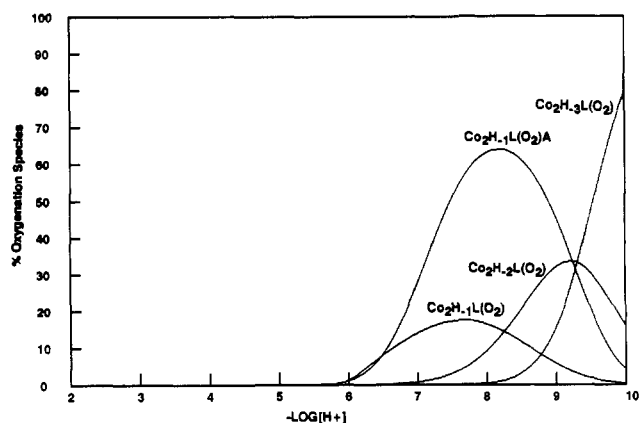


Figure 3. Distribution of dioxygen complex species present as a function of $p[H]$ in a solution containing a 1:1:2 molar ratio of BISDIEN (L), mesoxalic acid (H_2L), and cobalt(II), respectively, under 1.00 atm of dioxygen at 25.0 °C and $\mu = 0.100$ (KCl). $T_L = T_A = \frac{1}{2}T_{Co(II)} = 2.0 \times 10^{-3}$ M. Non-dioxygen-containing species are omitted for clarity. Negative subscripts indicate the number of hydroxo donor groups present, i.e., the number of protons abstracted from coordinated water molecules.

probably would form, but due to solubility product limitations of $Co(OH)_2$, coupled with the weakness of the reaction with the second cobalt(II) ion, cobalt(II) hydroxide precipitates, precluding the measurement of such a species.

Dioxygen Equilibria. Potentiometric equilibrium measurements were made in the presence of pure dioxygen in order to obtain data for the computation of the equilibrium constant for formation of the dioxygen complex of Co_2KMAL^{2+} . The reactions were found to be sluggish, requiring about 3–8 h per experimental point to reach equilibrium. The standard deviation was estimated at ~ 0.07 log units. During such long equilibration times it is possible that very slow oxidation reactions were proceeding to a minor extent but not enough to appreciably affect the measurements. A small correction was applied to compensate for the hydrogen ions released in the oxidation, and the resulting measurements are considered a slight approximation but reasonably close to the equilibrium values. The log equilibrium constant obtained is -7.01 for the following quotient:

$$K_{O_2} = \frac{[Co_2L(OH)AO_2^+][H^+]}{[Co_2LA^{2+}]P_{O_2}} \quad (6)$$

The units of K_{O_2} are $M \cdot atm^{-1}$. The oxygenation constant thus obtained is larger than that of the corresponding oxalate case, $\log K_{O_2} = -7.92$.² The pH range of significant concentration (i.e. $>20\%$) of the dioxygen complex is $\sim 7-9.5$, reaching maximum concentration of about 63% at pH 6.4. Figure 3 illustrates the formation and decline of the triply bridged dioxygen complex as

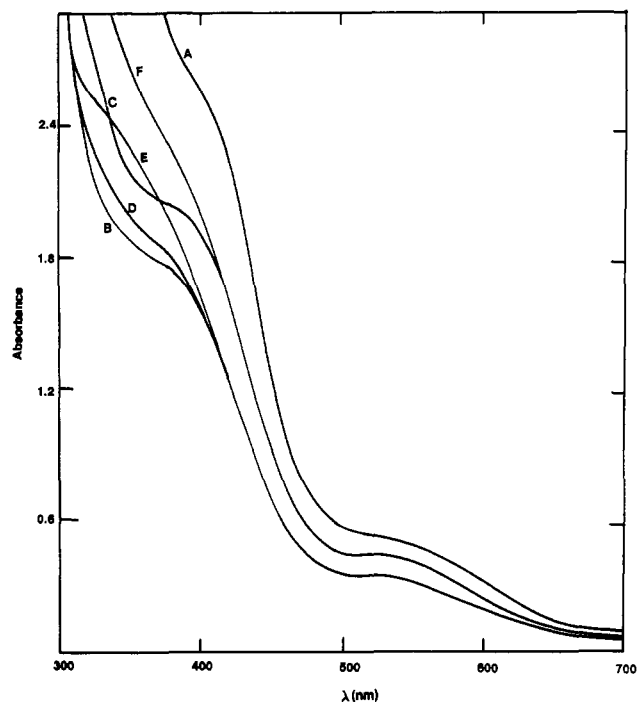
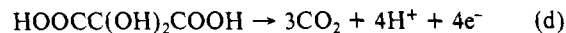


