transfer reactions of the type described in this and other papers.[16,17,19,20,30b,31] By using this terminology we have sought firstly to avoid the use of particular ligand structures and metal valence formalisms which may be ambiguous, as in $[NiS_4C_4R_4]^{0,-}$, or meaningless, as in $[VS_6C_6R_6]^0$, and secondly to eliminate the use of confusing abbreviations such as MNT or BDT. Complexes which cannot engage in electron-transfer reactions of the type discussed above are referred to by the normal "dithiolate" nomenclature and by the use of formal oxidation numbers; *e.g.*, $[ZnS_4C_4(CN)_4]^{-2}$ is described as the bis(*cis*-1,2-dicyanoethylene-1,2-dithiolate)zinc(II) anion.

It must be emphasized that the use of this terminology in no way represents a criticism, implied or real, of the existing discussion of the ground states of these unusual sulfur complexes.

Acknowledgments. We are grateful to Drs. J. B. Headridge, S. F. A. Kettle, and D. Pletcher of this department for useful advice and discussions, and to G. A. Wright and Miss J. Minnis for valuable experimental assistance.

The Kinetics of the Rapid Interaction of Bis(histidinato)cobalt(II) with Oxygen

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Abstract: Bis(L-histidinato)cobalt(II), $Co(L\text{-}h)_2$, reacts rapidly with molecular O_2 in aqueous solution to give a deep brown solution containing $[Co(L\text{-}h)_2]_2O_2$. The kinetics of formation and decomposition of the brown species have been studied spectrally by flow methods. Kinetic data support the mechanism: $Co(L\text{-}h)_2 + O_2 \rightleftarrows Co(L\text{-}h)_2O_2$ (k_1, k_{-1}) and $Co(L\text{-}h)_2O_2 + Co(L\text{-}h)_2 \rightleftarrows [Co(L\text{-}h)_2]_2O_2$ (k_2, k_{-2}). The formation rate is independent of pH (8–11) and insensitive to 1 M KNO_3 addition. It was studied by using excess $Co(L\text{-}h)_2$ from 0.12 to 15.0 mM, and from the results $k_1 = 5 \times 10^7 \exp(-5600/RT)$. At 4.0°, $k_1 = 1.6 \times 10^3\ M^{-1}\ sec^{-1}$ and $k_1k_2/k_{-1} = 5.5 \times 10^6\ M^{-2}\ sec^{-1}$. Addition of $EDTA^{4-}$, $S_2O_4^{2-}$, or low $[H^+]$ to the brown solution removes rapidly $Co(L\text{-}h)_2O_2$ and/or $Co(L\text{-}h)_2$ and leads to an identical first-order decomposition rate constant, $k_{-2} = 6 \times 10^{17} \exp(-26{,}000/RT) = 10^{-2}\ sec^{-1}$ at 4°. Addition of high $[H^+]$ produces an intermediate which decomposes at similar rates to the brown species. The over-all association constant K_{12} $(= k_1k_2/k_{-1}k_{-2})$, $5.5 \times 10^8\ M^{-2}$ at 4°, compares well with that $(3.5 \times 10^8)\ M^{-2}$ obtained previously by manometric measurements and with our own determinations $(3.0 \times 10^8\ M^{-2})$. The behavior of the cobalt(II)–DL-histidine–O_2 system is more complex. The value of k_1 is very similar whether one uses L- or DL-histidine, but some differences reside in the decomposition behavior of the brown species even though K_{12} is very similar for the two systems.

