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AMINO ACID COMPOSITION OF HEMOCYANIN OF THE CRAYFISH  
ORCONECTES LIMOSUS

Amino acid composition of hemocyanin of Orconectes limosus was studied. A high content of glutamate and aspartate was found. No differences were revealed in the amino acid composition of hemocyanins of the three crayfish species occurring in Poland viz. Astacus astacus, Astacus leptodactylus and Orconectes limosus.

Introduction

In the group of respiratory proteins, capable of reversible oxygen binding and transporting oxygen in aerobic organisms, a special role belongs to the copper protein hemocyanin.

Hemocyanin, a giant protein molecule of hemolymph of arthropods and molluscs may constitute up to 90% of hemolymph proteins and occurs in different aggregation states. The protein dissociates in alkaline media. Divalent cations affect specifically its structure and function [1, 4].

Hemocyanins are present in two animal types inhabiting both terrestrial environment (e.g. arthropod Porcelio levis) and as fresh (e.g. arthropod Astacus astacus) as marine waters. In spite of differences in living conditions, hemocyanins function in the same way in all these animals. As far as the oxygen concentration is concerned the water medium is less advantageous than terrestrial. One liter of distilled water contains 10 ml of oxygen at a temperature of 0°C and this value decreases with in-

creasing temperature and salinity. At a temperature of 20°C, one liter of marine water contains only 5 ml of oxygen.

It is assumed generally that the functional and structural variability of hemocyanins results from adaptation of one molecule to different ecological factors determining living conditions of individual animal species.

Polish fresh waters are inhabited by three crayfish species. Two of them are native: a) Astacus astacus (L.) and b) Astacus leptodactylus (Esch.). The third one, Orconectes limosus (Raf.) (Cambarus affinis Say) was brought in the beginning of this century from North America (Pennsylvania). This species was introduced in Europe after the crayfish "plague" epidemic that exterminated the two native species.

About 100 individuals of Orconectes limosus were introduced to Poland by Max von dem Borne from the Delaware river (Pennsylvania) and settled a small pond in Baranówek connected with the Myśla river, a right affluent of Odra. A next, higher amounts of these animals was brought in 1911 and settled in the basin of Wda and Brda rivers. This species as resistant to the plague were to substitute for the native species [6]. Due to its origin, Orconectes limosus is called commonly "American crayfish".

With respect to the native species, American crayfish is characterized by a smaller size and a much higher mobility. Now it has spread out into the whole territory of Poland and dislodges the other, more good tempered species.

One can suspect that while the European species origin from a common ancestor, it seems less probable for Orconectes limosus. It has been hypothesized that the fresh-water crayfish species can be derived from a marine forefather.

The aim of this paper was to compare the amino acid composition of hemocyanin of the two European species and of Orconectes limosus a species so distant from the standpoint of the original geographical distribution. Elucidation first of the amino acid composition and then of the amino acid sequence is indispensable for the understanding of functional properties of any protein.

### Material and methods

Hemolymph taken from males was kept for 12 h at 4°C for clotting. The clot was discarded and the serum was filtered through a 0.64 µm millipore filter and ultracentrifuged at 100 000 x g for 8 h in a Beckman centrifuge. Hemocyanin formed a blue sediment which was then washed twice and dissolved in a small amount of water. After 24 h dialysis against distilled water, the protein was characterized on the basis of absorption spectra and copper content.

Hemocyanin was precipitated from the solution with 5 volumes of cold acetone. Protein aliquots were subjected to acid hydrolysis with 3 N p-toluenesulfonic acid and 2% 3-/2-aminoethyl/indole [7]. Hydrolysis was run at 110°C for 22, 48 and 72 h, respectively. Amino acids in the hydrolysate were determined according to Stein and Moore [10] with a Jeol 6AH automatic analyzer.

### Results and discussion

Amino acid composition of hemocyanin of *Orconectes limosus* is shown in Tab. 1.

