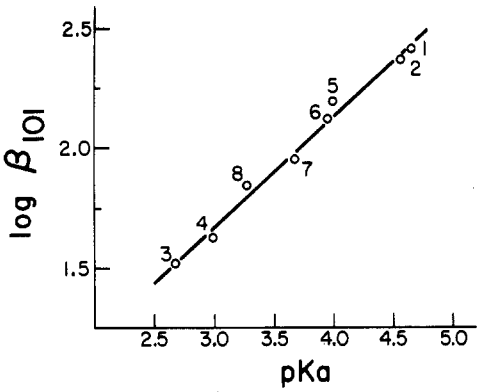

Figure 1. Relationship between the stability constant, β_{101}, for formation of SmL^{2+} and the acid constant, pK_a, of HL: (1) propionic acid; (2) acetic acid; (3) iodoacetic acid; (4) chloroacetic acid; (5) benzoic acid; (6) 4-fluorobenzoic acid; (7) 3-fluorobenzoic acid; (8) 3-nitrobenzoic acid.

The correlation in Figure 1 for the alkyl and aryl monocarboxylates reflects the validity of these interpretations in lanthanide–monocarboxylate complexation.

Figure 2 presents the thermodynamic parameters for different lanthanide–benzoate equilibrium systems. From the similar shapes of the curves for each complexed system we can infer that the complexation involves similar dehydration and bonding effects in all four systems (including the benzoate complexes). Unfortunately, enthalpy and entropy data are not reported[5] for the lanthanide complexation with aliphatic monocarboxylate ligands at an ionic stength of 0.1 M, so no comparison of enthalpy and entropy changes can be made. We can note in Figure 2 that there is some grouping in that, for both ΔH and ΔS, the benzoate and 4-fluorobenzoate curves fall closer together as do the 3-fluorobenzoate and 3-nitrobenzoate curves. Moreover, the relative values indicate slightly more dehydration in benzoate and 4-fluorobenzoate complexation than in the case of the other two ligands.

Smith et al.[7] have proposed values for predicting stability constants of lanthanide complexes. For saturated alkyl monocarboxylate ligands, they propose addition of a correction to the successive log β's of La(III). For example, for Nd(III) complexation, +0.33 is added to the log β_{101} value of La(III); for Sm(III), the correction is +0.45 for Dy(III), it is +0.10, and for Lu(III), it is +0.11. For the aromatic ligand systems of this study, the corrections result in log β_{101} values that are larger than the experimental values in all cases. For the Nd(III) complexes, for our ligands plus benzoate, the predicted log β_{101} value is 0.08–0.15 unit larger than the experimental value. For Sm(III), the average of the differences between predicted and experimental log β_{101} values for the four ligands is 0.17, while for Dy(III) complexation, it is 0.06, and for Lu(III), it is 0.07. For many purposes, such a deviation would not be troublesome.

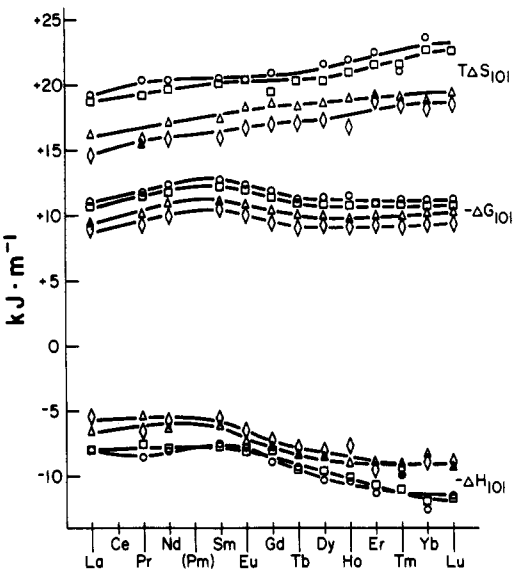

Figure 2. Thermodynamic parameters ($-\Delta G_{101}$, $-\Delta H_{101}$, and $T(\Delta S_{101})$) for formation of LnL^{2+} complexes: (○) benzoic acid; (□) 4-fluorobenzoic acid; (△) 3-fluorobenzoic acid; (◇) 3-nitrobenzoic acid.