Figure 4. Absorption spectra of a solution containing 1.00×10^{-4} BISDIEN, 1.00×10^{-4} M mesoxalic acid, and 2.00×10^{-4} Co(II) as a function of cycling of 1.00 atm of dioxygen and 1.00 tm of argon; ($t = 45.0$ °C, $\mu = 0.100$ M (KCl)): (A) dioxygen saturated; (B) after 260 min under argon; (C) after 12 min under dioxygen; (D) after 50 min under argon; (E) after 750 min under argon; (F) after 15 min under dioxygen.

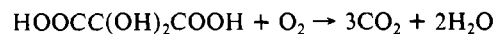
a function of pH , as well as the pH ranges and concentrations of the three hydroxodicobalt-BISDIEN-dioxygen complexes formed. The equilibrium constants governing the formation of these non-KMA dioxygen complex species have been reported earlier.²

Redox Reaction at 45.0 °C. Preliminary identification of the gaseous product of the redox reaction between coordinated dioxygen and coordinated KMA was made by running the reaction of a 1:1 molar ratio of KMA to Co_2L^{4+} under dioxygen in a sealed vessel for 24 h at 45.0 °C at an initial pH value of ~ 9 . After acidification through a septum with excess sulfuric acid, the gaseous phase was analyzed with an Alltech gas chromatograph set up to detect all atmospheric gases including hydrocarbons, carbon monoxide, and carbon dioxide. The results showed that carbon monoxide was not present and about 70% of the expected carbon dioxide was detected, thus providing the first evidence that the reaction yields only carbon dioxide. The purpose of the experiment was to identify the products, but there was no opportunity to adjust $p[H]$ during the course of the reaction because the reaction vessel was sealed. As the reaction proceeds with the evolution of carbon dioxide, the pH drops and eventually the reaction ceases by shifting of the species distribution to that of the region, $\sim pH$ 6, where the reactive intermediate, the dioxygen adduct of the KMA-bridged dinuclear Co(II)-BISDIEN complex, is not present (Figure 3).

Feliu et al.¹² have shown that mesoxalic acid quantitatively undergoes four-electron oxidation to carbon dioxide and hydrogen ions according to the reaction



Using dioxygen as the electron sink, we have



The carbon dioxide formed (in equilibrium with bicarbonate ion) is responsible for the observed lowering of the pH of the reaction mixture.

(12) Feliu, J. M.; Claret, J.; Muller, C.; Vazquez, J. L.; Aldaz, A. J. *Electroanal. Chem. Interfacial Electrochem.* **1984**, *178* (2), 271.

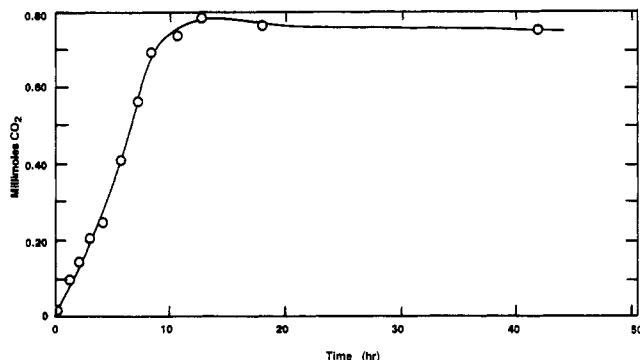


Figure 5. Measured (as CO_2) amounts of carbonate formed in the reaction between coordinated O_2 and KMA in the dinuclear BISDIEN-Co(II) complex at $45.0\text{ }^\circ\text{C}$ and $\mu = 0.100\text{ M}$ (KCl). Appropriate amounts of 1.00 M KOH were supplied as necessary to maintain the $\text{p}[\text{H}]$ constant at 8.5.

