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Blood Function in the Hydrothermal Vent Vestimentiferan Tube Worm

Abstract. *Extracellular hemoglobin in the whole blood of* Riftia pachyptila *has a high oxygen affinity (P_{50} = 1.8 millimeters of mercury at 3°C), a moderate decrease in oxygen affinity at higher temperatures (P_{50} = 2.7 millimeters of mercury at 14°C), a small effect of carbon dioxide on oxygen affinity ($\Delta \log P_{50}/\Delta$ pH = − 0.12), and a high oxygen carrying capacity (up to 11 milliliters of oxygen per 100 milliliters of blood). These characteristics are compatible with the high oxygen demand of chemoautotrophic metabolism in the variable vent environment.*

Giant vestimentiferan worms (up to 3 m in length) are found at a depth of 2500 m around the deep-sea hydrothermal vents (*1*). These sessile animals, *Riftia pachyptila*, live where the vent water (up to 22°C, 350 μ*M* H_2S, anoxic) is actively mixing with the surrounding water (2°C, no H_2S, 110 μ*M* O_2) and they are therefore exposed to conditions continually changing between these extremes (*1–3*). It has been postulated that these worms, or their symbionts, oxidize H_2S in the presence of oxygen for their metabolic energy source (*4*).

The vent worm has no mouth or gut but does have a ventral heart and a well-developed circulatory system that supply blood to the trophosome, the proposed site of autotrophic metabolism (*2, 4*). The red color of the animal is due to an extracellular hemoglobin, which occurs in both the vascular blood and the coelomic fluid, and is similar in structure to that of annelid worms (*5*). We examined the oxygenation characteristics of the whole blood of *R. pachyptila* to gain a better understanding of respiratory function of animals in this unusual habitat. We present data showing that the concentrated extracellular hemoglobin is suited to the needs of an autotrophic organism in this variable environment.

Vestimentiferan worms were collected by the submersible *Alvin* during November and December of 1979 at the Galápagos Rift valley sites Garden of Eden and Rose Garden (*1, 6*). As soon as the specimens were brought to the surface, blood was sampled and introduced into a chamber that was regulated to the same temperature and CO_2 concentration as the subsample whose oxygen binding characteristics were being studied. Changes in *p*H were monitored by the use of an E and K combination *p*H electrode calibrated with *p*H 4 and *p*H 7 buffers at each experimental temperature. The effect of 0 percent (air), 0.4 percent, and 2 percent CO_2 on the *p*H of the oxygen saturated blood was determined, and the *p*H of each curve generated was taken from these measurements. Oxygen equilibrium curves of a small subsample of whole blood (2 to 4 μl) were measured with a modified Hem-O-Scan (from 3° to 30°C) (*7*). The effect of various concentrations of CO_2 were examined at each temperature by generating oxygen equilibrium curves in the presence of 0 percent (air), 0.4 percent and 2 percent CO_2. Most data were collected at sea from fresh blood samples (the exceptions being some studies on dilution and Bohr effects) which showed no sign of denaturation or change in oxygen binding characteristics with time (up to 14 hours). All data reported are from vascular blood, except when coelomic fluid data are included for comparison.

The whole blood of *R. pachyptila* shows a small Bohr effect (a shift of the oxygen equilibrium curve to the right) with increased CO_2 concentrations and decreased *p*H ($\Delta \log P_{50}/\Delta$ *p*H = −0.12 ± 0.09; 95 percent confidence interval, n = 21) (P_{50} is the oxygen partial pressure at which the hemoglobin is half saturated with oxygen). Oxygen equilibrium curves were generated without CO_2 gas and with 2 percent CO_2 gas repeatedly at 8°C; *p*H values were determined from subsamples of whole blood equilibrated with these same gas mixtures. In general, for annelids containing extracellular hemoglobins, worms that inhabit permanent, well-ventilated burrows or tubes tend to have large Bohr effects (for *Diopatia*, −0.86); but transient burrow dwellers tend to have small Bohr effects and sometimes reverse Bohr effects (*Marphysa*, −0.25; *Nephtys*, +0.09) (*8*). *Riftia pachyptila*, although inhabiting a permanent tube, is unusual in that the whole blood has a small Bohr effect. This unusual characteristic may be important for an autotroph that transports CO_2 simultaneously with oxygen to the tissues.

The vascular blood and the coelomic fluid both showed a high affinity for oxygen. The P_{50} values for vascular blood increased with temperature and ranged from 1.2 mmHg at 3°C to 7.2 mmHg at 25°C (Fig. 1), while coelomic

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fluid ranged from 0.7 mmHg at 3°C to 3 mmHg at 25°C.

