Lobster haemocyanin

Influence of acclimatization on subunit composition and functional properties

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Haemocyanin from the lobster *Palinurus elephas* has been shown to change in its subunit composition according to the time of year. In contrast, in *Palinurus mauritanicus*, a lobster living at greater depth, no seasonal changes in subunit composition have been observed. The results obtained from a set of experiments performed on some *Palinurus mauritanicus* acclimatized in an aquarium have clearly indicated that modifications of haemocyanin subunit composition may be involved in the adaptation of arthropods to environmental change.

INTRODUCTION

Haemocyanins are the O₂ transporting molecules in many arthropods and molluscs. One of the most striking features of arthropod haemocyanins is their remarkable subunit heterogeneity, which has been widely documented in the literature. Intensive studies of several haemocyanins have shown intra-specific differences between subunits in electrophoretic mobility, immunogenicity, primary structure and O₂-binding function (Bonaventura & Bonaventura, 1980; Van Holde & Miller, 1982; Markl, 1986; Markl *et al.*, 1986; Stocker *et al.*, 1986; Makino, 1988; Makino & Kimura, 1988).

On the basis of what is known for haemoglobins, different types of subunits are probably required for the development of homotropic interactions, i.e. of the co-operativity between the oxygen-binding sites. It is also possible that some of them may serve as regulatory units which bind and respond to allosteric effectors such as bivalent cations and protons.

Another possibility, suggested by Sullivan *et al.* (1974), is that the functional diversity of the subunits may provide haemocyanin with a flexibility that could be useful to adapt the molecule to environmental changes. In other words, at the genetic level, a species may be able to manufacture one or more unique haemocyanins which allow adaptation to their own particular ecological, behavioural and physiological milieu. Thus, within an animal's lifetime, haemocyanin performance may be varied, with season or with habitat changes, either by modulation of the binding property of the existing molecule (McMahon, 1986; Brix *et al.*, 1988, 1989, 1990; Burnett *et al.*, 1988) or by the manufacture of one or other variant haemocyanin.

In a previous structural and functional characterization of lobster (*Palinurus elephas*) haemocyanin (Bellelli *et al.*, 1985, 1988; Giardina *et al.*, 1986) we described changes in its subunit composition according to the animal's sex and the time of year. The sexual difference, characterized by the presence of a specific subunit in the haemocyanin from female animals, did not appear to be linked to any evident functional property of the pigment related to O₂ transport. By contrast, the seasonally dependent structural variation appeared to be paralleled by functional changes which resulted mainly in a decrease in O₂ affinity and in an increase of the co-operativity during the summer. Interestingly, preliminary results obtained with haemocyanin from *Palinurus mauritanicus*, a lobster phylogenetically related to *P. elephas*, but normally living at a greater depth (500 m as against 70 m) in the same marine region, did not show any significant sexual and

seasonal differences in subunit composition (Giardina *et al.*, 1986; Bellelli *et al.*, 1988). This difference in behaviour was tentatively attributed to the different environmental conditions in the habitat of the two species.

With the aim of testing this hypothesis, we have carried out a more detailed characterization of haemocyanin from *P. mauritanicus* lobsters freshly caught during the whole year and a set of experiments on some *P. mauritanicus* (11 animals, five of which survived for the entire observation period) which have been acclimatized in an aquarium at atmospheric pressure and room temperature for a period of 2 years.

The results, which have been compared with those previously obtained from freshly caught *P. elephas* lobsters, strongly support the hypothesis that modifications of haemocyanin subunit composition may be involved in the adaptation of arthropods to environmental changes.

A preliminary account of some of this work was presented at the Symposium on Invertebrate Dioxygen Carriers held in Leuven, Belgium, in July 1989.

MATERIALS AND METHODS

Specimens of *P. mauritanicus*, 11 in all, were caught at a depth of 500 m, brought to the Institute of Zoology, Cagliari, and placed in outdoor tanks. The water of the aquarium was cleaned and changed in the usual way. The lobsters were fed on teleosts, crustaceans and molluscs throughout the season.

Blood samples were taken regularly over the season from each animal. The results reported concern the coldest month, February (water at 13 °C), the warmest month, October (water at 18 °C), and the intermediate month, May. Blood samples from specimens caught at 500 m depth (13 °C, no light) were used as a control.

Haemocyanin was prepared as described by Bellelli *et al.* (1985) from the haemolymph of single adult lobsters. Protein concentration was calculated from the absorbance at 278 nm by using the absorption coefficient ($A_{1\text{ cm}}^{1\%}$) for *P. vulgaris* haemocyanin of 13.8 cm.