Figure 4 presents the absorption spectra of a solution containing BISDIEN, KMA, and Co(II) in 1:1:2 molar ratio, initially prepared under dioxygen and measured as a function of time as the system was cycled between an argon atmosphere and a dioxygen atmosphere. The initial curve A is characterized by a shoulder at $\sim 400\text{ nm}$. Flushing the solution with argon for 260 min dropped the absorbance considerably, resulting in curve B, with a shoulder at 380 nm . After 12 min of dioxygen treatment the spectrum stabilized in the intermediate position shown by curve C. Argon treatment for 50 min reduced the absorbance as shown by curve D, whereupon continued heating under argon for 750 min more gave a new higher curve E. Curve E has a shoulder at $\sim 340\text{ nm}$ and thus is different from the other absorbance spectra. However, treatment with dioxygen once again enhanced the spectrum to that shown by curve F. This series of measurements illustrates two points: (1) the reactions taking place produce Co(II) intermediates, and (2) the redox reaction takes place even in the absence of dioxygen. This means that it is the dioxygen complex of dicobalt-KMA-BISDIEN that is undergoing the redox reaction and does not involve the additional free gaseous or dissolved dioxygen.

In view of the above observations, it was decided to measure the kinetics of the reaction on the basis of quantitative measurement of the carbon dioxide released as the reaction progresses at $45.0\text{ }^\circ\text{C}$. Since it had already been shown that the reaction is catalytic (i.e. more than 1 mol of KMA decomposes/mol of dicobalt(II)-BISDIEN present), the redox reaction of a 10:1 molar ratio of KMA to Co_2L was followed at $\text{pH } 8.5$. Throughout the reaction the color of the solution remained reddish, and only when no further carbon dioxide formation occurred did the solution turn brown, indicating that KMA was no longer present and that there was final conversion of the binuclear complex to the non-KMA-bridged dicobalt(III)-BISDIEN dioxygen complex as the predominant species remaining in solution.

Figure 5 shows the progress of carbon dioxide formation in a 10:1 substrate to catalyst system. The formation of carbon dioxide increases steadily and then levels off near 10 h, remaining relatively constant for an additional 24 h. The measured GC peak areas are proportional to the carbon dioxide contained in the reaction vessel, and on this basis the total millimoles of carbon dioxide formed were calculated. The amount of CO_2 observed (in 12 h) to be formed (in 12 h) was 0.79 mmol . This is 7.6 times the number of millimoles of catalyst ($0.79/0.104$), indicating that before the system became inactive, 2.5 times the theoretical stoichiometric yield of carbon dioxide (3 mol of carbon dioxide/mol of KMA; reactions a-c) were produced per mole of catalyst. This result represents much more than 2.5 turnovers based on the intermediate triply bridged dioxygen complex because it has been shown (Figure 3) that the maximum amount of the intermediate formed in a single pass is 63% of the theoretical stoichiometric amount and probably becomes much less than that as the catalytic reaction proceeds. The ultimate fate of the catalytic system is indicated by the absorption spectrum in Figure 6, which is that