Temperature has only a moderate effect on the oxygen affinity of *R. pachyptila* hemoglobin, as mean P_{50} values showed only small changes from 3° to 14°C (Fig. 1; the enthalpy of oxygenation, ΔH, was −9.2 kcal/mole for Rose Garden animals, 3° to 14°C; and −7.7 kcal/mole for Garden of Eden animals, 3° to 14°C). This reduced response of hemoglobin to temperature has been reported in other worms containing hemoglobin in solution, notably *Arenicola* (ΔH = −5.3 kcal/mole) (*8*). Oxygen equilibrium curves at 25° and 30°C showed a difference in response of the whole blood from animals from the two different vent areas to temperature. These data are reported but are difficult to interpret, since they are limited in quantity (Fig. 1).

Oxygen binding by the vascular blood showed a high degree of cooperativity, indicating considerable heme-heme interaction that would facilitate the unloading of oxygen from the hemoglobin molecule at low partial pressures of oxygen (mean Hill cooperativity coefficient, n, was 2.8 ± 0.2; 95 percent C.I., n = 11) (*9*). Reversible oxygen binding may also be augmented in this animal by the varied temperature environment in which it lives. At Rose Garden the water at the base of the worms may be as warm as 22°C, which would produce a lower affinity of hemoglobin for oxygen in the posterior portion of the worm (the location of the trophosome organ), whereas water at the plume may be less than 10°C, allowing increased oxygen affinity where oxygen is loaded (*3*).

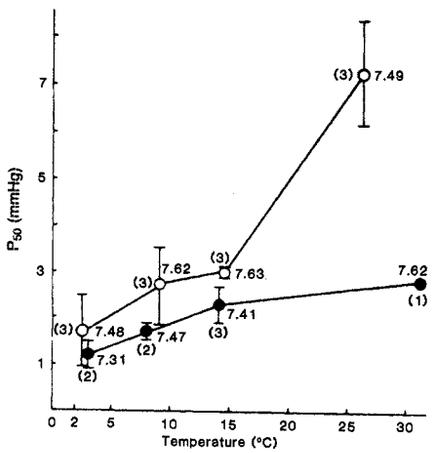

Fig. 1. Oxygen affinity of whole, vascular blood as a function of temperature; data points are means ± standard deviation: (●) Rose Garden and (○) Garden of Eden; numbers in parentheses are numbers of animals tested; *p*H values of oxygen saturated blood samples are included over or under the data points. Oxygen equilibrium curves were generated in the absence of CO_2.

Absorption spectra of dilute fresh blood from one Rose Garden specimen and three Garden of Eden specimens showed an absorbance pattern typical of hemoglobin, with α and β peaks at wavelengths similar to those of annelid worms [at *p*H 7.8, λ (Soret) = 415 nm; λβ = 540 nm; λα = 576 nm]. There was no indication of methemoglobin, as the α bands were consistently greater or equal to the β bands, and there was no peak near 635 nm. The spectra of the fresh blood also showed no presence of sulfhemoglobin, as there was no absorbance peak around 620 nm.

In a report on the oxygen binding characteristics of the respiratory pigment of an animal related to the vent vestimentiferan worm (*10*), the oxygen equilibrium curve for the hemoglobin of *Siboglinum ekmani*, a deep-sea pogonophoran, was generated spectrophotometrically with the use of a highly dilute hemoglobin solution (approximately $1 \times 10^{-6}M$ heme in $0.1M$ phosphate buffer, *p*H = 6.5) showing a P_{50} value of 0.085 mmHg at 20°C (*10*). To compare the oxygen affinity of these two pogonophorans' blood and to check the accuracy of the Hem-O-Scan at low partial pressures of oxygen, oxygen equilibrium curves of dilute blood were produced with the Hem-O-Scan at 25°C and compared to oxygen equilibrium curves from tonometer spectrophotometric studies of dilute blood. Agreement was good and showed a substantial increase in oxygen affinity upon dilution, probably indicating that the hemoglobin molecule is breaking up into subunits, as further supported by the decrease in cooperativity with dilution (Table 1).

The stability of fresh blood over time suggests that the subunit dissociation is a product of dilution and that the fragility of this hemoglobin molecule reported by others occurs only in dilute solutions (*5, 11*). The high affinity of dilute vestimentiferan hemoglobin may explain the difficulty in deoxygenating dilute solutions as reported by Terwilliger *et al.* and suggests that the moderately high affinity of the whole blood measured with the Hem-O-Scan is more representative of actual hemoglobin function in the animal than would be higher affinities generated with the dilute solutions necessary for traditional spectrophotometric methods (*5*). This large dilution effect indicates that the high affinity reported for *S. ekmani* hemoglobin may be due to the dilute samples used.

