Integrated Function of the Respiratory Pigment Hemocyanin in Crabs

LOUIS E. BURNETT
Department of Biology, College of Charleston, Charleston, South Carolina 29424

SYNOPSIS. A large number of factors are now known to influence the oxygen affinity of hemocyanins in crabs. In this article I review several case studies in which a number of different variables, including pH, CO₂, calcium ions, lactate, urate, dopamine, and temperature, act together on hemocyanin oxygen affinity to bring about adaptive changes in oxygen transport. These changes act in tandem with ventilatory and cardiovascular adjustments to stabilize oxygen uptake during hypoxia or to increase oxygen uptake during exercise in a swimming crab. It is important, however, that the effects of the different modulators be examined at very low concentrations, where their effects are large, to assess their importance in resting crabs where their concentrations may be low. Temperature effects among hemocyanins in crabs are variable and the magnitude may be inversely related to the magnitude of the Bohr effect. Several examples are presented of acclimation effects where oxygen affinity increases significantly at cold temperatures. The acclimation response may be important in partially regulating the metabolic responses of crabs to seasonal changes in temperature.

INTRODUCTION

The number of papers published on the hemocyanins has greatly increased in recent years. Interest in the crustacean hemocyanins has risen dramatically since the publication of a short paper by Tru الفت in 1980 that demonstrated that L-lactate at physiological concentrations increased oxygen affinity in the crab Carcinus maenas. This exciting discovery indicated that the oxygen binding characteristics of the hemocyanins, much like the hemoglobins, were affected by organic molecules. Furthermore, analysis of dialyzed samples of hemocyanin made it clear that other factors underscribed were controlling oxygen affinity. These findings led investigators to search for the mechanisms by which L-lactate interacted with and controlled oxygen affinity (Graham et al., 1983) and to search for the identity of the "unknown factors" controlling oxygen affinity (e.g., Morris et al., 1985). The functional importance of these findings and their placement in a historical context have been reviewed recently by Morris (1990).

In this paper I will focus on some of the variables that allow hemocyanin to function as part of a set of integrated cardiovascular and ventilatory responses of crabs to environmental perturbations. I will do this by examining a number of situations that occur in crabs where our information is fairly complete. These "case studies" will illustrate how a single factor can affect the oxygen affinity of hemocyanin in a way that either opposes or supports the effect of one or more other factors. Mention will also be made of how the oxygen binding properties of hemocyanin act in tandem with adjustments in cardiac output to achieve an adaptive result. I will ignore the effects of these variables on the cooperativity of oxygen binding, not because they are unimportant, but because the effects, where they occur, are complex and their function less well understood (e.g., Morris and Bridges, 1989).

438
Establishing Oxygen Affinity in "Resting" Crabs

Organic factors such as lactate and urate may or may not affect oxygen affinity (Morris, 1990). When oxygen affinity is sensitive to a variable, however, it is important to appreciate the magnitude of this sensitivity as well as the distribution of the sensitivity as a function of concentration. In most cases where this has been documented, the greatest sensitivities, i.e., the largest changes in oxygen affinity, occur at the lower "factor" concentrations. Thus, the oxygen affinity of crab hemocyanin in the "normal" or "resting" animal is indeed established by the presence of a factor that is present at a low concentration. For example, Lailler and Truchot (1989) present a bar graph showing the contribution of lactate, urate and pH in modulating oxygen affinity in crabs exposed to hypoxia. The bar graph shows in Figure 1 is adapted from Lailler and Truchot (1989) and shows clearly that all three variables are important in bringing about an adaptive increase in oxygen affinity during hypoxia. However, it should also be recognized that lactate and urate play an important role in establishing the oxygen affinity of the hemocyanin in normoxic animals. This situation is represented for crabs held in normoxia in Figure 1 by showing that low levels of lactate and urate serve to raise oxygen affinity to its measured value. It is entirely possible that minor changes in the concentrations of these factors in the normoxic crab may have a quantitatively greater effect than the environmentally stimulated change.

Variables Affecting Oxygen Affinity

Even a cursory survey of the literature reveals that a large number of factors affect hemocyanin oxygen affinity in crabs. In this article I will focus on an important subset of these factors to illustrate the integrated effects of modulators of hemocyanin to bring about important and adaptive changes to oxygen affinity when the oxygen transport system is stressed.