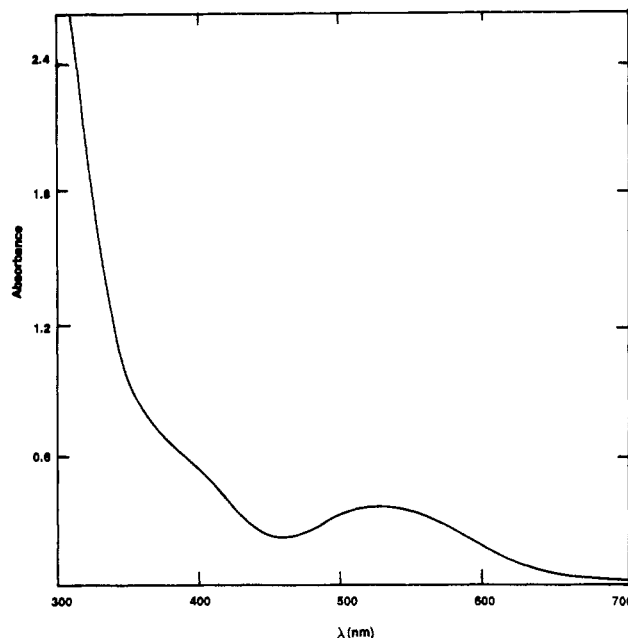


Figure 6. Absorption spectrum of the final redox reaction product after 96 h at $45.0\text{ }^\circ\text{C}$. Initial conditions: $1.00 \times 10^{-4}\text{ M}$ BISDIEN; $1.00 \times 10^{-4}\text{ M}$ mesoxalic acid; $2.00 \times 10^{-4}\text{ M}$ cobalt(II); $\text{p}[\text{H}] = \sim 8.5$; $\mu = 0.100\text{ M}$ (KCl).

of the hydroxo-bridged dicobalt(III)-BISDIEN complex.

$^{18}\text{O}_2$ Tracer Studies. It is of interest to determine the source of the oxygen needed for complete conversion of mesoxalate to carbon dioxide, as indicated by the chemical eq a suggested above. If this reaction applies, the oxygen atom may derive from coordinated dioxygen by direct insertion within the macrocyclic complex in which both dioxygen and mesoxalate are coordinated bridging groups between the same two metal ions, 3. If such an insertion reaction does not take place, but eq b applies, the additional oxygen atom may come from water, through deprotonation of the hydrated form of mesoxalic acid. Unfortunately, it is not possible to trace the pathway of oxygen from dioxygen to carbon dioxide by a conventional $^{18}\text{O}_2$ tracer experiment because the oxygen of carbon dioxide rapidly equilibrates with water under the reaction conditions employed, so that CO_2 does not retain its label long enough for separation and analysis. Therefore, the use of $^{18}\text{O}_2$ would produce only a slight increase in the $^{18}\text{O}_2$ content of both water and CO_2 , regardless of whether the redox reaction proceeds by eq a or b, since in both cases the major quantity of the label would end up in the solvent. The $^{18}\text{O}_2$ supplied to the system would thus result in a slight increase over the natural abundance (0.2%) of ^{18}O in both bicarbonate and water and to the same extent. With the procedure described in the Experimental Section, 21.5 mL of 0.06 M dicobalt-BISDIEN catalyst and 0.12 M mesoxalate treated with an excess (100 mL) of $^{18}\text{O}_2$ at 1 atm pressure gave a 25% increase over the 0.20% natural abundance of ^{18}O in natural O_2 . At infinite time ($\sim 96\text{ h}$), the solutions revert to a cobalt(III) type of spectrum previously described in the work on oxalate. This is indeed what happens with Co(II)-BISDIEN-dioxygen complexes, which undergo very slow irreversible metal-centered degradation at $45.0\text{ }^\circ\text{C}$, forming inert Co(III) complexes, characterized by a broad peak above 500 nm and a shoulder below 400 nm.

Discussion

KMA. The monobasic acids included in Table II are intended to point out structure-activity relationships between constitution and basicity. One trend relates to the number of hydroxy substituents, while a second indicates the effects of keto and hydrated keto (dihydroxy) groups. It is seen that the 2-ketopropionic acid, which is in the predominantly keto form, is much more acidic than the weaker (more hydrated) mesoxalate ion by 1.3 log units, while both the corresponding monohydroxy and the parent unsubstituted acids differ only by about 0.6 log units. This latter difference is

the normal statistical difference in the pK's of mono- and dicarboxylic acids. For malonic acid and its derivatives, increasing the number of hydroxyls from 0 to 2 gives decreases in basicity by 1.0 and 0.7 orders of magnitude, in line with expectations. Substitution of one hydroxy group in the 2-position of propanoic acid weakens the basicity by 1.0 log *K* unit. Further substitution (with a keto group) decreases the pK by 1.4 log units, indicating the greater oxo polarization effect. These observations indicate that ketomalonic acid has the properties expected for dihydroxy malonic acid (the hydrated form) in aqueous solution.

