



## Structure and function of hydrothermal vent crustacean haemocyanin: an update

François H. LALLIER<sup>1</sup>, Lionel CAMUS<sup>1</sup>, Fabienne CHAUSSON<sup>1</sup> and Jean-Paul TRUCHOT<sup>2</sup>

<sup>1</sup> Equipe Ecophysiologie, Station Biologique, UPMC - CNRS - INSU, BP 74, 29682 Roscoff cedex, France

Fax: (33) 2 98 29 23 24 - e-mail: lallier@sb-roscoff.fr

<sup>2</sup> Laboratoire de Neurobiologie et Physiologie Comparées, CNRS URA 1126, Pl. Dr Peyneau, 33120 Arcachon, France

### Introduction

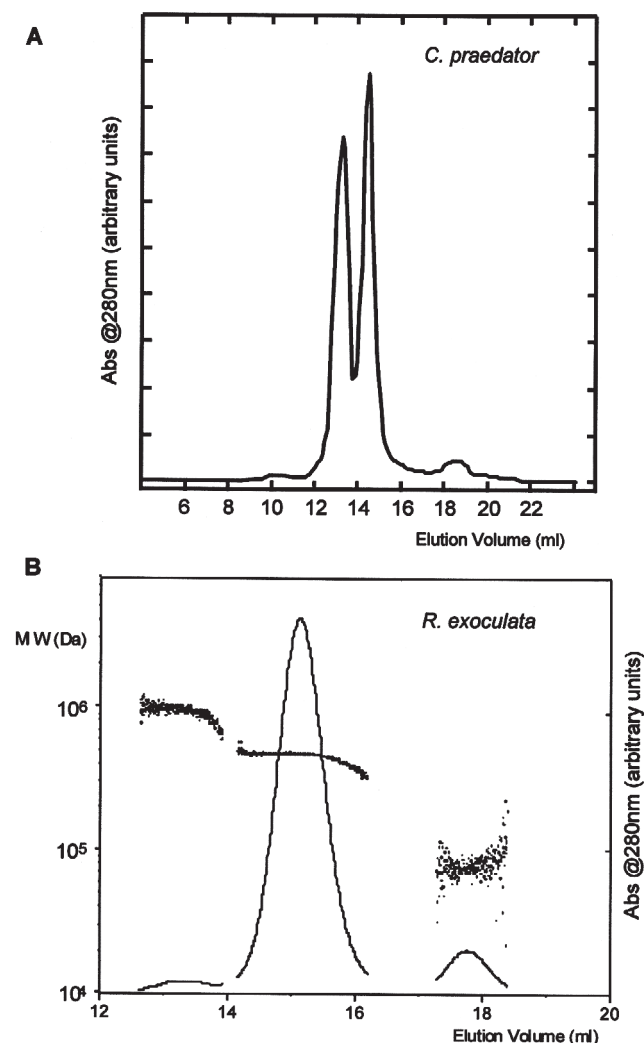
Deep-sea hydrothermal vents, first discovered in 1977, are characterized by variable and extreme conditions, in particular by a low level of oxygen, a low pH, a high temperature, a high sulphide content, and a high level of carbon dioxide. Decapod crustaceans, which are found in all the hydrothermal vents yet known at the East Pacific Rise (e.g. *Bythograea thermydron* Williams, 1980, *Cyanagraea praedator* de Saint-Laurent, 1984, *Alvinocaris lusca* Williams & Chace Jr., 1982) and at the Mid-Atlantic Ridge (e.g. *Rimicaris exoculata*, Williams & Rona 1986, *Chorocaris chacei* (Williams & Rona, 1986), *Segonzacia mesatlantica* Williams, 1988), had to develop adaptation mechanisms, in particular respiratory adaptations to survive in this environment.

The respiratory pigment of decapod crustaceans is haemocyanin that binds reversibly oxygen to a pair of copper atoms. It is a large extracellular protein, with a blue colour, which has a typical hexameric structure (Markl & Decker, 1992) and a large potential for functional plasticity (Truchot & Lallier, 1992).