I will present three different case studies to illustrate the functional importance of
some of the variables listed in Table 1. The case studies will focus on the blue crab Callinectes sapidus for which there exists a large body of information. To illustrate the integrative properties of hemocyanin in C. sapidus I have chosen three situations that challenge the oxygen transport system: 1) chronic hypoxia, 2) air exposure, and 3) exercise. I will then discuss the different patterns of responses of hemocyanin to both acute and long-term effects of temperature. Finally, I will explore briefly the importance of dopaminine, recently found to modulate oxygen affinity, on oxygen transport.

CHRONIC HYPOXIA

Crabs respond typically to short-term hypoxia by increasing the ventilation of waste over their gills and this has been shown to induce an adaptive alkalosis in the hemolymph (Truchot, 1975; Burnett and Johannsen, 1981; Lallier and Truchot, 1989). The blue crab C. sapidus is no exception to this pattern. Penn and deFur (1986) demonstrated that C. sapidus underwent a sustained respiratory alkalosis for 5 days when exposed to moderate hypoxia. The alkalosis is helpful to crabs with normal Bohr shifts in maintaining the oxygenation of post-brachial hemolymph when PO2 is falling. A separate study (deFur et al., 1980) on the same species showed no evidence of an alkalosis after 7 and 25 days of moderate hypoxia. Reasons for the different results obtained in the two studies (Penn and deFur, 1986; deFur et al., 1990) are unclear. However, a number of other important changes occurred in the 7 and 25 day study that illustrate adaptive adjustments in the properties of the oxygen transport system. There was a significant increase in the concentration of hemocyanin circulating in the hemolymph after 25 days from 3.1 ± 0.4 g/100 mL to 4.4 ± 0.1 g/100 mL. In addition, oxygen affinity was increased by several factors. Hemocyanin lactate and urea concentrations increased slightly resisting in a small increase in oxygen affinity of less than 1 torr for each modulator. Hemocyanin calcium concentration also increased, and deFur et al. (1990) maintained that this also results in an increase in oxygen affinity.

The most interesting result of this study, however, is the intrinsic change in oxygen affinity due to a change in the subunit composition of the hemocyanin. Hemocyanin in C. sapidus can be separated electrophoretically into 6 distinct monomers (Kang and Raineer, 1988). The contributions of each subunit to the total number of subunits can therefore be quantified. Shifts in the concentrations of individual subunits can result in changes in the overall oxygen affinity of the pigment. deFur et al. (1990) report that the levels of subunits 3, 5, and 6 decrease, bringing about an increase in oxygen affinity of about 5 torr.

The results of all of these changes is a net movement of the oxygen equilibrium curve upward as a result of the increase in hemocyanin concentration and to the left as a result of intrinsic and extrinsic factors (Fig. 2). While measurements of prebranchial PO2 during chronic hypoxia are unavailable, the values are likely to be low. All these factors act in concert to maintain levels of oxygen uptake similar to those found in the normoxic animal as shown by the results of the product of cardiac output and the post-brachial-prebranchial oxygen concentration difference (Fig. 2). Thus, during sustained moderate hypoxia oxygen uptake is regulated by a combination of adjustments in convection, i.e., circulation and ventilation, and in the chemical properties of the oxygen transporter.

AIR EXPOSURE

Many crabs are exposed to bouts of air exposure whether they are uncovered by an outgoing tide, actively seek to move into the air, or both (Burnett and McMahon,
The responses of crabs and a variety of other organisms are similar in that CO₂ tends to accumulate in the hemolymph (Burnett and McMahon, 1987; Burnett, 1988). In crabs the accumulation of CO₂ leads to an acidosis which is almost always compensated to some degree. Recently, Maughan and Burnett (1986) surveyed a number of hemocyanins, including crab hemocyanins, and determined that some pigments had a specific sensitivity to CO₂ while others were insensitive. The possibility exists, therefore, that a CO₂-sensitive pigment plays a role in preserving the respiratory function of hemocyanin during air exposure when PCO₂ rises.