Although a large variety of cobalt(II) complexes with amines, amino acids, and dipeptides have been shown to take up oxygen reversibly,[1] those of histidine and diglycine have been most thoroughly studied.[2–5] The cobalt(II)–histidine complex reacts readily with molecular O_2 to form an amber-colored species which is, however, rapidly decomposed with the release of oxygen, *e.g.*, by the addition of acid or by flushing the solution with nitrogen. The equilibrium involved has been very carefully studied[2] and the amber-colored species, shown to be diamagnetic,[2,6,7] contains a $Co:h:O_2$ ratio of 2:4:1 being probably a binuclear species with an –O–O– unit linking two $Co(histidine)_2$ residues.[5] We shall refer to this as the oxygenated species. No other oxygen-containing complex has been detected in the oxygenated solution.[2] Subsequent to the rapid reversible uptake of oxygen, there occurs a slow complex irreversible uptake of more oxygen leading eventually to dark pink cobalt(III) complexes. This slower oxygenation is being studied currently.[8] The present paper is concerned with the kinetics of formation and decomposition of the oxygenated species using a spectral stopped-flow apparatus. We have investigated mainly the behavior of L-histidine (with which the bulk of reported work is concerned) and then briefly the more complicated DL-histidine–cobalt(II)–oxygen system. These types of systems afford us an opportunity eventually to compare and contrast the behavior of the synthetic chelates with the reversible oxygen carriers of biological importance[9] although any analogy should not be over-emphasized.

Experimental Section

Materials. All chemicals used were reagent grade. The source of L- and DL-histidine (Mann Research Laboratories, Nutritional Biochemicals Corp.) had no effect on the results. Solutions of cobalt(II) nitrate were standardized by titration with EDTA using a murexide indicator.

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(7) C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc.*, **85**, 3736 (1963).

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(9) Q. H. Gibson, *Progr. Biophys.*, **9**, 1 (1959).

Table I. Formation of Oxygen Adduct from Bis(L-histidinato)cobalt(II)

Temp, °C	[Co(L-h)$_2$], mM	k_{obsd}, sec^{-1}	k_1, M^{-1} sec^{-1}
4.0	0.125[a]	0.060	...
4.0	0.25	0.18	...
4.0	0.80	0.87	...
4.0	1.5	1.9	...
4.0	4.5	6.9	1.5×10^3
4.0	10.0	16	1.6×10^3
4.0	15.0	28	1.8×10^3
15.0	4.5	12	2.7×10^3
26.0	1.5	3.5	...
26.0	4.5	15	3.3×10^3
26.0	10.0	35	3.5×10^3
26.0	13.0	39	3.0×10^3
26.0	15.0	55	3.7×10^3
4.0	5.0[b]	5.8	1.2×10^3
15.0	5.0[b]	9.2	1.8×10^3
26.0	5.0[b]	14	2.7×10^3[c]
26.0	10.0[b]	26	2.6×10^3

[a] $[O_2] \simeq 3 \times 10^{-6}$ M; in other experiments $[O_2] \sim 1 \times 10^{-4}$ M. [b] Using DL-histidine. [c] Mean of several separate experiments.

Table II. Decomposition of Oxygen Adduct from Bis(L-histidinato)cobalt(II)

Temp, °C	[Decomposing agent], mM	k_{obsd}, sec^{-1}
	$EDTA^{4-}$	
4.0	10.0	0.009
15.5	10.0	0.086
26.0	1.0	0.35
26.0	5.0	0.35
26.0	10.0	0.29
15.0	10.0	0.020[a,b]
26.0	10.0	0.048[a]
	$S_2O_4^{2-}$	
5.0	10.0	0.010
15.0	10.0	0.10
25.0	10.0	0.24
3.5	20.0	0.0042[a]
16.5	20.0	0.016[a]
26.0	20.0	0.046[a]
	H^+	
26.0	2.5×10^{-6}	0.43
5.0	1.0×10^{-4}	0.0096
15.0	1.0×10^{-4}	0.09
25.0	1.0×10^{-4}[c]	0.33
26.0	1.0×10^{-2}	0.22
26.0	0.50	0.09[d]
26.0	1.0	0.065
25.0	0.50	0.013[a]

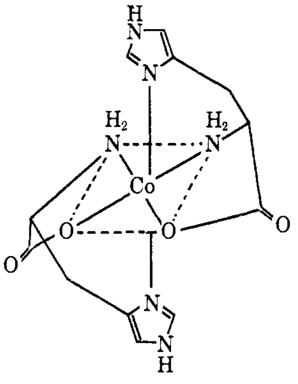
[a] Using DL-histidine. [b] Similar result using oxygen meter. [c] Similar result with $CH_3COOH-CH_3COO^-$ buffer. [d] k = 0.086 with 1.0 M KNO_3 added.