Table 1

Amino acid composition Hc - *Orconectes limosus*

Skład aminokwasowy Hc - *Orconectes limosus*

Аминокислотный состав гемоцианина рака - *Orconectes limosus*

| Amino acid | 22 h<br>µM/mg     | 48 h<br>µM/mg     | 72 h<br>µM/mg     | $\bar{x}$ | % weight | Amino acid residue<br>80 000 MW |
|------------|-------------------|-------------------|-------------------|-----------|----------|---------------------------------|
| 1          | 2                 | 3                 | 4                 | 5         | 6        | 7                               |
| Trp        | 0.0675<br>±0.0096 |                   |                   | 0.0675    | 1.06     | 5.40                            |
| Lys        | 0.3952<br>±0.0291 | 0.4134<br>±0.0076 | 0.3626<br>±0.0381 | 0.3904    | 6.14     | 31.23                           |

Table 1 (contd.)

| 1   | 2                 | 3                 | 4                 | 5      | 6     | 7     |
|-----|-------------------|-------------------|-------------------|--------|-------|-------|
| His | 0.4184<br>±0.0322 | 0.4131<br>±0.0076 | 0.3916<br>±0.0427 | 0.4077 | 6.41  | 32.62 |
| Arg | 0.2800<br>±0.0157 | 0.3041<br>±0.0050 | 0.3081<br>±0.0328 | 0.2974 | 4.67  | 23.79 |
| Asp | 0.8797<br>±0.1032 | 0.9459<br>±0.0213 | 0.8597<br>±0.0918 | 0.8951 | 14.07 | 71.61 |
| Thr | 0.3132<br>±0.0112 | 0.3042<br>±0.0278 | 0.2687<br>±0.1197 | 0.3400 | 5.34  | 27.20 |
| Ser | 0.3569<br>±0.0397 | 0.3452<br>±0.0185 | 0.2721<br>±0.0125 | 0.3800 | 5.97  | 30.40 |
| Glu | 0.6263<br>±0.0530 | 0.6404<br>±0.0081 | 0.6121<br>±0.0621 | 0.6263 | 9.85  | 50.10 |
| Pro | 0.3126<br>±0.0227 | 0.3166<br>±0.0030 | 0.3039<br>±0.0309 | 0.3110 | 4.89  | 24.88 |
| Gly | 0.4332<br>±0.0317 | 0.4312<br>±0.0184 | 0.4110<br>±0.0526 | 0.4251 | 6.68  | 34.01 |
| Ala | 0.3976<br>±0.0342 | 0.4231<br>±0.0049 | 0.4161<br>±0.0450 | 0.4123 | 6.48  | 32.98 |
| Cys | 0.0132<br>±0.0023 | 0.0130<br>±0.0022 | 0.0010<br>±0.0005 | 0.0091 | 0.14  | 0.73  |
| Val | 0.3223<br>±0.0214 | 0.3765<br>±0.0092 | 0.4075<br>±0.0409 | 0.3688 | 5.80  | 29.50 |
| Met | 0.0315<br>±0.0016 | 0.0283<br>±0.0024 | 0.0017<br>±0.0008 | 0.0205 | 0.32  | 1.64  |
| Ile | 0.2140<br>±0.0205 | 0.2710<br>±0.0027 | 0.3020<br>±0.0291 | 0.2623 | 4.12  | 20.98 |
| Leu | 0.4879<br>±0.0409 | 0.5256<br>±0.0017 | 0.5559<br>±0.0548 | 0.5231 | 8.22  | 41.85 |
| Tyr | 0.2282<br>±0.0098 | 0.2585<br>±0.0167 | 0.2454<br>±0.0250 | 0.2440 | 3.84  | 19.52 |
| Phe | 0.3483<br>±0.0256 | 0.3983<br>±0.0159 | 0.3936<br>±0.0406 | 0.3801 | 5.98  | 30.41 |

The data represent mean values from 3 determinations expressed as  $\mu$ moles of amino acid per mg protein, as well as the per cent abundance. The content of serine and threonine was calculated by extrapolation to zero time. Tryptophan does not suffer destruction under the above hydrolysis conditions [7].