Previously the complexation of lanthanides by *o*-, *m*-, and *p*-methoxybenzoates was reported. The stability constants were larger than predicted from the log β_{101} vs. pK_a correlation in Figure 1. This enhanced stability was attributed to an increased negative charge on the carboxylate group when bound to the lanthanide cation. Such increased charge could be explained by inductive and/or resonance effects. In the ligands studied in this work, resonance effects are unlikely and both F and NO_2 are such strong electron-withdrawing groups that the lanthanide cations cannot achieve the charge polarization that seemingly occurs with the methoxy group.

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Registry No. 3-Fluorobenzoic acid, 455-38-9; 4-fluorobenzoic acid, 456-22-4; 3-nitrobenzoic acid, 121-92-6.

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Notes

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Studies Relating to the Specificity of the Hemerythrin Active Site

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Hemerythrin from sipunculid marine worms consists of an octamer form (M_r 108 000) present in the coelomic fluid, each subunit of which contains a binuclear Fe active site capable of binding a single O_2 molecule.[1-4] A monomer form is present in the retractor muscle, and the situation appears closely analogous to that of hemoglobin and myoglobin. The active site of deoxyHr A has two Fe(II)'s, one of which is six-coordinate and the other five-coordinate.[5] Uptake of O_2 at the vacant position is accompanied by a 2e redox change to give two Fe(III)'s, and peroxide, which is bound in an unusual manner (B).[7-9] The metHr form

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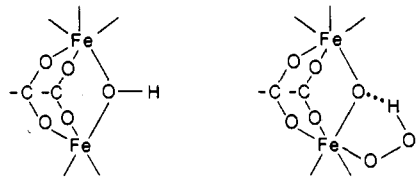
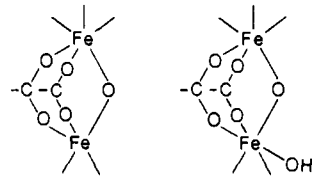


C is known to coordinate OH^- to give D in a process given by eq 1; $pK_a \approx 7.8$.[10,11]



$$\text{metHr} + H_2O \rightarrow \text{metHr(OH)} + H^+ \quad (1)$$

From extensive kinetic studies on the redox interconversion of deoxyHr and metHr forms with 1-equiv redox partners, it is now clear that such processes are extremely complicated. At 25 °C oxidation of octameric deoxyHr is a two-stage process (the second independent of the identity and concentration of oxidant), which requires ~3 h to reach completion.[3,12] Similarly, reduction of metHr is a three-stage process (the last two stages independent of reductant) which requires ~12 h.[3,13] It appears from these studies that octameric deoxyHr is designed to retain an O_2-binding capability in the presence of adventitious oxidants.

Previously we have determined rate constants for the reaction of the octamer of *Themiste zostericola* deoxyHr with O_2: k(25 °C) = 7.5 × 10^6 M^{-1} s^{-1}; I = 0.10 M (Na_2SO_4).[14] Clearly a facile 2e redox process occurs, generating formally an $Fe^{III}_2O_2^{2-}$ species. It was of interest to us whether other 2e redox reagents gave similar behavior, and we therefore selected as oxidants H_2O_2, BrO_4^-, and BrO_3^- and as reductant the bacterial 2[4Fe-4S] ferredoxin from *Clostridium pasteurianum* (M_r 6000, charge −10 at pH 7.5). Two-equivalent reactions of H_2O_2 (→2 OH^-)[15] and BrO_3^- have been reported.[16] In the case of BrO_4^- the kinetic barrier to redox change is less than with ClO_4^-,[17] and BrO_4^- (like BrO_3^-) is potentially able to release O atoms in 2-equiv processes, (2) and (3).

