troleums using appropriate compounds characterized in the above-mentioned matrices as well as testing other bonded ligands.

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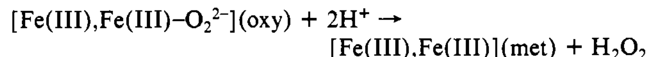
A Cytochrome b_5 from Erythrocytes of *Phascolopsis gouldii*. One Component of a Potential System for Reduction of Methemerythrin in Vivo

Sir:

Hemerythrin (Hr), the oxygen-carrying protein found in erythrocytes of sipunculan worms, is the counterpart to hemoglobin in mammalian erythrocytes. In contrast to hemoglobin, Hr contains a non-heme binuclear iron center as the oxygen-binding site.[1,2] This site can be stabilized in four distinct states: [Fe(II),Fe(II)](deoxy), [Fe(III),Fe(III)–O_2^{2-}](oxy), [Fe(II),Fe(III)](semi-met), and [Fe(III),Fe(III)](met). All four states exhibit antiferromagnetic coupling between iron atoms, which is mediated by an oxo or hydroxo bridge.[3-5] Up to now only the former two states have been established as physiologically relevant, since only these two have been implicated in reversible binding of O_2. Purified Hr from erythrocytes of the sipunculid *Phascolopsis gouldii* undergoes autooxidation according to the reaction



$$[\mathrm{Fe(III),Fe(III)}{-}\mathrm{O_2^{2-}}](\mathrm{oxy}) + 2\mathrm{H^+} \rightarrow [\mathrm{Fe(III),Fe(III)}](\mathrm{met}) + \mathrm{H_2O_2}$$

At 25 °C, pH 7.0, and 0.3 M Cl^-, $t_{1/2}$ for autooxidation is 18.5 h.[1] However, very little metHr is found in freshly isolated erythrocytes. Therefore, a system apparently exists within the erythrocyte that either prevents or reverses autooxidation.

Hemoglobin also undergoes autooxidation to a met form. In normal mammalian erythrocytes methemoglobin accounts for less than 1% of the total hemoglobin.[6,7] This low steady-state level is maintained by a reductase system in which electrons are transferred to methemoglobin in the sequence NADH → cytochrome b_5 reductase → cytochrome b_5 → methemoglobin.[8,9]

Despite the differences between mammalian and sipunculan erythrocytes, we have discovered what appears to be a similar system in *P. gouldii*. Herein we report preliminary characterization of a cytochrome b_5 (*P. gouldii* cyt b_5) isolated from the soluble fraction of *P. gouldii* erythrocytes and the possible role of *P. gouldii* cyt b_5 in reduction of metHr in vivo. Table I compares the properties of *P. gouldii* cyt b_5 with those of human erythrocyte cyt b_5.[10] As can be seen, these properties are quite similar. Figures 1 and 2 show the absorption and EPR spectra, respectively, of *P. gouldii* cyt b_5. The EPR parameters are typical of proteins having low-spin ferric heme with axial bis(histidine) ligation.[12] *P. gouldii* cyt b_5 in either the oxidized or reduced form

Table I. Comparison of Properties of Cytochromes b_5 from Human and *P. gouldii* Erythrocytes

	human[a]	*P. gouldii*[b]
mol wt	13 700	14 000[d]
pI	4.3	3.8
EPR *g* values (oxidized)	3.03, 2.21, 1.39[c]	3.07, 2.22, 1.4
Soret max (oxidized; reduced), nm	412; 423	412; 422
α-band max (reduced), nm	556	555
β-band max (reduced), nm	526	526

[a] Reference 9. [b] This work. [c] Reference 13. [d] Determined in 6 M guanidine hydrochloride by HPLC using an Altex Spherogel-TSK 300-PW column and commercial samples of *C. pasteurianum* ferredoxin (6000), horse heart cytochrome *c* (13 000), and sperm whale myoglobin (17 800) as molecular weight markers.