### Results and discussion

Haemocyanins of *Cyanagraea praedator* and *Rimicaris exoculata*, studied by gel filtration, are composed of

hexamers and dodecamers (Fig. 1). The proportion of dodecamers in these shrimps is very low, as generally observed in members of the Caridea. For the crabs, there is a larger proportion of dodecamers but the hexameric form



**Figure 1.** Elution profiles of native haemolymph obtained from *Cyanagraea praedator* (A) and *Rimicaris exoculata* (B). Elution was performed by FPLC with a Superose 6, 30x1 cm column using a saline solution as eluant (NaCl, 400 mM, MgSO<sub>4</sub>, 32, CaCl<sub>2</sub>, 11, KCl, 3 and BTP, 50, pH 7), and monitored at 280 nm. For *R. exoculata*, the eluate was also assayed for molecular mass using a multi-angle laser light scattering (MALLS) as detailed in Lallier & Truchot, 1997. Reprinted by permission of John Wiley & Sons, Inc. All Rights Reserved.

still predominates, whereas in littoral species native haemocyanin is mostly in the dodecameric form. The subunit composition, studied by PAGE electrophoresis, differs between species but in all cases the molecular weight of the subunits ranged between 70 and 85 kDa. These quaternary structure characteristics are typical of decapod crustacean haemocyanins and no major particularity could be correlated with the hydrothermal environment.

Functional properties of haemocyanin oxygen-binding were investigated with a diffusion chamber using the step by step procedure. Values of affinity, cooperativity, Bohr effect and temperature effect of native haemolymph are presented in Table 1. For the four species, oxygen affinity is high, in particular for *C. chacei* and *B. thermydron*. The majority of non-hydrothermal species shows a lower oxygen affinity, with  $P_{50}$  values often above 10 Torr (Truchot, 1992). The Bohr effect is normal, i.e. affinity decreases with decreasing pH, and reached rather large values when compared with other crustaceans. The combination of these two properties, a high affinity and a large Bohr effect, confer an advantage to species living in potentially anoxic medium: high Hc-O<sub>2</sub> affinity allows a better extraction of oxygen whereas a large Bohr effect favours oxygen release to the tissues, assuming pH is lower in the vicinity of active tissues.

**Table 1.** Affinity ( $P_{50}$ ) and cooperativity ( $n_{50}$ ) of haemocyanin measured at pH 7.5, 15°C on native hemolymph from four species of hydrothermal decapod crustaceans. The effect of protons (Bohr effect,  $\Delta \log P_{50}/\Delta \text{pH}$ ) is measured at 15°C for the pH range 7.2-7.8, and the effect of temperature (apparent heat of oxygenation,  $\Delta H$ ) at pH 7.5 between 15 and 35°C. The effect of lactate is measured at 15°C with lactate concentrations varying from 0 to 10 mM.

|                              | $P_{50}$<br>(Torr) | $n_{50}$ | Bohr<br>effect | $\Delta H$<br>(kJ.mol <sup>-1</sup> ) | Lactate<br>effect |
|------------------------------|--------------------|----------|----------------|---------------------------------------|-------------------|
| <i>Rimicaris exoculata</i>   | 6.8                | 1.2      | -1.8           | -1.23                                 | -0.12             |
| <i>Chorocaris chacei</i>     | 1.2                | 1.2      | -1.7           | -13.96                                | -0.16             |
| <i>Cyanagraea praedator</i>  | 9.7                | 1.5      | -1.8           | +9.17                                 | -0.03             |
| <i>Bythograea thermydron</i> | 3.4                | 2.0      | -1.8           | -8.36†                                | -0.06*            |

