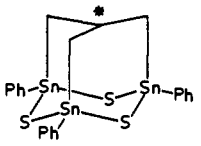
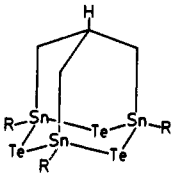
carbon is 129.1 Hz, intermediate between the values observed for adamantane (133.5 Hz)[13] and tri-*tert*-butylmethane (124 Hz).[14] In addition, flattening of the bridgehead carbon atom in stannaadamantane **1** presumably favors transfer of hydrogen by minimizing the amount of additional strain incorporated in the hypothetical radical or cationic intermediate **2**.[15] Related compounds containing even longer bonds, like tristannatritelluraadamantane **3**,[16,17] should have bridgehead carbon atoms that are even more deformed, and we expect that the central carbon–hydrogen bonds of these compounds will be even more reactive.



2



3

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Registry No. 1, 87922-35-8; adamantane, 281-23-2.

Supplementary Material Available: Refined temperature factors, hydrogen coordinates, bond lengths, bond angles, least-squares planes, structure factor table, and experimental procedures (24 pages). Ordering information is given on any current masthead page.

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Cooperativity in Oxygen Binding to *Lingula reevii* Hemerythrin: Spectroscopic Comparison to the Sipunculid Hemerythrin Coupled Binuclear Iron Active Site

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Among the metalloprotein dioxygen carriers, hemoglobin and hemocyanin are found to be cooperative in oxygen binding under many conditions,[1,2] while hemerythrin is generally regarded as noncooperative.[3,4] In addition, hemoglobin and hemocyanin typically have marked Bohr effects,[5,6] while in sipunculid hemerythrins these pH effects are absent.[3] Hemerythrin and hemocyanin have binuclear iron and copper active sites, respectively, and the binding of oxygen in both cases is a redox process with the oxygen bound as peroxide.[7,8] Spectroscopic and chemical investigations have shown the peroxide to be bound differently in the two cases, with a μ-1,2-peroxo bridge in hemocyanin (I) and end-on binding to a single iron(III) in hemerythrin (II).[10,11]

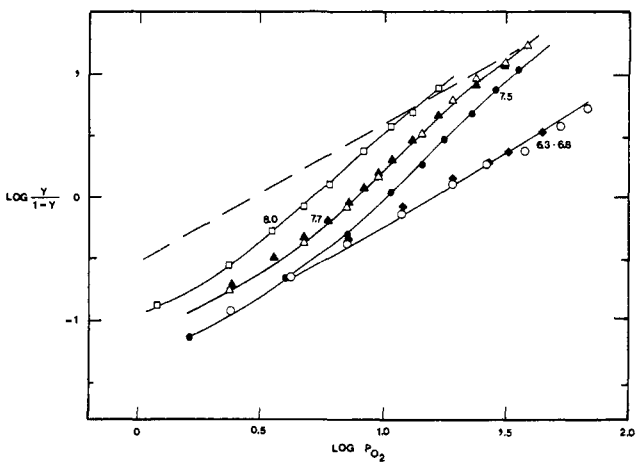
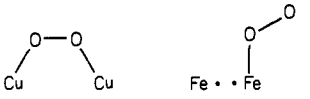


Figure 1. Hill plots for oxygen binding to *Lingula reevii* hemerythrin (solid lines). The pH values are indicated in the figure (μ 0.2 phosphate buffer). The dashed line is the binding curve for *Phascolopsis gouldii* hemerythrin measured under the same conditions (pH 6.3–8.0). Temperature 23 °C.



I II

It has been suggested that the allosteric control of oxygen affinity in hemocyanin could be accomplished via steric interactions involved in formation of the exogenous peroxide bridge.[12] A central question is then whether the binuclear non-heme active site in hemerythrin is intrinsically capable of engaging in the homotropic allosteric interactions necessary for cooperative ligand binding. In this report we document the oxygen-binding properties of the coelomic hemerythrin from the brachiopod *Lingula reevii* (hereafter *L.r.* Hr) and characterize its spectroscopic properties for comparison to other hemerythrins.[13] The results show that at pH ~7–8 *L.r.* Hr exhibits cooperativity in dioxygen binding at an active site very similar to that of other known hemerythrins and that the end-on bonding mode is retained.

Oxyhemerythrin was obtained from live specimens of *Lingula reevii* collected in shallow waters off Oahu, HI. The protein was isolated from erythrocyte lysate by dialysis against phosphate buffer (pH 7.7, μ 0.2) and gel chromatography. We have estimated the molecular weight of the native protein to be 105 000 ± 5000 and the subunit MW to be 13 000 ± 1000 D using the sedimentation equilibrium method and gel chromatography. The native protein appears to be octameric, as is usually found for other coelomic hemerythrins.[3,14]

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Table I. Spectral Comparison of *Lingula reevii* and *Phascolopsis gouldii* Hemerythrins