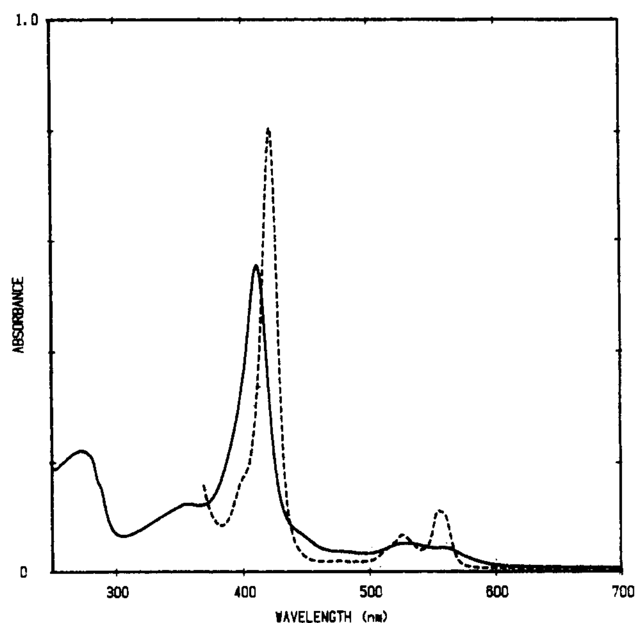


Figure 1. UV–visible spectra of oxidized (—) and reduced (- -) *P. gouldii* cyt b_5.

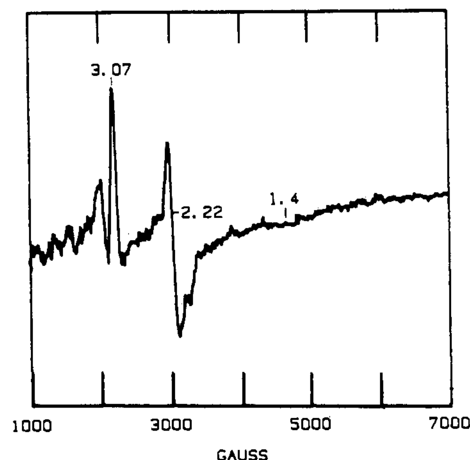


Figure 2. First-derivative EPR spectrum of *P. gouldii* cyt b_5. Spectral conditions: temperature, 4 K; frequency, 9.42 GHz; power, 20 mW; modulation, 16 G at 100 kHz; time constant, 0.15; gain, 3.2 × 10^4. Positions of the *g* values reported in Table I are indicated.

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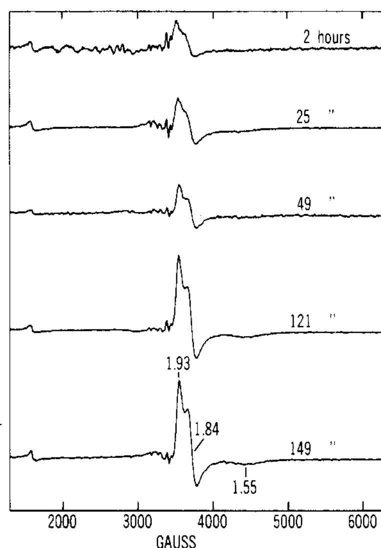

Figure 3. First-derivative EPR spectra of erythrocytes from *P. gouldii*. "Deoxygenated" (see text) coelomic fluid was filtered through cheesecloth. The erythrocytes were then spun down, resuspended in artificial seawater, and incubated aerobically for the indicated times at 4 °C. Zero time is that of removal of the fluid from the coelom. Spectral conditions: temperature, 4 K; frequency 9.57 GHz; power, 0.2 mW; receiver gain, 1.6×10^5. Other conditions are as given in the caption to Figure 2. Double integration against a $CuSO_4$ standard gave the following ratios of [semi-metHr]/[total Hr]: 25 h, 0.0038; 49 h, 0.0042; 121 h, 0.011; 149 h, 0.015.[23]

shows no reaction with 10 mM CN^- or gaseous CO.