$$BrO_4^- + 2e^- \rightarrow BrO_3^- + O^{2-} \quad (2)$$

$$BrO_3^- + 2e^- \rightarrow BrO_2^- + O^{2-} \quad (3)$$

Experimental Section

Materials. Procedures for the isolation and handling of Hr from *T. zostericola* (purchased live from Pacific Bio-marine Laboratories, Venice, CA)[13] and 2[4Fe-4S] ferredoxin from *C. pasteurianum* (PHLS, Microbiological Supplies, Porton, U.K.)[18] were as previously described. Hydrogen peroxide (Fisons 100 Vol, SLR solution), sodium bromate (BDH, Analar), and potassium perbromate (generously provided by Professor H. Gamsjäjer, University of Leoben, Leoben, Austria) were used as obtained.

Buffers. The pH of 0.05 M solutions of 2-morpholinoethanesulfonic acid (Mes, Sigma; pH 6.3) and tris(hydroxymethyl)aminomethane (Tris, Sigma; pH 7.9–8.6) and 0.25 M sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$, BDH; pH 9.58) was adjusted by addition of 1 M NaOH (Mes and borate) or 1 M H_2SO_4 as required. The ionic strength was adjusted to 0.15 M with Na_2SO_4 (BDH, AnalaR).

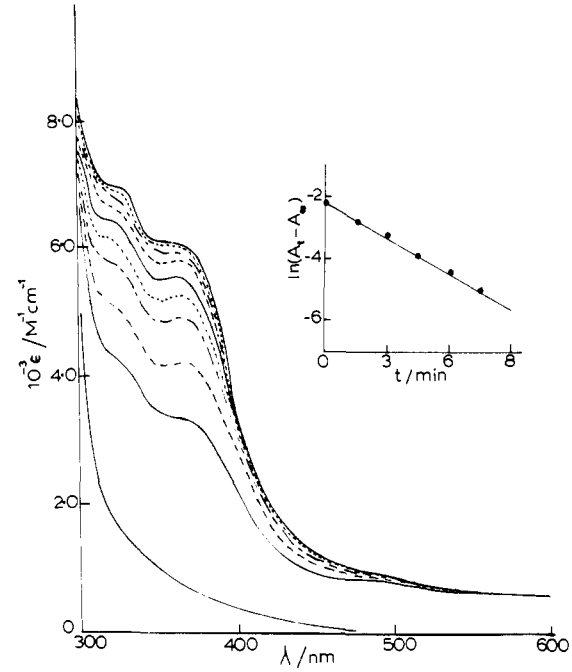


Figure 1. Scan spectra at 1.5-min intervals recorded during the reaction of *T. zostericola* deoxyhemerythrin (5 × 10^{-5} M) with H_2O_2 (1.18 × 10^{-3} M) at 25 °C, pH 6.3, and I = 0.15 M (Na_2SO_4). The insert shows the corresponding first-order plot, the slope of which corresponds to k_{obsd}.

Table I. First-Order Rate Constants k_{obsd} (25 °C, Except As Stated) for the Oxidation of *T. zostericola* Deoxyhemerythrin, (5.0–8.2) × 10^{-5} M, by H_2O_2, I = 0.15 M (Na_2SO_4)

pH	$10^3[H_2O_2]$, M	10^3k_{obsd}, s^{-1}	pH	$10^3[H_2O_2]$, M	10^3k_{obsd}, s^{-1}
6.30	0.71	4.8	8.35	2.50	13.6
	1.18	6.3		2.50	22.5 (35 °C)
	2.35	14.4		3.44	18.8
	4.70	25.6		5.00	13.6 (5 °C)
7.96	1.50	8.3		5.00	19.3 (15 °C)
8.35	0.67	3.5		5.00	27.2
	1.67	9.0		5.00	49.4 (35 °C)
	2.50	8.9 (5 °C)	8.60	1.50	8.3
	2.50	8.9 (15 °C)	9.58	1.50	8.3