The specific association constants given in the last column of Table III for the combination of mesoxalate with the protonated forms of BISDIEN are the largest in magnitude for the hexaprotonated macrocycle and the smallest for the monoprotated form. A suggested hydrogen-bonding mode for the binding of KMA to one of the protonated BISDIEN species, H₆L⁶⁺, is illustrated by formula 4. There are two special factors to be considered. One is that the values of *K*_{n^{H,L}A} do not monotonically decrease. The fact that the mesoxalate bridging complex of the tetraprotonated form seems to form much less readily than do the complexes of KMA with the tri- and pentaprotonated BIS-TREN species indicates that the symmetrical species H₄L⁴⁺ with maximally separated positive charges is relatively unsuited to bridging of mesoxalate relative to the less protonated form, H₃L³⁺, or the more protonated form, H₅L⁵⁺ (this in spite of the fact that MH₄LA²⁺ is formed to a greater extent than both MH₃LA³⁺ and MH₂LA⁴⁺). It is surprising that even the monoprotated HL⁺ species interacts measurably and significantly with mesoxalate A²⁻. This can be rationalized in terms of complex formation through both hydrogen bonding and charge neutralization as factors in molecular association of mesoxalate with the macrocycle. The degree of mesoxalate binding can be visualized throughout the pH range studied by the species distributions in Figure 1. The most important observation is that, at all pH values below 10, BISDIEN combines with mesoxalate in increasing proportions as the p[H] is increased, with the concentration of each binary complex at least twice that of free BISDIEN.

Binding of KMA to Co(II)-BISDIEN Complexes. At this point it is interesting to compare mesoxalate binding constants of BISDIEN to those of oxalate. Both anions have the same charge, but the corresponding constants *K*_{n^{H,L}A} for *n* = 6, 5, and 4 (5.15, 4.52, and 3.65, respectively) are larger than those of oxalate (4.68, 3.59, and 2.06). While the tri-, di-, and monoprotated forms of the BISDIEN-Co(II) complex combine with mesoxalate, they do not associate with oxalate. Since both anions have the same negative charge, the relative strengths of interaction can only be understood in terms of the differences in distances between the coordinating carboxylate oxygens and the fact that mesoxalate is much more flexible than the completely rigid oxalate dianion.

There are several interesting trends in the values of log *K* reported in the second column of Table IV, defined by eq 5. To begin with, the value 5.28 for the binding of KMA to the 1:1 Co(II)-BISDIEN complex is considerably higher than 2.42, which is log *K* for the binding of KMA to the Co(II) aquo ion. Also the log binding constants 6.45 and 6.51 for combination of KMA with the mono- and diprotonated Co(II)-BISDIEN complexes are very close in magnitude. Third, the binding constant of KMA to the binuclear Co(II)-BISDIEN complex is much higher than any of the other KMA binding constants listed.

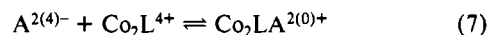
With respect to the first observation, it seems that the cobalt(II) ion encapsulated by the deprotonated BISDIEN ligand becomes a better acceptor for the mesoxalate anion through secondary stabilization interactions with the ligand, an example of "supramolecular" chemical bonding. This effect could involve hydrogen bonding of the hydroxyl groups of the hydrated mesoxalate to the macrocyclic nitrogens. The loss of an additional proton from this complex to give CoLAOH⁻ could also involve the loss of a hydrogen ion from hydrated mesoxalate, [CoH₁A]⁻, thus increasing its binding to the metal center. Neither of these effects is possible in the analogous oxalate-bridging,² giving rise to the interpretation that hydrogen bonding, bridging ligand charge, and specific effects involving the geometry of the KMA

anion contribute to the previously unexpected strong binding of KMA to CoL²⁺.