I will next present another case study that is somewhat atypical to illustrate how a CO₂ sensitive hemocyanin may be adaptive to a crab that is air exposed. In my atypical example I again use the blue crab, C. sapidus. C. sapidus is not noted for its ability to venture onto land. However, as a commercial commodity the blue crab is well known to be able to survive hours and days when air exposed on its way to the market. Survival is especially high when the animals are kept cool. I have observed another species of Callinectes, Callinectes bocconius in the Gulf of California dash out of shallow tide-pools onto land in a seconds-long pursuit of small fiddler crabs. While it is possible that specimens can be isolated on dry land for longer periods, such situations probably represent the exception and not the rule. Nevertheless, the case study of C. sapidus is instructive.

When C. sapidus is exposed to air for 4 hr, hemolymph lactate increases and pH decreases (deFur et al., 1988). The increase in lactate does not usually accompany air exposure in crabs (Burnett, 1988). Postbranchial hemolymph PCO₂ decreases significantly from about 100 torr to 24.4 torr and while PCO₂ was not measured in this study, it is safe to assume that it increases in a pattern similar to the other crabs studied (Fig. 3). The large change in hemolymph pH from 7.7 to 7.5 upon emersion would, in isolation, result in a large right shift of the oxygen equilibrium curve (dotted line in Fig. 3). However, a pronounced lactate effect shifts the curve back to the left. In addition, the oxygen affinity increases in the presence
of molecular CO₂ in this species (Mangum and Burnett, 1988). Assuming that hemolymph PCO₂ increases to about 10 torr (see examples in Burnett, 1988), then P_{IO} decreases (oxygen affinity increases) by about 3 torr. Therefore, the modulating effect of both CO₂ and lactate return the oxygen equilibrium curve (dashed line in Fig. 3) to approximately the pre-emersion position. In the case of the blue crab, resisting a decrease in oxygen affinity allows the circulating hemolymph to continue to be well oxygenated despite the decline in postbranchial hemolymph P_{O₂}. Clearly, in the blue crab, there is some disruption of oxygen supply to the tissues as evidenced by the small increase in hemolymph lactate. deFur et al. (1988) have shown that gill lamellae collapse onto one another during air exposure removing them as effective gas exchange organs and perhaps altering or disrupting the flow of hemolymph through the gills. The disruption of access to oxygen of the normally well-ventilated and well-oxygenated gills may result in the production of small amounts of lactate. On the other hand, if the gills are an important site for the oxidation of lactate (Morris and Greenaway, 1989), the reduction of circulation through the collapsed gills during emersion might easily result in the accumulation of small amounts of lactate in the hemolymph. It would be interesting to measure oxygen uptake in emersed blue crabs.

**Exercise**

One of the best examples of the integrated response of the oxygen transport system to a challenge is in swimming blue crabs. *Callinectes sapidus* is known for its ability to migrate long distances (e.g., Joly and Dudley, 1970). When *C. sapidus* is stimulated to swim in the laboratory, a severe acidosis in the hemolymph is observed where the prebranchial pH changes from 7.6 to 7.1 (Booth et al., 1982). This acidosis is thought to arise largely from the metabolic production of lactic acid, which increases from 0.7 to 9.8 mmol × liter^{-1} after 30 minutes of sustained swimming. The effect of the acidosis on decreasing the hemocyanin oxygen affinity (dotted curve in Fig. 4) is offset largely by the significant increase in hemolymph lactate, which increases the oxygen affinity. The lactate effect greatly attenuates the otherwise large right shift in the oxygen equilibrium curve (dashed curve in Fig. 4). Hemocyanin continues to be highly satu-
rated with oxygen during exercise. The large venous reserve is tapped effectively, and this, in combination with an increase in cardiac output, results in a large increase in oxygen delivery. The integrated response of the mechanical and chemical components of the oxygen transport system can best be seen in Figure 4 by comparing the product of cardiac output and the postbranchial-prebranchial oxygen concentration difference, i.e., the surface areas (~oxygen uptake) before and immediately after exercise. This illustration depicts the mechanisms by which the large increase in oxygen uptake during swimming is accomplished. While, postbranchial PaO₂ decreases slightly, the hemocyanin remains 84% oxygenated. Prebranchial PaO₂ on the other hand, remains the same. In this case, the small net shift of the oxygen equilibrium curve to the right functions to tap the venous oxygen reserve. The larger postbranchial-prebranchial oxygen concentration difference during exercise coupled with a significant increase in cardiac output serves to increase oxygen uptake by 2.6 times.