Results and Discussion

At pH 6.3 and 8.3 the product of the H_2O_2 oxidation of deoxyHr is hydroxometHr, D, with a peak at 333 nm (ϵ = 6450 M^{-1} cm^{-1} per active site).[13] On addition of sodium azide (22 mM) to the product, formation of the azidomet form peak at 325 nm (ϵ 7450 M^{-1} cm^{-1}) is observed. Oxidation of deoxyHr by 2 equiv of $[Fe(CN)_6]^{3-}$ followed by addition of N_3^- (22 mM) generated spectra in excellent agreement with those produced by H_2O_2 oxidation. In this respect the work differs from a previous study of Bradič et al.,[19] which suggested that a different met form was produced by H_2O_2 oxidation. The kinetics of the reaction of deoxyHr (5 × 10^{-5} M) with H_2O_2 (>10-fold excess) were monitored at 360 nm, and a uniphasic absorbance change was observed.

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First-order plots of ln $(A_t - A_\infty)$ against time were linear to at least 3 half-lives and gave rate constants k_{obsd} (Table I), which exhibit a first-order dependence on $[H_2O_2]$. No dependence on pH was observed over the range pH 6.3–9.5 investigated, consistent with H_2O_2 (pK_a 11.67)[20] being the sole reactant. At 25 °C, k was found to be 5.5 ± 0.2 M^{-1} s^{-1} in good agreement with the value previously reported (6.6 M^{-1} s^{-1}).[19] From the temperature dependence $\Delta H^\ddagger$ = 7.6 ± 0.6 kcal mol^{-1} and $\Delta S^\ddagger$ = −2.9 ± 2.0 cal K^{-1} mol^{-1}. These values compare with $\Delta H^\ddagger$ = 4 kcal mol^{-1} and $\Delta S^\ddagger$ = −11 cal K^{-1} mol^{-1} for the reaction of deoxyHr with O_2.[14] The higher $\Delta H^\ddagger$ value is consistent with more extensive structural changes in the case of the H_2O_2 reaction. In support of the pH independence of rate constants Bradič et al. have reported that the protonated forms of NCO^-, N_3^-, and F^- are involved in complexation reactions with deoxyHr.[21]

Of particular interest is the formation of hydroxomet, D, characterized by two absorbance maxima in the 300–400-nm region at pH 6.3 as well as at pH 8.3. Subsequent absorbance changes corresponding to the D → C conversion are not as rapid or complete as we would at first have expected, which may be related to the recently proposed existance of cis and trans hydroxo forms.[11b] In a duplicate experiment in which D was generated by an alternative route and the pH then adjusted from 8.2 to 6.3, comparable absorbance time changes were observed. Also changes are not as well defined as we would like for the whole process since some denaturation of Hr occurs at the higher $[H_2O_2]$ required to separate the two kinetic stages. It is possible that spectra reported previously[19] differ from those in the present work due to such denaturation, which is difficult to avoid at pH 6.3. At pH 8.3 a further slow reaction of D with H_2O_2 (0.12 M) is also observed with decay of absorbance bands in the 300–400-nm region in a first-order process, $t_{1/2} \approx 2$ h. This probably corresponds to denaturation as observed with $[IrCl_6]^{2-}$ and $[Mn(Cydta)(H_2O)]^-$ as oxidants[22] and in other studies described by Harrington and Wilkins.[23,24] In contrast to the earlier work[19] spectra similar to that of oxyHr were not observed. Since deoxyHr, A, is μ-hydroxo (or a closely related form) and metHr is μ-oxo, the $H_2O_2 \rightarrow 2OH^-$ change can be represented as in (4), with release of H_2O and retention of coordinated OH^- as in structure D.

$$\text{deoxyHr}(\mu\text{-OH}) + H_2O_2 \rightarrow \text{metHr(OH)}(\mu\text{-O}) + H_2O \quad (4)$$