Increasing KMA binding, as protonation of the 1:1 Co(II)-BISDIEN complex, 5, is increased, was expected because of the increased hydrogen bonding between mesoxalate and one of the protonated BISDIEN amino groups. However, the increase in binding of the diprotonated over the monoprotated form is marginal. This indicates that, in the binding of KMA to CoLH₂⁴⁺, there must be an added effect, such as a change in conformation, which interferes with coordination of the secondary ligand, which in this case must occupy a bridging position between the Co(II) at one side of the macrocycle and the protonated amino nitrogens at the other (formula 5). While the oxalate affinity for the monoprotated Co(II)-BISDIEN complex is ~2 orders of magnitude lower than the mesoxalate binding, these two anions have similar affinities for diprotonated Co-BISDIEN with low *K*'s of 6.50 (oxalate) and 6.51 (mesoxalate).

The binding constant of mesoxalate to the binuclear Co(II)-BISDIEN complex, Co₂L⁴⁺ (6), is greater than for any of the mononuclear protonated forms but is very much lower than the previously reported oxalate binding log value of 9.06. Therefore, the binuclear Co(II)-BISDIEN complex is much better suited to oxalate bridging than to bridging of the larger mesoxalate anion.

At this point it is of interest to compare oxalate and mesoxalate binding constants to those recently reported for catechol binding to the binuclear Co(II)-BISDIEN complex. The log stability constants for the bridging reaction (eq 7) where A²⁽⁴⁾⁻ is cate-



cholate(2-), tironate(4-) (3,5-disulfocatechol), oxalate(2-), and mesoxalate(2-) are 12.3, 13.0, 9.06, and 7.60, respectively. The tironate binding is slightly stronger than that of catechol because of its higher negative charge, while oxalate is more strongly bound than mesoxalate probably due to a better fit in bridging the cobalt(II) centers.

As bridging data accumulate for mononuclear (protonated) and binuclear BISDIEN complexes, it becomes apparent that these BISDIEN complexes are able to serve as excellent hosts for a variety of secondary bridging donor groups, making possible the development of many new catalytic systems. In the present case, summation of the species present in solution (Figure 2) shows that virtually all of the KMA is complexed (as a bridging anion) above pH 7.

Dioxygen Complexes. Qualitatively, the KMA equilibria with BISDIEN under dioxygen resemble the oxalate system studied earlier. There were 35 equilibrium species in the computation, and without an efficient computer program such as BEST this calculation would not have been possible. As in the oxalate case, the mesoxalate dioxygen complex was converted to the Co₂(OH)₃LO₂⁺ complex above pH 9, as indicated in Figure 3, which shows the species distribution of the dioxygen complex formed with the bridging KMA as a function of p[H], as well as those of other dioxygen complexes present that do not contain bridging KMA.

Another interesting observation is that the complexes in the present study possess quite different UV-visible spectra compared to those measured in the previous oxalate study.² In this study, the ~525-nm band is prominent whereas the oxalate-bridged dioxygen complex showed a continuum in this region. Distinctly different also is the above-mentioned shoulder at 380 or 400 nm, which is actually one of two peaks, the other being near 320 nm in the oxalate case. There is no reason to suspect that the oxalate and mesoxalate anions are bound in any conformation other than binuclear, but it is quite likely that significant structural and bonding differences exist in KMA- and oxalate-bridged dioxygen complexes. Further information on this important question will be sought by attempting to obtain crystals of these binuclear complexes suitable for X-ray structure determinations.

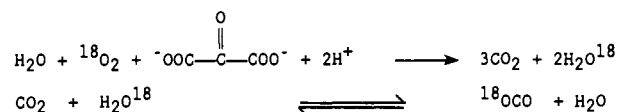
Oxidation of Coordinated Mesoxalate by Coordinated Dioxygen. It is noted that termination of the oxidation of mesoxalate in the catalytic system is signaled by a change in absorbance to the typical color of the dicobalt(II)-BISDIEN-dioxygen complex.

Thus, it seems that when excess substrate is present, the mesoxalato-bridged complex predominates, as indicated by its characteristic absorption spectrum. The fact that not all of the excess substrate in the solution is converted to carbon dioxide suggests that some of the mesoxalate may also decompose through another, nonredox process, before it has time to react with the regenerated dinuclear cobalt-dioxygen complex. We are now investigating the possibility that the substrate is undergoing simultaneous metal ion catalyzed decarboxylation to glyoxylic acid, a reaction type that has been observed in other systems with analogous substrates.