† data from Sanders et al., 1988 obtained on a dialysed sample at pH 7.4, 2-20°C

\* data from Sanders & Childress, 1992

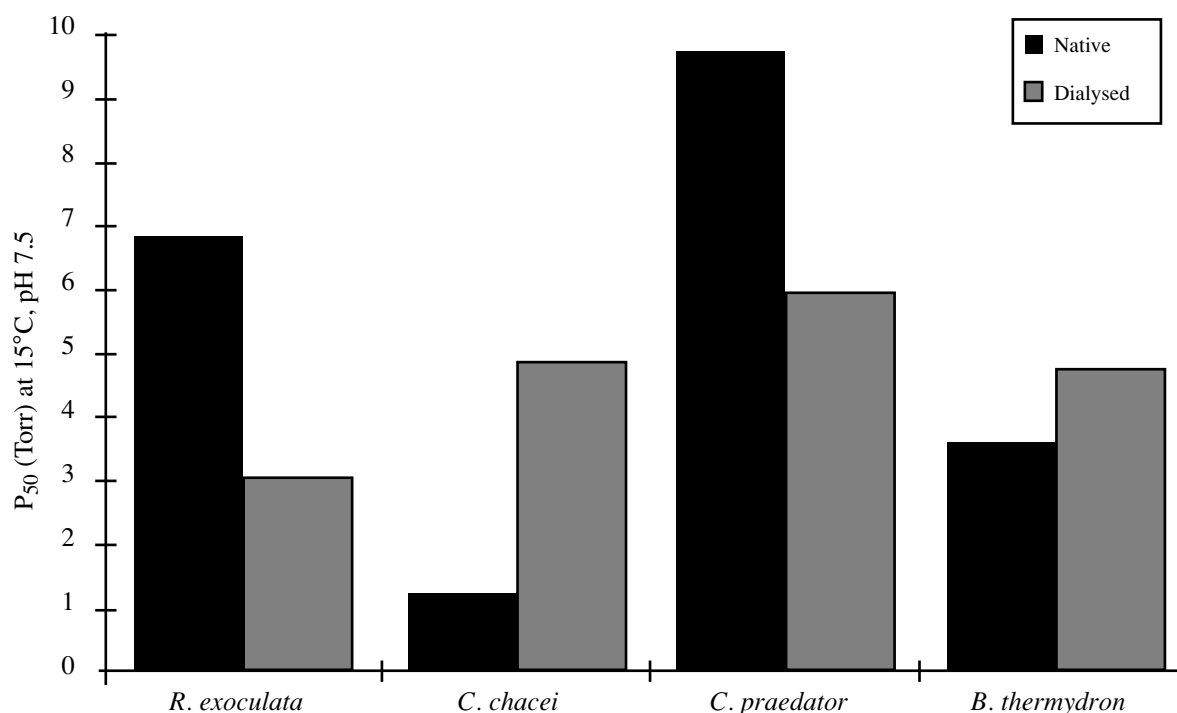
Cooperativity is in the lower range for crustacean haemocyanin (Truchot, 1992), with values of the Hill coefficient below 2 for all the species studied. Non-hydrothermal species show a higher cooperativity with the Hill coefficient generally well above 2. However, all

samples from hydrothermal species were frozen before use and it is known that freezing affects haemocyanin oxygen-binding cooperativity.

Generally arthropod haemocyanin oxygen affinity decreases with increasing temperature. The effect of temperature was studied between 15°C and 35°C for *R. exoculata*, *C. chacei* and *C. praedator* (Table 1). It appears that temperature has little or no effect on Hc-O<sub>2</sub> affinity, cooperativity and Bohr effect for *C. chacei*, *R. exoculata* and *C. praedator*. For *B. thermydron*, affinity increases between 2 and 10°C but decreases between 10 and 20°C, as reported by Sanders et al. (1988). In the hydrothermal environment, temperature and oxygen content are negatively correlated. Therefore, the loss of a temperature effect, or its reversal as observed in *B. thermydron*, could be interpreted as an advantage in an environment typified by oxygen/temperature fluctuations as at the vents.

