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Structural properties, conformational stability and oxygen binding properties of *Penaeus monodon* hemocyanin

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Abstract

Hemocyanin sequences allineament shows the presence of highly invariant regions especially in the active site and in the tight intersubunits interaction sites. Comparing the aminoacids in contact regions between monomers is possible to interpret the stability of hexamers.

Keywords: Hemocyanin; Quaternary structure; Arthropods; Homology modelling

1. Introduction

The availability of several sequences of arthropod Hcs in the PDB has stimulated a variety of evolutionary studies and has provided, in combination with the X-ray crystallographic data, the possibility to also trace some structural patterns that are relevant for the description of tertiary and quaternary structures for Hcs that have not yet been solved. The strategy used to rationalize the high stability of Peneid Hc oligomer(s) on a structural basis involves a comparison of the amino acid positions within the crustacea Hcs, whose sequences are available in PDB, in the contact regions between subunits.

2. Materials and methods

The SwissProt and NCBI accession numbers for the complete amino acid sequences of 15 crustacean hemocyanins are: HCYA_PANIN; HCYB_PANIN; HCYC_PANIN; Q9NGL5; U48881; Q8IFT5; Q95P19; Q95P17; Q95P18; HCY_PALVU; Q9NFR6; P83180; Q8MUH8; Q26180. (Original references are available from these entries). For the elaboration and the analysis of Hc primary structures, the tools provided by ExPASy Molecular Biology Server of the Swiss Institute of Bioinformatics (http://www.expasy.ch) were used. A multiple alignment was carried out with ClustalX, and the result was controlled considering the two conservative binding-site regions.

From these alignments we were able to compare the amino acids in the positions involved in the interaction areas between monomers, which are responsible for final stability of the hexameric oligomers (Volbeda and Hol, 1989).

3. Results and discussion

The complete sequences of crustacea Hcs available in PDB have been aligned to compare the inter-subunit interaction regions. This areas have been precisely defined on the basis of the X-ray crystallographic map obtained on the *Panulirus interruptus* hexamer, made by subunits a and b (Volbeda and Hol, 1989). To explore the structural basis of the hemocyanin stability we focused on the *Penaeus vannamei* sequence, the only one available for Peneid shrimps. Table 1 was created by considering the positions for amino acids involved in interactions between pairs of subunits and the different residues present in the various crustacea Hcs. Some positions are strictly conserved (columns marked with C in Table 1), indicating that both the kind of interaction and steric factors are of crucial importance. Among these, there are positions where charged residues are involved (Asp273, Arg295, Lys360, Asp438, Arg634) as partners in ion–ion or ion–dipole interactions and positions where hydrophobic (Phe256, Pro272), polar (Asn176, His302) and polar/hydrophobic (Tyr155, Tyr304) are involved. Other positions appear to be mainly controlled by the helicogenicity and low steric hindrance of the amino acid residue (Gly255, Gly310).

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Table 1
Result of the multiple alignment of the indicated Hc sequences