Kinetic Experiments. The formation of the brown oxygenated species was studied by mixing deoxygenated solutions of bis(histidinato)cobalt(II) abbreviated Coh_2, 0.1–15.0 mM at pH 8–10 with oxygen-saturated water $[O_2]$ = 2.5×10^{-4} M at 25°[10] in a glass Lucite stopped-flow apparatus[11] and following the optical density increase in the region of the absorption peak at 385 mμ. First-order rate plots were linear over at least three half-lives (rate constant = k_{obsd}). The results are shown in Table I. For the decomposition experiments, ~0.5 mM adduct was prepared by air-saturating a 4 mM solution of Coh_2, and the solution was mixed with the decomposing solution. This consisted of 10 mM $[EDTA^{4-}]$ or $S_2O_4^{2-}$ at pH ~10, or an acid pH generated by citric acid–Na_2HPO_4 buffer or hydrochloric acid. A rapid small decrease in optical density arose from the dilution perturbation of the equilibrium which exists in the initial Coh_2–O_2 solutions. This did not interfere with accurate observation of the subsequent slower decomposition. In solutions containing only L-histidine, one first-order decomposition rate was observed, but for O_2 complex solutions prepared from DL-histidine distinct curvature of the first-order plot was obtained. The results in Table II under the DL entry refer specifically to the slower reacting portion of the curve. Some of the slower decomposition experiments were also followed with a Beckman laboratory oxygen sensor, which measures by a polarographic method only the dissolved oxygen and has a 90% response in 10 sec.

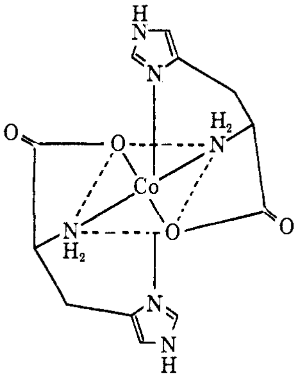
Oxygen Uptake. The stability constant of the O_2 adduct was measured using the Beckman oxygen sensor. A 0.2 M solution of boric acid was adjusted to pH 9.0 and air-saturated at the measurement temperature. The system was sealed off from the atmosphere, and the free oxygen in solution measured with the meter. Aliquots of a Coh_2 solution were then added, and the free $[O_2]$ remeasured after each addition. The results enable the calculation of K_1 (= $[(Coh_2)_2O_2]/[Coh_2]^2[O_2]$). Spectra were measured by a Cary 14 and a Cary spectropolarimeter 60.

Results and Discussion

All bonding sites in histidine are coordinated to cobalt in the bis(histidinato)cobalt(II) species, thereafter abbreviated Coh_2, in the solid state[12] and in solution.[7] The imidazole groups are *trans* to one another in the crystal structure,[12] and, if this favorable arrangement persists in solution, the carboxylate groups *must* be *cis* in Co(L-h)$_2$ (I) and *trans* in Co(L-h)(D-h) (II) due simply to the enantiomeric conformation of the ligand.[5] It has been suggested that the brown species is a binuclear peroxy species [$h_2Co-O-O-Coh_2$] in which one carboxylate group has been severed from each cobalt to allow the formation of the peroxy bridge. Although this is of secondary importance, we shall assume this structure in our discussion of the kinetics.