	UV-vis, nm (M^{-1} cm^{-1})[a]	circular dichroism, nm (M^{-1} cm^{-1})[a]	Raman, cm^{-1} [b]
oxy-Hr			
L.r.	507 (2100), 332 (6000)	795 (−0.4), 518 (−3.5), 340 (−5.3)	505 (Fe-O), 844 (O-O)
P.g.	500 (2300), 326 (6900)	791 (−0.36), 520 (−2.5), 336 (−3.8)	505, 844
metazido-Hr			
L.r	448 (3900), 329 (6900)	500 (−5.2), 367 (−12.0)	376 (Fe-N), 511 (FeOFe), 2052 (N_3^-)
P.g.	446 (3800), 327 (7200)	500 (−4.1), 370 (−9.0)	376, 510, 2054

[a] Principal band maxima. Intensities are based on concentration of iron dimers (2 Fe/subunit) as determined by atomic absorption. Intensities for *P.g.* Hr are quoted from ref 15. [b] Frequencies have been measured relative to sulfate at 984 cm^{-1}.

Results of our tonometric oxygen-binding studies are summarized in Figure 1.[15] The binding is cooperative and shows the Root effect,[16] with the maximum Hill slope[17] highest at pH 7.7 (n_{max} = 2.0) and decreasing at higher and lower pH values (n_{max} = 1.1 at pH 6.3–6.6). Manwell studied the oxygen equilibrium of *Lingula unguis* hemerythrin[18] and reports that n_{max} = 1.7 at pH 7.6 (phosphate) with the value of n_{max} decreasing to ~1 at pH 6.7–6.8.

The oxygen-binding curves have been fit by using a modified relaxed-tense model.[19] It is notable that the oxygen affinity of sipunculid coelomic hemerythrins (~3 mmHg)[3] is close to that estimated for the relaxed form of *L.r.* Hr (K_R = 2 mm). This implies the presence of structural constraints[20] in the tense form (K_T = 20 mm) of *L.r.* Hr. The allosteric energy, given by the difference in free energies of R and T oxygen binding, is ~1.5 kcal/mol.

The absorbance, CD, and resonance Raman data from *L.r.* oxy-Hr and metazido-Hr are correlated to analogous results for *Phascolopsis gouldii* Hr in Table I. All spectral features of *L.r.* Hr are extremely similar to those of *P. gouldii*, and two are particularly characteristic of the peroxide binding mode. Single-crystal polarized spectral studies[10] of the $O_2^{2-} \rightarrow$ Fe(III) charge-transfer band in *P. gouldii* oxy-Hr were initially used to determine that the peroxide is bound end-on (II), and the similar energy (507 nm) and intensity of the band in *L.r.* Hr implies no significant differences in bonding geometry. In the resonance Raman spectrum the Fe–(O_2^{2-}) stretching frequency of oxy-Hr is expected to be sensitive to the peroxide binding mode. A normal coordinate analysis predicts that the terminally bound Fe–O stretch observed at 505 cm^{-1} for *P. gouldii* oxy-Hr would shift to ~350 and 456 cm^{-1} for μ-1,1 and -1,2 bridging geometries, respectively.[21]

The comparison of spectroscopic properties in Table I strongly implies that the terminal exogenous ligand binding mode of sipunculid Hr is maintained in *L.r.* Hr. This demonstrates that the hemerythrin binuclear non-heme iron site is capable of engaging in cooperative interactions and that a relaxed-tense conformational change in the protein quaternary structure can control the affinity of the active site for oxygen. Thus, cooperativity has been found in coupled binuclear metalloprotein oxygen carriers that have either end-on or bridging peroxide geometries. Although the μ-peroxo geometry may be important in homotropic allosteric effects of hemocyanins, the present results indicate that other mechanisms can contribute significantly to cooperativity in this type of active site.

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Iron–Histidine Stretching Raman Lines of the aa_3-Type Cytochrome Oxidases

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Cytochrome oxidase (EC 1.9.3.1), a terminal oxidase of the respiratory chain, couples cytochrome *c* oxidation with dioxygen reduction and simultaneously translocates protons across an energy-transducing membrane (see ref 2 for review). The aa_3-type cytochrome oxidase is composed of two active centers, namely, cytochrome *a* and a_3. The former consists of a low-spin heme *a* and an EPR-active copper, and the latter contains a high-spin heme *a* and an EPR-silent copper antiferromagnetically coupled with the high-spin iron.[3] The a_3 heme serves as the catalytic site for dioxygen reduction, but for the resting enzyme the occupation of a sulfur ligand in the catalytic site is proposed from the EXAFS study.[4] Thus, elucidation of the iron coordination environments of the a_3 heme is a matter of current spectroscopic concern. We report here the iron–histidine stretching Raman line of the reduced a_3 heme of mammalian, yeast, and bacterial cytochrome oxidases.

The Fe–His stretching Raman line of the five-coordinate ferrous high-spin hemoproteins[5,6] is strongly intensity enhanced upon excitation around 442 nm, and its frequency is noticeably sensitive to a state of the coordinated histidine.[7] Presence of appreciable strain in the Fe–His bond of the low-affinity deoxy-Hb was first revealed by the observation of the Fe–His stretching Raman line,[8] and now its frequency difference between the α and β subunits is discussed.[9,10] The structural implication of the frequency difference between the Fe–His stretching modes of peroxidases and oxygen carriers was also interpreted satisfactorily.[11] A large

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