The rapid equilibration of the oxygen of dissolved CO₂ (as CO₂ + H₂O ⇌ H₂CO₃ ⇌ HCO₃⁻ ⇌ CO₃²⁻) with the solvent water molecules prevents the use of tracers from distinguishing between the two reaction sequences for oxygen atom transfer in the redox process. The ¹⁸O label may go directly to water, and then to CO₂, or simultaneously to both water and CO₂, as



or



While the tracer experiment does not distinguish between these two pathways, it does demonstrate that the oxidation products, CO₂ and H₂O, are derived from molecular oxygen through dioxygen complex formation and formation of the mixed-bridged (tribridged) intermediate, 3.

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Stability and Characterization of Iron(III) and Iron(II) Heme Peptides Encapsulated in Aqueous Detergent Micelles: ¹H NMR and UV-Vis Spectroscopic Studies

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Proton NMR and optical spectral studies on a heme undecapeptide in aqueous SDS micelles at different pHs are reported. In a micellar solution the monodispersed heme peptide is found to be encapsulated inside the hydrophobic micellar cavity. pH dependence of the ferric heme peptide inside the SDS micelle shows equilibrium conversion from the diaqua ferric heme at low pH (~2) to the mono-aqua monohistidine complex at pH ~6, which on further increase in pH undergoes deprotonation to give the monohistidine monohydroxo heme peptide complex. pH titration by optical spectroscopy shows a pK of 7.2, corresponding to the aqua (high-spin) ⇌ hydroxo (low-spin) transition. The cyano complex of the heme peptide in micellar solution shows a large spread of the heme methyl signals compared to the signals in the cyano protohemin complex, and its spectrum is similar to those of cyano heme proteins. Ferrous heme peptide complexes have also been stabilized inside the SDS micelles. Optical as well as NMR spectra of the ferrous complex suggest a five-coordination geometry. The line widths of the heme methyl resonances for these heme peptide complexes are much broader compared to those of the corresponding protoheme complexes but are similar to those found in the spectra of heme proteins.

1. Introduction

Heme peptides are obtained by enzymatic degradation of cytochrome *c*.^{1,2} Pepsin degradation of cytochrome *c* gives a heme undecapeptide, in which the heme is bound to an eleven amino acid peptide chain, while trypsin digestion of cytochrome *c* gives a heme octapeptide, in which the heme is bound to an eight amino acid peptide chain of known sequence (Figure 1). Both the heme peptides contain an iron(III) porphyrin complex covalently linked to the peptide through two thioether linkages involving cysteine residues of the peptide and the α-carbons of the saturated vinyl groups on two pyrrole positions of the porphyrin ring (Figure 1). The N-terminal valine, lysine, and glutamic acid residues on the heme undecapeptide are absent in the heme octapeptide. The effects of the protein environment on the electronic structure and properties of the heme prosthetic group in heme proteins have been investigated by different techniques in substituted porphyrin model compounds.³⁻⁵ Heme peptide complexes in aqueous solutions have therefore been studied by ¹H NMR,^{6,7} circular dichroism,⁸⁻¹⁰ and UV-visible^{1,2,11} spectroscopy as models for cytochromes and other heme proteins. Further interest in the study of these heme peptides arises from the knowledge that they show peroxidatic activity^{1,12-15} similar to that of heme peroxidases.

Previous studies^{6,7} in aqueous solutions show that the spectroscopic properties of the heme peptides have general similarity to those of the cytochrome *c* complexes, but some subtle differences do exist. In the case of low-spin cyano as well as pyridine complexes of heme octapeptide, the NMR resonances of the heme methyl protons are quite sharp and their spread is smaller compared to the spectra of the corresponding cytochrome *c* complexes.⁷ Moreover, NMR spectra of heme peptides in aqueous solutions show significant pH dependence,⁶ associated with a spin-state transition similar to that observed in metmyoglobins. Tempera-

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