Comparison of amino acid composition of hemocyanins of the



Table 2

Amino acid composition of Hc three crayfish species

Skład aminokwasowy Hc trzech gatunków raków

Аминокислотный состав гемоцианина трех видов раков

| Amino acid | Astacus astacus (9)     |       |                              | Astacus leptodactylus (5) |       |                              | Orconectes limosus      |       |                              |
|------------|-------------------------|-------|------------------------------|---------------------------|-------|------------------------------|-------------------------|-------|------------------------------|
|            | $\mu\text{M}/\text{mg}$ | %     | amino acid residue 80 000 MW | $\mu\text{M}/\text{mg}$   | %     | amino acid residue 80 000 MW | $\mu\text{M}/\text{mg}$ | %     | amino acid residue 80 000 MW |
| Trp        | 0.0731                  | 1.10  | 5.85                         | 0.0675                    | 1.06  | 5.40                         | 0.0675                  | 1.06  | 5.40                         |
| Lys        | 0.3699                  | 5.55  | 29.59                        | 0.3770                    | 5.90  | 30.16                        | 0.3904                  | 6.14  | 31.23                        |
| His        | 0.4144                  | 6.21  | 33.15                        | 0.4193                    | 6.56  | 33.54                        | 0.4077                  | 6.41  | 32.62                        |
| Arg        | 0.3149                  | 4.72  | 25.19                        | 0.2963                    | 4.64  | 23.70                        | 0.2974                  | 4.67  | 23.79                        |
| Asp        | 1.0522                  | 15.78 | 84.18                        | 0.9139                    | 14.30 | 73.11                        | 0.8951                  | 14.07 | 71.61                        |
| Thr        | 0.3900                  | 5.85  | 31.20                        | 0.3400                    | 5.32  | 27.20                        | 0.3400                  | 5.34  | 27.20                        |
| Ser        | 0.3600                  | 5.40  | 28.80                        | 0.3600                    | 5.64  | 28.80                        | 0.3800                  | 5.97  | 30.40                        |
| Glu        | 0.6660                  | 9.99  | 53.28                        | 0.6293                    | 9.85  | 50.34                        | 0.6263                  | 9.85  | 50.10                        |
| Pro        | 0.3302                  | 4.95  | 26.42                        | 0.3125                    | 4.89  | 25.00                        | 0.3110                  | 4.89  | 24.88                        |
| Gly        | 0.4258                  | 6.39  | 34.06                        | 0.4240                    | 6.64  | 33.92                        | 0.4251                  | 6.68  | 34.01                        |
| Ala        | 0.4079                  | 6.12  | 32.63                        | 0.4174                    | 6.53  | 33.39                        | 0.4123                  | 6.48  | 32.98                        |
| Cys        | 0.0164                  | 0.25  | 1.31                         | 0.0218                    | 0.34  | 1.74                         | 0.0091                  | 0.14  | 0.73                         |
| Met        | 0.0531                  | 0.80  | 4.25                         | 0.0443                    | 0.69  | 3.54                         | 0.0205                  | 0.32  | 1.64                         |
| Val        | 0.3707                  | 5.56  | 29.66                        | 0.4006                    | 6.27  | 32.05                        | 0.3688                  | 5.80  | 29.50                        |
| Ile        | 0.2598                  | 3.90  | 20.78                        | 0.2544                    | 3.98  | 20.35                        | 0.2623                  | 4.12  | 20.98                        |
| Leu        | 0.5022                  | 7.53  | 40.18                        | 0.4845                    | 7.58  | 38.76                        | 0.5231                  | 8.22  | 41.85                        |
| Tyr        | 0.2555                  | 3.83  | 20.44                        | 0.2333                    | 3.65  | 18.66                        | 0.2440                  | 3.84  | 19.52                        |
| Phe        | 0.4059                  | 6.09  | 32.47                        | 0.3920                    | 6.14  | 31.36                        | 0.3801                  | 5.98  | 30.41                        |