**TEMPERATURE**

Nearly all crabs encounter temperature changes in their environment. The responses of the oxygen transport system to temperature changes can occur easily in each of the three case studies mentioned above. And yet few studies have dealt directly with the effects of temperature integrated with other changes in environmental variables. There are enough studies available on the effects of temperature on hemocyanin oxygen affinity that some generalities of temperature effects independent of other effects can be made.

The influence of temperature on hemocyanin oxygen affinity is somewhat variable in crabs. However, when the phenomenon is measured in crabs over a sufficiently large temperature range, some patterns are discernible. Four general responses to acute temperature changes have been documented: 1) temperature insensitivity, e.g., Burnett et al. (1988), 2) temperature sensitivity with decreasing sensitivity as temperature increases (Fig. 5A), e.g., Burnett et
The functional meaning of the different patterns is not yet clear. There may be an inverse relationship between the magnitude of temperature sensitivity and the magnitude of the Bohr effect (Burnett et al., 1988). These authors suggest that this may be important to minimize the effect of temperature due to the thermal sensitivity of hemolymph pH. The result of this interaction is to minimize the change in oxygen affinity due to temperature.

The oxygen binding properties of hemocyanin are also influenced by acclimatization temperature. Mauro and Mangum (1982) have shown that the oxygen affinity of *C. sapidus* hemocyanin varies seasonally. The oxygen affinity of “winter” crabs is significantly higher than the oxygen affinity of “summer” animals at a common temperature. These authors speculated that the lower oxygen affinity of the stummer animals plays an important role in the natural history of the animal in downshifting its overall metabolism to the “hibernating” state with the onset of winter temperatures (7°C). They argued that in the absence of the ability of the crab to make cardiovascular and ventilatory adjustments to change hemolymph P02, less and less oxygen is transported by hemocyanin as temperatures begin to fall resulting in lower rates of oxygen uptake.

With the shift to the higher oxygen affinity “winter” hemocyanin and the lower hemolymph PO2, one would predict that oxygen uptake could be maintained. But, as Mauro and Mangum (1982) state, “the oxygen affinity of the carrier remains so high that very little oxygen can be extracted by the tissue” and oxygen uptake at 5°C is only 9% of what it is at 23°C.

During the spring, the high affinity “winter” hemocyanin becomes quantitatively important as temperature and hemolymph PO2 rise (Mauro and Mangum, 1982). As temperatures continue to rise, however, there is a gradual decrease in the intrinsic oxygen affinity to “summer” hemocyanin. This change assures a functional oxygen equilibrium curve with an adequate venous reserve of oxygen at 20°C.
DOPAMINE

Finally, the recent discovery by Morris and McMahoon (1989) that dopamine can be an important modulator of hemocyanin oxygen affinity has broadened further our definition of an integrated response of an oxygen transport system in crabs. Morris and McMahoon (1989) demonstrated that dopamine increases oxygen affinity in the hemocyanin of the Dungeness crab Cancer magister at concentrations that are presumably physiological. This effect adds to the lactate effect in this species and presents the possibility of an additional method of physiological control of hemocyanin oxygen affinity. The importance of dopamine as a modulator will become clear with the documentation of its precise physiological levels in the hemolymph and how these levels change in response to stressors. Morris and McMahoon (1989) speculate that dopamine, in addition to its interaction with hemocyanin, may stimulate ventilation and circulation. Thus, the importance of dopamine may lie in its great potential to enhance the chemical properties of the oxygen transport system and also affect directly the dynamics of the hemolymph and water convection systems.

SUMMARY

We now know of many variables that are important in rendering the oxygen binding properties of crab hemocyanins labile and responsive to change in the environment and the behavior of crabs. Complete studies of the oxygen transport systems of crabs must take into account not only the interactions between the ventilatory and cardiovascular systems of crabs, but also the thermal acclimation history and the chemical properties of hemocyanin, which may respond to a variety of extrinsic factors. In addition, environmentally induced structural modifications of hemocyanin can render interesting and adaptive changes in its intrinsic properties.

ACKNOWLEDGMENTS

The work on this manuscript was done while the author was supported by NSF Grant DCD-8802291, Physiological Processes.

REFERENCES