The oxygen carrying capacity of the whole blood was determined by a modified Scholander technique, and five determinations were made for each specimen, except in two cases when the size of the sample was too small (*12*). The oxygen carrying capacity of *R. pachyptila* is high for an invertebrate and reflects the presence of a concentrated respiratory protein. Similarly high oxygen carrying capacities have been reported in worms containing hemoglobin in solution (up to 11 ml of O_2 per 100 ml of blood for *Arenicola* and up to 23 ml of O_2 per 100 ml of blood for *Terebella*) (*13*). The vascular blood of *R. pachyptila* has a higher oxygen carrying capacity than coelomic fluid, reflecting a higher hemoglobin concentration. Oxygen carrying capacity was 8.4 ± 1.8 ml of O_2 per 100 ml of blood (mean ± standard deviation, n = 5) for the vascular blood, and 5.6 ± 1.4 ml of O_2 per 100 ml of blood (mean ± standard deviation, n = 4) for the coelomic fluid ($.02 < t < .04$) (*14*).

This high oxygen carrying capacity might allow this animal to rely on stored oxygen reserves during periods of low oxygen. Approximate calculations based on preliminary respiratory rate for *R. pachyptila* indicate the total oxygen stored in an animal that was 80 percent coelomic fluid would only be enough to

Table 1. Oxygen affinity and cooperativity of vascular blood and coelomic fluid of one specimen of *Riftia pachyptila* at varying dilutions. Oxygen dissociation curves were constructed from previously frozen blood samples (25°C, P_{CO_2} = 0 mmHg). Dilutions were made with $0.1M$ phosphate buffer, *p*H = 6.4. All data were collected with the Hem-O-Scan except as indicated.

Hemoglobin as heme (M)	P_{50} mmHg	n*
Vascular blood		
Undiluted†	4.5	3.1
8.7×10^{-4}	4.5	2.8
4.4×10^{-4}	3.5	2.6
1.7×10^{-4}	2.2	2.5
8.7×10^{-5}	2.4	2.2
4.4×10^{-5}	1.6	2.4
2.2×10^{-5}	1.0	1.2
2.2×10^{-5}‡	0.8	
Coelomic fluid		
Undiluted§	4.5	2.4
8.7×10^{-4}	3.5	2.3
4.4×10^{-4}	3.3	2.2
1.7×10^{-4}	2.7	2.0
8.7×10^{-5}	2.2	1.7
4.4×10^{-5}	1.1	1.9
2.2×10^{-5}	1.2	

*Hill cooperativity coefficient. †Undiluted vascular blood had a concentration of $4.4 \times 10^{-3}M$ heme. ‡This value was calculated from a curve produced by spectrophotometric methods, tracing the spectrum from 650 to 380 nm. §Undiluted coelomic fluid had a concentration of $2.3 \times 10^{-3}M$ heme. [Comparable data collected and contributed by J. B. Wittenberg showed that a dilution of $2 \times 10^{-5}M$ heme had a P_{50} value of 0.7 mmHg, n = 2.5, and a dilution of $1.3 \times 10^{-5}M$ heme had a P_{50} value of 0.7 mmHg, n = 2.8 (at 24°C, P_{CO_2} = 0 mmHg). Dilutions were made in $0.05M$ potassium phosphate buffer with 1 mM EDTA, *p*H = 7.5.]

last 20 minutes (*15, 16*). Clearly this animal cannot be utilizing its stored oxygen as a long-term reserve, but may be drawing on it to withstand the short periods of anoxia encountered in the rapidly fluctuating vent environment. The high metabolic rate of *R. pachyptila* and the high concentrations of hemoglobin in its blood may reflect a high demand for oxygen for H_2S metabolism.

The high oxygen carrying capacity, high hemoglobin cooperativity, and high oxygen affinity of the whole blood of *R. pachyptila* should enable the animal to load oxygen into the blood and to transport it to the trophosome tissue at a substantial rate. This high concentration of oxygen in the blood may also serve as a short-term oxygen store for use during adverse conditions. The moderate thermal stability of the hemoglobin oxygen affinity is useful as the pigment is thermally tolerant enough so that temperature changes will not radically alter its properties, and yet it is sensitive enough so that the higher temperatures, which are found at the base of the animal, may allow for enhancement of oxygen unloading at the trophosome. The small interaction between CO_2 concentration and oxygen affinity is of adaptive value for an animal which must transport CO_2 to the tissues along with oxygen. In summary, the blood of *R. pachyptila* has a combination of characteristics that would be supportive of a chemoautotrophic animal in an unusually variable environment.