I



II

We have confirmed previous observations[2] that the rapidly attained equilibrium between bis(L-histidine)cobalt(II) and oxygen involves only

$$2Co(L\text{-}h)_2 + O_2 \rightleftarrows [Co(L\text{-}h)_2]_2O_2 \quad (1)$$

and by using an oxygen sensor have obtained values for $K_1(24°)$, $K_1(15°)$, and ΔH_1 of 7.2 ± 0.5 × 10^6 M^{-2}, 3.1 ± 0.3 × 10^7 M^{-2}, and −36 kcal mole^{-1}, respectively. These compare well with the corresponding values of 5.0 × 10^6 M^{-2}, 3.0 × 10^7 M^{-2}, and −38 kcal mole^{-1} obtained by observations on oxygen uptake using a Warburg apparatus.[2] A few experiments in which DL-histidine was used showed that the uptake of oxygen by the bis complex was only slightly greater (~8% at 15°) than that of the L-histidine complex.

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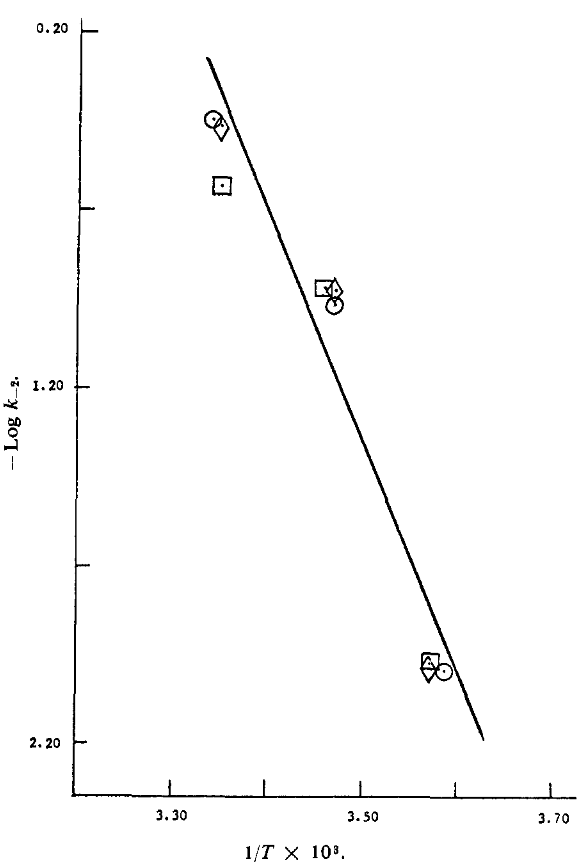

Figure 1. Arrhenius plot for decomposition of $[Co(L\text{-histidine})_2]_2O_2$ by $S_2O_4^{2-}$ ⊡, $EDTA^{4-}$ ⊙, and citric acid-Na_2HPO_4 buffer ◇.

The bis(L-histidinato)cobalt(II)–O_2 complex has absorption peaks at 385 mμ (ϵ_M 7.1 × 10^3) and 350 mμ (ϵ_M 7.5 × 10^3). Although the peak positions are in agreement with the literature,[2] our extinction coefficients are considerably higher than reported. However, only rough estimates of the O_2 content in solution were used in previous experiments. The complex solution obeys Beers law. The oxygen complex formed from DL-histidine has a similar spectra except that $\epsilon_{385m\mu}$ is greater than $\epsilon_{330m\mu}$. In spite of the strong colored solution we have been able to measure the ORD of $[Co(L\text{-}h)_2]_2O_2$. It has a positive Cotton effect and $[\alpha]^{M}_{390}$ 6.2 × 10^4. The oxygen adduct produced from DL-histidine is optically inactive.

Formation Reaction with L-Histidine. The formation of the oxygenated species was studied at 385 mμ and in the majority of experiments at pH 10.0. A large excess of bis(histidinato)cobalt(II) compared with the oxygen concentration (5×10^{-6}–2×10^{-4} *M*) ensured (a) pseudo-first-order conditions and (b) substantially complete consumption of molecular oxygen thus obviating tedious kinetic treatment of a reversible reaction. We were able to study a wide range of Coh_2 concentrations (0.12–15 m*M*) best at lowered temperatures (4°), and the kinetic data are readily accommodated with the scheme

$$Co(L\text{-}h)_2 + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} Co(L\text{-}h)_2O_2 \qquad (1a)$$

$$Co(L\text{-}h)_2O_2 + Co(L\text{-}h)_2 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} [Co(L\text{-}h)_2]_2O_2 \qquad (1b)$$