The low isoelectric point listed in Table I means that near pH 7, *P. gouldii* cyt b_5 will have a net negative charge, while Hr, with an isoelectric point of ~8,[14] will have a net positive charge. Thus, a complex should form between hemerythrin and cytochrome b_5 at low [salt]. This putative complex provides an interesting contrast to that formed between human cytochrome b_5 and hemoglobin.[15] In the latter case a heme to heme one-electron transfer occurs, whereas in the case of Hr a heme to non-heme iron electron transfer would occur and two electrons are required to reach deoxyHr from metHr.

Like all other known cytochromes b_5, reduced *P. gouldii* cyt b_5 is rapidly oxidized by atmospheric oxygen and slowly autooxidizes under anaerobic conditions. Most importantly, reduced *P. gouldii* cyt b_5 rapidly reduces metHr. Under anaerobic conditions the rate constant for this reaction is 650 ± 50 M^{-1} s^{-1} in 10 mM phosphate, pH 7.2, 0.5 mM EDTA at 20 °C.[16] This rate constant is much higher than that reported for reduction of *P. gouldii* metHr by deoxymyoglobin (0.25 M^{-1} s^{-1} at pH 7.0 and 25 °C[17]), which could reflect the lower reorganizational energy required for electron transfer from low-spin heme.[18] On the basis of absorbance changes at 555 nm, a mixture of 0.019 mM reduced *P. gouldii* cytb_5 and 0.010 mM metHr in anaerobic buffer (pH 7.2) resulted in oxidation of 39% of the cyt b_5 within 2 min.[19] An EPR spectrum at 4 K of this mixture indicates that semi-metHr is the product of reduction, having *g* values at 1.95, 1.86, and 1.67. These *g* values are typical of (semi-met)$_R$, the form produced by one-electron reduction of metHr.[22]

If reduction of metHr proceeds in this fashion within the erythrocyte, similar semi-met EPR signals should be observed. In fact, we observe no significant EPR intensity at 4 K from oxygenated erythrocytes even after 150 h incubation at 4 °C. However, if the worms are kept in seawater (20 °C) under a N_2 atmosphere for 12 h prior to isolation of the erythrocytes, which effectively deoxygenates the coelomic fluid, a characteristic semi-met EPR signal *is* observed to build up with time upon subsequent oxygenation as shown in Figure 3. At the longest incubation time the intensity of this signal accounts for less than 2% of the total Hr.[23] The *g* values reported in Figure 3 are similar to but not identical with those of (semi-met)$_R$ (vide supra).[24] A possible explanation for these results is that prolonged oxygen deficiency followed by rapid reoxygenation induces increased levels of metHr and/or *P. gouldii* cyt b_5 within the erythrocytes. We have in fact noticed a significant (≥5-fold) increase in the yield of *P. gouldii* cyt b_5 from "deoxygenated" erythrocytes.

We have also isolated and partially purified a cytochrome b_5 reductase from the membrane fraction of *P. gouldii* erythrocytes. This reductase, when combined with *P. gouldii* cyt b_5, will catalyze the reduction of metHr to deoxyHr by NADH. The reactions between these various components and the role of the entire system in reduction of metHr in vivo are currently under study.

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Registry No. Cytochrome b_5, 9035-39-6.

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(24) That the EPR signal of Figure 3 is due to intracellular Hr was verified by spinning down a portion of the erythrocytes after the longest incubation time and examining the supernatant by EPR at 4 K. No signal was observed.

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Synthesis and Characterization of the Polyoxothioanions $TaW_5O_{18}S^{3-}$ and $NbW_5O_{18}S^{3-}$

Sir:

Attempts to isolate early-transition-metal d^0 polyoxothioanions from polyoxoanions by oxygen–sulfur exchange are frequently frustrated by metal center reduction and/or metal–oxygen framework degradation.[1] In order to circumvent these difficulties, we have sought to introduce sulfur into polyoxoanions such as tantalo- and niobotungstates that contain labile metal–oxygen subunits embedded in relatively substitution-inert polyoxoanion

(1) See Do, Y.; Simhon, E. D.; Holm, R. H. *Inorg. Chem.* **1985**, *24*, 1831 and references cited therein.

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