Since a number of inorganic and organic molecules affect oxygen-binding properties of crustacean haemocyanin (Truchot, 1992), it is usual practice to study these properties on dialysed samples. In almost all species which have been studied, haemolymph dialysis induces a decrease of the Hc-O<sub>2</sub> affinity, due to the removal of L-lactate and urate for example. This is the case for *C. chacei* and *B. thermydron* (Fig. 2), in which dialysis induces an increase of  $P_{50}$  probably related to the existence of a specific effect of L-lactate (see below). However, for *R. exoculata* and *C. praedator*, dialysis brings on an increase of affinity (Fig. 2) which tends to indicate that O<sub>2</sub> affinity in native haemolymph is depressed by a low molecular weight factor, removed by dialysis. For *R. exoculata*, a partial reconstitution of dialysed haemolymph with an ultrafiltrate of native sample partially restores the initial affinity (Lallier & Truchot, 1997).

L-lactate is the end product of anaerobic metabolism in crustaceans and high levels of lactate are present in the haemolymph of hydrothermal vent crustaceans (see e.g. Lallier & Truchot, 1997). We therefore tested the effect of lactate on haemocyanin-O<sub>2</sub> affinity (Table 1). For *C. chacei*, *R. exoculata* and *B. thermydron*, the specific effect of lactate is significant and independent of pH variations. For *C. praedator*, L-lactate seems to have no effect on O<sub>2</sub> affinity. As long as in vivo determination of Hc-O<sub>2</sub> affinity and lactate concentrations are missing, the adaptative value of a lactate effect can only be speculated. However, environmental hypoxia is one of the situations in which an increase of lactate concentration has been shown to regulate in-vivo oxygen transport in the haemolymph of shallow water crabs (Lallier & Truchot, 1989). Therefore, a lactate effect, when present, could help maintain proper oxygenation of the haemolymph at the gill level in the hypoxic vent environment.



**Figure 2.** Effect of dialysis on haemocyanin-oxygen affinity of *Rimicaris exoculata*, *Chorocaris chacei*, *Cyanagraea praedator* and *Bythograea thermydron*. Dialysis buffer composition is derived from actual measurements of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup> in native haemolymph. Measurements were made at pH 7.5, 15°C.

Additional cofactors may play an adaptive role in hydrothermal vent crustaceans. Indeed, Sanders & Childress (1992) have shown that, in *B. thermydron*, thiosulphate increases Hc-O<sub>2</sub> affinity. This effect has been interpreted as a specific adaptation to the high-sulphide and low-O<sub>2</sub> concentrations of the hydrothermal vent environment. However, to date, it has not been assayed in other hydrothermal vent crustaceans.

## Conclusion

Although in vivo data would be necessary to infer true physiological adaptations to the vent environment, it is tempting to speculate from the in vitro characteristics of haemocyanin presented in this paper. The quaternary structure seems to be essentially correlated with the taxonomy of the studied species: the two shrimps (*C. chacei* and *R. exoculata*) exhibit the usual predominance of hexameric haemocyanin, whereas the crabs (*B. thermydron* and *C. praedator*) possess a larger proportion of dodecamers. The subunit composition apparently shows a similar pattern to other crustaceans (Markl & Decker, 1992) but it should be more accurately determined using modern techniques such as mass spectrometry. The functional properties of oxygen binding, however, appear to be related to the respective habitat of each species. *R. exoculata* and

*C. praedator*, which are believed to live in warmer waters, close to the vent chimneys, exhibit some common functional properties like a moderate Hc-O<sub>2</sub> affinity due to one or several unknown factors which decrease this affinity. This unique potential to lower affinity could eventually prove beneficial in the large physico-chemical gradient in which they live. Conversely, *C. chacei* and *B. thermydron* which live in cooler waters, among *Bathymodiolus* and *Riftia* respectively, exhibit an higher Hc-O<sub>2</sub> affinity due to a more pronounced lactate effect. This tentative scheme needs to be substantiated with detailed information on the microenvironment of each species, which in turn would allow in vivo experiments to be conducted in appropriate conditions.

## Acknowledgements

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