There were no detectable amounts of any rapidly formed intermediate absorbing in the region 380–600 mμ and assuming steady-state conditions.

$$d/dt\ (\text{peroxy species}) = \frac{k_1k_2[Coh_2]^2[O_2]}{k_{-1} + k_2[Coh_2]} = k_{obsd}[O_2]$$

The plot of $[Coh_2]/k_{obsd}$ against $1/[Coh_2]$ is linear. The value of the slope is k_{-1}/k_1k_2 and of the intercept $1/k_1$. At moderately high concentrations of Coh_2, $k_{-1} \ll k_2[Coh_2]$, a simple second-order reaction is obtained and this condition pertains in our experiments at $[Coh_2] \geqslant 5$ m*M*. At the higher temperatures, only a value for k_1 could be easily obtained. The results are shown in Table III. The second-order rate constant did not vary with pH from 8 to 11 nor was it modified by the addition of 1 *M* KNO_3. The irreversible oxygenation is very slow and did not interfere with our experiments.

One coordinated carboxylate group in bis(histidinato)cobalt(II) is considered to be cleaved when the 1:1 adduct with O_2 is formed in (1a). This adduct then reacts with another molecule of cobalt(II) complex to form the brown oxygenated species (1b). The values for k_1 and ΔH_1^* are reasonably close to those which can be predicted for the replacement of a cobalt(II)-coordinated carboxylate by a neutral ligand.[13] It can be estimated, for example, that the reaction of $Co(EDTA)^{2-}$ with a neutral ligand, such as ammonia, will have second-order rate constant and enthalpy of activation values of the order 10^5 M^{-1} sec^{-1} and 7 kcal $mole^{-1}$. The somewhat smaller rate constant for the oxygen reaction results from a larger negative entropy of activation, this possibly reflecting strict orientation requirements for the O–O grouping with respect to the cobalt complex in the transition state.[14] The only comparison which can be made is with the study of the kinetics of formation of the cobalt(II)–glycylglycine (GG)–oxygen complex.[3] A brown intermediate $[Co(GG)_2(OH)]_2O_2$, formally analogous to the histidine species, can be observed at pH $\gtrsim$ 10 and its formation is believed controlled by a reaction

$$Co(GG)(OH)_2^{2-} + O_2 \rightleftarrows Co(GG)OH \cdot O_2^{-} + OH^{-}$$

in which now a hydroxy group is replaced by the O_2 entity. The forward rate constant for this reaction was estimated indirectly from the rate–pH profile at lower alkalinity. Its value, 1.0 × 10^3 M^{-1} sec^{-1} at 25°, is interestingly (and from our ideas predictably) similar to k_1 (3.5 × 10^3 M^{-1} sec^{-1}) for the reaction we have studied.

Decomposition Reactions with L-Histidine. The freshly formed brown species can be easily decomposed by a number of reagents, with the complete regeneration of oxygen.[2] Decomposition studies enable us to measure the value of k_{-2}. The loss of brown color on addition of EDTA to an oxygenated cobalt(II)–histidine solution is first order. The rate constant is almost independent of the concentration of EDTA thus ruling out substantial *direct* attack of EDTA on the peroxy species. Separate experiments show that EDTA reacts rapidly

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(13) M. Eigen and R. G. Wilkins, Advances in Chemistry Series, No. 49, American Chemical Society, Washington, D. C., 1965, p 55.

(14) Large negative entropies of activation accompany reactions between peroxide and many nucleophiles: J. O. Edwards, "Inorganic Reaction Mechanisms," W. A. Benjamin, Inc., New York, N. Y., 1964, p 75; M. Orhanovic and R. G. Wilkins, *J. Am. Chem. Soc.*, **89**, 278 (1967).

with Coh$_2$ to form (with our relative concentration conditions) a mixed species which is not oxygen sensitive so that the EDTA acts by removing free Coh$_2$ and possibly Co(h$_2$)O$_2$ present in equilibria 1a and 1b. The concurrent release of oxygen into solution can be measured by the polarographic oxygen meter at lowered temperature and the rate constants for color loss and oxygen release agree well.