Positions	I*	I	I	C	+	+	I	C	−	C	C	C	C
	59	62	64	155	159	160	161	176	254	255	256	272	273
P. interruptus sub A	E	D	R	Y	M	T	Q	N	E	G	F	P	D
P. interruptus sub B	E	D	R	Y	M	T	Q	N	E	G	F	P	D
P. interruptus sub C	D	D	R	Y	M	T	N	N	E	G	F	P	D
Callinectes sapidus	E	D	R	Y	M	T	Q	N	E	G	F	P	D
Cancer magister	E	E	R	Y	M	T	Q	N	E	G	F	P	D
P. elephas	E	D	R	Y	M	T	H	N	E	G	F	P	D
P. vulgaris sub 1	E	D	R	Y	M	T	H	N	E	G	F	P	D
P. vulgaris sub 3	E	D	R	Y	M	T	H	N	E	G	F	P	D
P. vulgaris sub 2	E	D	R	Y	M	T	H	N	E	G	F	P	D
P. vulgaris	K	D	R	Y	M	T	H	N	E	G	F	P	D
Homarus americanus	E	D	R	Y	M	T	Q	N	E	G	F	P	D
Pontastacus leptodactylus	E	D	R	Y	M	T	Q	N	E	G	F	P	D
Pacifastacus leniusculus	E	D	R	Y	M	T	Q	N	D	G	F	P	D
P. vannamei	D	D	K	Y	Q	K	Q	N	D	G	F	P	D
Positions	C	I*	C	C	C	C*	C*	I	C	I	C	I	C
	295	300	302	304	310	339	340	359	360	363	438	443	634
P. interruptus sub A	R	I	H	Y	G	Y	Y	G	K	L	D	I	R
P. interruptus sub B	R	I	H	Y	G	Y	Y	G	K	L	D	I	R
P. interruptus sub C	R	I	H	Y	G	F	Y	G	K	L	D	I	R
Callinectes sapidus	R	I	H	Y	G	Y	Y	G	K	L	D	V	R
Cancer magister	R	T	H	Y	G	Y	Y	G	K	L	D	V	R
P. elephas	R	I	H	Y	G	Y	Y	G	K	M	D	V	R
P. vulgaris sub 1	R	I	H	Y	G	Y	Y	G	K	M	D	V	R
P. vulgaris sub 3	R	I	H	Y	G	Y	Y	G	K	M	D	V	R
P. vulgaris sub 2	R	I	H	Y	G	Y	Y	G	K	M	D	I	R
P. vulgaris	R	I	H	Y	G	Y	P	G	K	M	D	V	R
Homarus americanus	R	I	H	Y	G	Y	Y	G	K	M	D	V	R
Pontastacus leptodactylus	R	L	H	Y	G	Y	Y	H	K	M	D	V	R
Pacifastacus leniusculus	R	V	H	Y	G	Y	Y	H	K	M	D	V	R
P. vannamei	R	I	H	Y	G	Y	Y	G	K	L	D	I	R

The aminoacids found in the indicated positions of various Hc are listed. For details see text. I, isofuntional substitutions; C, conserved residues; ±, gain/loss of positive charges in *P. vannamei* subunit.

Again, other positions (columns marked with I in Table 1) are also conserved as far as isofunctional residues are involved, maintaining either the charge or the dipole moment (Asp/Glu59, Arg/Lys64, Gln/Asn,His161) or the hydrophobic character (Ile/Lue/Val300, Ile/Val443). It is worth noting the presence of 'sporadic substitutions', namely substitutions that occur in one case in a position that is otherwise conserved (column C*) or isofunctional (column I*). In columns C* we have the presence of Phe (in *P. interruptus* Hc sub C) and Pro (in *Palinurus vulgaris*) in positions 339 and 340, respectively, that are occupied by Tyr both in the former and latter positions. In columns I* we have the positively charged Lys59 in *P. interruptus* Hc in a position where isofunctional negatively charged residues are otherwise present and the presence of a polar residue (Thr300, *Cancer magister*) in a position typically occupied by hydrophobic residues. The 'sporadic mutations' in C* are not likely to markedly affect the position. In contrast, the sporadic mutation in I* are more significant.

From the overall evaluation of the amino acids present in the different positions we can single out positions 159 and 160 where *Penaeus* Hc exhibits peculiar features with respect to the other proteins. In position 159, the presence of Gln in *Penaeus* against Met in the other crustacean Hcs introduces a H-bond donor group substituting a hydrophobic residue. Moreover, the presence of Lys160 versus Thr introduces a positive charge competent for an ion–ion interaction. These peculiar structural features of *Penaeus* Hc correlate with the high resistance of this protein to dissociate, *Penaeus* Hc dissociates only after denaturation, and with the high cooperativity and affinity when oxygen binding.

Reference

Volbeda, A., Hol, W.G.J., 1989. Crystal structure of hexameric haemocyanin from *Panulirus interruptus* refined at 3.2 A resolution. J. Mol. Biol. 209, 249–279.