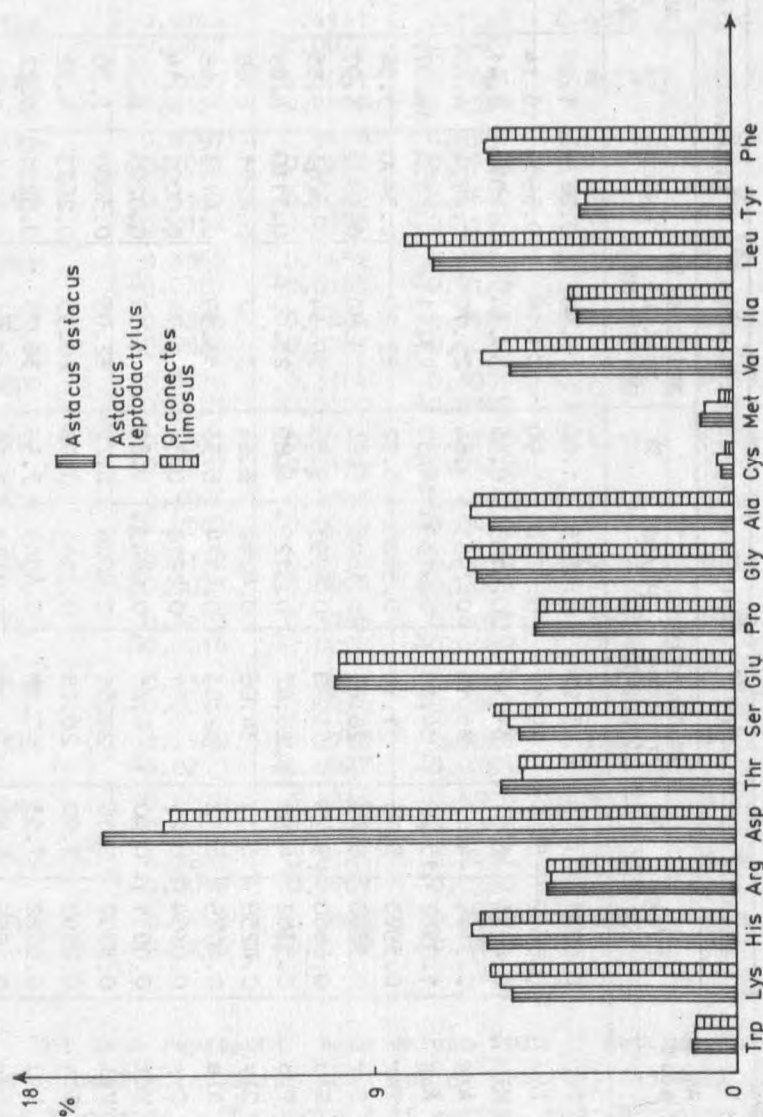


Fig. 1. Per cent content of amino acids for hemocyanin of three crayfish species  
 Procentowa zawartość aminokwasów w hemocyjaninie trzech gatunków raków  
 Процентный аминокислотный состав гемоцианина трех видов раков

three crayfish species is given in Tab. 2. The data are mean values for 3 hydrolysis times.

Results of Tab. 2 are demonstrated graphically in Fig. 1.

No significant differences in amino acid composition were found between hemocyanins of the three species studied. Acidic amino acids viz. glutamate and aspartate were dominating in all cases (about 25%). The next most abundant amino acids were: glycine, alanine, leucine and phenylalanine. Sulfur amino acids were present in smallest amounts. The amino acid composition of crayfish hemocyanins does not differ therefore from that reported hitherto for other animal species [2, 3, 8, 11].

Table 2 shows, too, the number of amino acid residues per smallest functional subunit of hemocyanin (5 S) of estimated molecular weight 80 000 D. This subunit is formed by 509 amino acid residues.

The similarity of amino acid composition of the three hemocyanins demonstrated in this study suggests that hemocyanins of the species studied originate from a common protein ancestor.

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SKŁAD AMINOKWASOWY Hc RAKA PRĘGOWANEGO (AMERYKAŃSKIEGO)  
(ORCONECTES LIMOSUS)

Oznaczono skład aminokwasowy hemocyjaniny raka pręgowanego. W wymienionej Hc stwierdzono znaczną zawartość kwasu glutaminowego i asparaginowego (25%).

Nie stwierdzono różnic w składzie aminokwasowym hemocyjaniny trzech gatunków raków żyjących w Polsce: Astacus astacus, Astacus leptodactylus i Orconectes limosus.

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СОСТАВ АМИНОКИСЛОТНЫЙ ГЕМОЦИАНИНА РАКА ORCONECTES LIMOSUS

Исследовался аминокислотный состав гемоцианина рака Orconectes limosus. Нашли высокое содержание глутамата и аспартата. Не обнаружили различий в аминокислотном составе гемоцианинов трех видов раков живущих в Польше: Astacus astacus, Astacus leptodactylus и Orconectes limosus.