Dithionite also decomposes the oxygen adduct by reacting rapidly with O$_2$ and once again the decomposition rate constant is independent of the concentration of decomposing agent and identical with the value from the EDTA experiments. The oxygen meter shows on addition of $S_2O_4^{2-}$ a rapid loss of the small amount of free oxygen in the equilibrated solution and thereafter remains registered at zero. Finally, the oxygenated species can be decomposed by plunging it into a buffered solution at pH 3–5. The associated Arrhenius plot for this and the other modes of decomposition is shown in Figure 1 and strikingly illustrates that k_{-2} is being measured by these means. In no case is there any sign of an intermediate during the decomposition. This is in marked contrast to the decomposition of the brown species at higher acidities ($\geqslant 0.5\ M$) when a paler reddish brown intermediate is rapidly formed within mixing times, with no release of oxygen, and which then decomposes slightly more slowly than the brown species and there is parallel release of oxygen. The pale-colored intermediate is presumably a protonated peroxy complex. We have not investigated the number or sites of protonation.

A value for k_{-1}/k_1k_2 ($1.8 \times 10^{-7}\ M^2$ sec) can be easily obtained only at 4°. However, this value, taken in conjunction with the value of k_{-2} obtained by decomposition studies (9×10^{-3} sec^{-1}), yields an over-all K_1 ($= k_1k_2/k_{-1}k_{-2}$) = $5.5 \times 10^8\ M^{-2}$ which is in quite satisfactory agreement with the estimated from manometric measurements ($3.5 \times 10^8\ M^{-2}$)[2] and our own determinations ($3.0 \times 10^8\ M^{-2}$).

Studies with DL-Histidine. When DL-histidine is added to cobalt(II), the solution contains Co(D-h)$_2$, Co(L-h)$_2$, and Co(D-h)(L-h) in amounts slightly favoring the mixed form (62% at room temperature).[7] The value of k_1 for the uptake of oxygen by this mixed system is only slightly less, and the value for ΔH^* slightly more, than for the reaction of Co(L-h)$_2$, barely outside experimental error (Table III). This result might be expected since only one carboxylate group is severed in I or II in forming the 1:1 Co:O$_2$ intermediate.

Table III. Kinetic Data for the Cobalt(II)–Histidine–O$_2$ System at 25°

System	k_1 (k_{-2}, sec^{-1}), M^{-1} sec^{-1}	ΔH_1^* (ΔH_{-2}^*), kcal mole^{-1}	ΔS_1^* (ΔS_{-2}^*), eu
L-Histidine	$3.5 \pm 0.4 \times 10^3$	5 ± 1	-25 ± 3
	(0.47 ± 0.03)	(30 ± 2)	($+38 \pm 6$)
DL-Histidine	$2.6 \pm 0.3 \times 10^3$	6 ± 1	-23 ± 3
	(0.043 ± 0.004)	(16 ± 2)	(-9 ± 6)

The main differences in the L and DL systems are seen in the decomposition behavior. First-order decomposition plots are curved, a small portion (~20%) corresponding to [Co(L-h)$_2$]$_2$O$_2$ and [Co(D-h)$_2$]$_2$O$_2$ with a slower linear portion which must refer to Co(D-h)(L-h)–O$_2$–Co(L-h)$_2$ and Co(D-h)(L-h)–O$_2$–Co(D-h)$_2$ or [Co(D-h)(L-h)]$_2$O$_2$ or an indistinguishable mixture. The relative disposition of the histidine groups in the various binuclear species relative to the peroxy linkage might be expected to affect the ease of Co–O–O bond breakage and hence the value of k_{-2}, but it is difficult at this stage to attempt to rationalize these small differences. We are currently investigating other Co(II)–amino acid and peptide systems.

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