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16. With a respiratory rate of 0.13 ml of O_2 per 100 g (wet weight) per hour, a hypothetical, 100-g animal would have an oxygen consumption rate of 13 ml of O_2 per hour. If 80 percent of the animal is coelomic fluid with an oxygen carrying capacity of 5.4 ml of O_2 per 100 ml of blood, the total amount of oxygen the animal would be able to store would be 4.5 ml (80 percent × 5.4 ml of O_2 per 100 ml of blood). With a respiratory rate of 13 ml of O_2 per hour this is only enough O_2 to last about 20 minutes (4.5 ml of O_2 per 10 ml of O_2 per hour = 0.35 hour, ~ 20 minutes).
17. This research was carried out with the following vessels funded by NSF: D.S.R.V. *Alvin*, R.V. *Lulu*, and R.V. *New Horizon*. It was supported by NSF grants OCE78-08852 and OCE78-08933 to J.J.C. and OCE78-10458 to J. F. Grassle. This work was made possible by the physical and intellectual efforts of many people, including the captains and crews of the vessels named above. In particular we thank J. F. Grassle for serving as chief scientist and R. Ballard for help in locating the vents. We also thank R. Hollis, G. Ellis, K. Smith, A. Arsenault, S. Witherow, G. Somero, and H. Felbeck for help and encouragement. M. L. Jones was helpful with the collection of blood and advice on the anatomy and taxonomy of the vestimentiferan worm. T. J. Mickel has made many contributions. J. B. Wittenberg contributed the data cited above and read an early draft of the manuscript. This report is contribution No. 10 of the Galápagos Rift Biology Expedition.

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Hemoglobin Kinetics of the Galápagos Rift Vent Tube Worm *Riftia pachyptila* Jones (Pogonophora; Vestimentifera)

Abstract. *Kinetics of the reactions of* Riftia pachyptila *hemoglobin with oxygen were followed spectrophotometrically by stopped-flow and laser flash photolysis techniques. The rate of oxygen dissociation increases eightfold over the range of 5° to 20°C* ($k = 2.2\ sec^{-1}$ *at 10°C). Oxygen recombination after flash photolysis was biphasic. The rates of both slow and fast phases of the reaction were independent of temperature from 0° to 20°C* ($k'_{fast} = 7 \times 10^6$; $k'_{slow} = 1 \times 16^6\ liter\ mole^{-1}\ sec^{-1}$). *As the oxygen affinity is relatively temperature independent, analysis in terms of the two-state model of cooperativity requires that the conformational equilibrium constant* L *decrease by about 50-fold between 3° and 15°C.*

Submarine thermal springs and hydrothermal vents along the central valley of the Galápagos Rift, at a mean ocean depth of about 2500 m (*1*), are the sites of numerous animals. Conspicuous among these is the tube worm, *Riftia pachyptila* Jones (*2*), belonging to the order Vestimentifera of the phylum Pogonophora, which exceeds 1.5 m in length and lacks both mouth and gut (*2*). The blood in these worms functions primarily as a carrier of oxygen and hydrogen sulfide to the trophosome where symbiotic bacteria generate energy by the oxidation of hydrogen sulfide (*3–6*).

Electron microscopy reveals that the hemoglobin molecules are two-tiered hexagonal arrays of submultiples of molecular weights 15,000 and 30,000, typical of annelid hemoglobins (*7, 8*). The expected molecular weight is about 3×10^6 but *Riftia* hemoglobin seems to dissociate in dilute solution, the apparent molecular weights being 1.7×10^6 and 0.4×10^6 (*7*).

The oxygen affinity is moderately high, is independent of *p*H and carbon dioxide concentration, and is weakly dependent on temperature (*6*). Oxygen binding is cooperative with a Hill constant of $n = 2.5$ to 3.0 (*6*). We examine here some of the reactions of *Riftia* hemoglobin with oxygen.

A specimen of *Riftia pachyptila* (USNM 59968) was collected by the submersible *Alvin* on 15 December 1979. Blood was drawn from the dorsal vessel and was found to contain 5200 μmole of heme per liter (*9*). The heme content of the blood is equivalent to an oxygen binding capacity of 11.6 percent (by volume), about half that of human blood (*6*).

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