Electrophoresis of subunits was carried out on 5% (w/v) polyacrylamide gels in 50 mM-glycine/1 mM-EDTA at pH 9.6, after overnight dialysis against the same buffer (Markl *et al.*, 1979). The proportion of the bands of the stained gels was assessed by a laser densitometer 2202 Ultrosan (LKB, Bromma, Sweden).

The fractional O₂ saturation of haemocyanin was calculated from the absorbance change at 335 nm. O₂-binding isotherms

were recorded spectrophotometrically as described by Giardina & Amiconi (1981).

RESULTS AND DISCUSSION

As far as the freshly captured lobsters are concerned, in agreement with previously reported data (Bellelli *et al.*, 1988) samples of *P. mauritanicus* haemocyanin collected in different months show identical O₂-dissociation curves, as illustrated by Fig. 1, where the effect of pH on the overall O₂ affinity of haemocyanin from *P. mauritanicus* caught in different months is reported.

Moreover (see Fig. 1), *P. mauritanicus* haemocyanin displays an O₂ affinity which is higher than that of *P. elephas* haemocyanin obtained from animals caught in October, the period of the year in which this respiratory protein has the lower O₂ affinity and the higher co-operativity (Bellelli *et al.*, 1988).

In agreement with the functional results, alkaline electrophoresis of fully dissociated haemocyanin from freshly caught *P. mauritanicus* have not indicated any appreciable seasonal variability of subunit composition (Table 1).

By contrast, experiments carried out on samples extracted from single acclimatized animals indicate that the relative pro-

Fig. 1. O₂ Böhrr effect of lobster haemocyanin at 20 °C in 0.1 M-Bistris or -Tris buffer containing 10 mM-CaCl₂.

●, Haemocyanin samples from *P. elephas* captured in October of three different years; open symbols, haemocyanin samples obtained from *P. mauritanicus* captured in October (○), February (△) and May (□). In this Figure and Fig. 2, P₅₀ is the overall O₂ affinity of haemocyanin. Note: 1 mmHg ≈ 133 Pa (SI unit).

Table 1. Percentage subunit composition of *P. mauritanicus* haemocyanin collected in winter and summer from acclimatized and freshly caught lobsters

The relative proportions were obtained from a laser-densitometric analysis of stained alkaline gels.

<i>P. mauritanicus</i>	Month	Subunit composition (%) (average values)				
		I	II	III	IV	V
Captured	February	88	8	2	2	0
	October	87	8	2	2	1
From aquarium	February	77	23	0	0	0
	October	67	17	8	4	4

Fig. 2. Effect of pH on the overall O₂ affinity of lobster haemocyanins at 20 °C in 0.1 mM-Bistris or -Tris buffer plus 10 mM-CaCl₂.

(a) Haemocyanin samples collected in October from *P. mauritanicus* lobsters acclimatized in aquarium (●) and *P. elephas* lobsters freshly caught (○). (b) Haemocyanin samples collected in October from *P. mauritanicus* acclimatized in aquarium (●) and from *P. mauritanicus* freshly caught in (○) October, (△) February and (□) May.

portion of the subunits assembled into the native hexamer is strikingly variable during the year. In fact, for acclimatized animals, quantitative determinations of the various components (see Table 1) reveal the presence of five electrophoretic peaks in October and only two in February. Freshly captured lobsters, by contrast, showed no appreciable seasonal variability of subunit composition. This is in line with what has been shown by Mangum & Rainer (1988) on the subunit composition of haemocyanin from the blue crab (*Callinectes sapidus*), which is dependent on environmental factors; it has been suggested that this variation may be a way of optimizing O₂ transport.

O₂-equilibrium experiments, performed on haemocyanin samples collected from acclimatized animals, indicate that the seasonal dependence of the relative proportions of the subunits observed in the electrophoretic patterns is paralleled by functional differences. In particular, co-operativity and O₂ affinity are lower in summer than in winter.

This difference is evident from the data presented in Fig. 2(b), where the O₂ Böhrr effect of *P. mauritanicus* haemocyanin collected in October from acclimatized animals is presented in comparison with that obtained from the same types of lobsters freshly captured during the whole year. Furthermore, the Böhrr effect seems to approach that showed by *P. elephas* lobsters (see Fig. 2a).

The finding that the seasonal variability of haemocyanin from *P. mauritanicus* may be induced by keeping the animals in an aquarium is, in our opinion, of general importance, since it indicates that *P. mauritanicus* does possess the same physiological mechanisms (normally silent) as those which are operative in *P. elephas* and in *Callinectes sapidus* with respect to season, or, in a more general way, to some environmental factors, and that these mechanisms may be 'switched on' or 'switched off' depending on the external conditions.

These observations call for further elucidation of the molecular mechanism and the physiological meaning of the phenomenon.

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