A very much slower uniphasic first-order process is observed in the oxidation of deoxyHr by BrO_4^- (3.3–13.3 mM) at pH 6.1–6.6. Rate constants k_{obsd} are independent of $[BrO_4^-]$ and first order in $[H^+]$, giving a second-order rate constant k_H = (1.1 ± 0.1) × 10^3 M^{-1} s^{-1}, and metHr (C) with a peak at 345 nm is formed. A similar process is observed with BrO_3^- (3.3–13.3 mM), $k_H \approx 21$ M^{-1} s^{-1}. Both processes (25 °C; I = 0.15 M Na_2SO_4) therefore display characteristics previously identified by Wilkins and colleagues[21a] and assigned to the slow anion-catalyzed conversion of deoxyHr to metHr in the presence of traces of O_2.

To further test the 2e redox capability of the Hr active site, we investigated the reduction of metHr (10^{-5} M) with (1–3) × 10^{-5} M 2[4Fe-4S] in the fully reduced state (rr) at 25 °C, pH 6.3, and I = 0.15 M (Na_2SO_4). Consumption of reductant was monitored at 420 nm, ε for 2[4Fe-4S] (rr) being 2.7 × 10^4 M^{-1} cm^{-1}, for the fully oxidized form (oo) 1.1 × 10^4 M^{-1} cm^{-1}, and for the half-oxidized (or) midway between these values. At this wavelength the Hr absorbance contributes <5% to the total change. From an average of 10 determinations one electron (0.95 ± 0.08) was consumed per metHr, corresponding to formation of an $Fe^{II}Fe^{III}$ semimetHr form. Further slow reduction of this semimet product was not studied due to the extreme sensitivity of reduced 2[4Fe-4S] to traces of O_2 over long periods. It can be concluded that reaction occurs in 1e steps and there is no direct 2e conversion of Fe(II, II) to Fe(III, III).

It is clear that the specificity of the Hr active site for O_2 is linked to the 2e change $Fe^{II}_2 \rightarrow Fe^{III}_2$ and the availability of a proton from the OH^- bridging the two Fe(II)'s in A to give B. Almost accidentally H_2O_2 is able to utilize these same features in the 2e change $H_2O_2 \rightarrow 2OH^-$, with concomitant uptake of a proton from the bridging OH^- and formation of (bound) OH^- and (unbound) H_2O. With BrO_4^- and BrO_3^- as oxidants for deoxyHr the reactions proceed by alternative routes. Schematically 2e changes are possible, but these require inner-sphere O atom transfer, the implication of our results seems to be that neither of these reactants gains access to the active site due to their charge and/or size. With the 2[4Fe-4S] ferredoxin (reduction potential −400 mV)[25] access to the active site is not even a remote possibility, and no 2e reduction is observed. A direct 2e reduction of the met active site would probably require proton donation to the μ-O bridging ligand, which in this instance is more difficult to achieve.

Overall the Hr active site appears to be highly specific for a 2e change, which may be linked to the ability of the redox partner to accept/donate a proton from the bridge. Studies to date have shown that in contrast reactions of Hr with 1e redox reagents are extremely complex. A notable exception (which we do not fully understand) is the reaction with deoxymyoglobin, which accomplishes reduction of metHr in a single step.[26] The specificity of deoxyHr for neutral molecules is noted, and the observation that metHr will bind only small and/or linear anions such as OH^-, N_3^-, and NCS^- is of interest. We also note that the O_2^{2-} in oxyHr is bound in a bent (end-on) fashion, B, whereas CO, which binds in a linear manner to deoxyhemoglobin (and binds also to hemocyanin), does not bind to deoxyHr.[27]

Acknowledgment. We thank the U.K. Science and Engineering Research Council for a studentship (to G.D.A.).

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Preparation and Characterization of the μ-Oxo Dimer and Hydroxo Complexes of (Tetrakis(pentafluorophenyl)porphinato)iron(III)

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On oxidation of ferrous tetraphenylporphyrin complexes with dioxygen or metathesis of the chloro ferric complexes with hydroxide,[1-6] bulky substituents such as methyl, methoxy, or ethoxy,

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