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2. Białka oddechowe
Respiratory Proteins
Отдыхательные белки

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HEMOLYMPH PROTEINS OF SOME *DECAPODA*

Applying the method of polyacrylamide gel electrophoresis, hemolymph proteins of three crayfish species occuring in Poland were compared.

Per cent content of hemocyanin in the hemolymph and isoelectric point of hemocyanin subunits were determined.

The effect of storage of hemolymph at -20°C on hemolymph proteins was also studied.

Among the proteins present in the hemolymph of *Decapoda*, the respiratory protein hemocyanin, is the most important with respect to the performed physiological role. The structure and function of hemocyanin has been understood relatively poorly, up to now, as compared with e.g. hemoglobin or myoglobin. There are different respiratory proteins present in invertebrates, as erythrocruorin, chlorocruorin and hemerythrins. Hemocyanin is the only natural copper protein, capable of reversible binding and transport of oxygen. It occurs only in two invertebrate phyla *Mollusca* and *Arthropoda*.

MATERIAL AND METHODS

The studied material consisted of hemolymph taken from crayfishes supplied by Fish Distributors in Warsaw. Hemolymph was obtained from males of the same molt stage.

In this study, hemolymph proteins of three crayfish species occuring in Poland were compared: *Orconectes limosus*, *Astacus leptodactylus* and *Astacus astacus*. The proteins were separated by polyacrylamide gel electrophoresis according to F a i r b a n k s [1], applying 5% gel in 0.1 M Tris buffer, pH 8. Electrode vessels were filled with a buffer of the same composition but diluted twofold. The electrophoretic separations were run for 2 h, at a current of 7 mA per gel. Protein bands were visualized by staining in a Coomassie Blue solution in perchloric acid. After destaining, gels were densitometrred in a TLD-100 densitometer. In parallel, glycoproteins were visualized by staining with the Schiff reagent according to C a p i t a n y [2] and lipoproteins by staining with Sudan Black B [3]. Identification of the hemocyanin fractions of the hemolymph was based on the selective staining of copper after G o u l d and K a r o l u s [4].

The presence of carbohydrate components in the hemolymph was confirmed by gas chromatography.

Hemocyanin was isolated from hemolymph as follows. Hemolymph was centrifuged at 10 000 x g for 15 min. in order to remove the clot and morphotic elements, and then hemocyanin was sedimented by spinning at 100 000 x g for 8 ha. For estimation of isoelectric point and per cent content of hemocyanin in the hemolymph of the three studied crayfish species, the method of isoelectric focusing was applied as described by F i n l a y s o n and C h r a m b a c h [5].

Ampholine of pH range of 3.5-10.0 was used. The gels were stained for protein and for copper in parallel.

In this study, the effect of a several-month storage of hemolymph preparations at -20°C on their protein electrophoregram pattern was also examined. This experiment was important due to the necessity of accumulation and storage of hemolymph resulting from the existance of a closed season during which the collection of crayfishes is forbidden. Electrophoretic separation of proteins of fresh and stored hemolymph was performed in 5% gel, with a Britton-Robinson buffer, pH 7.5. Also in this case, parallel staining for protein, copper, glycoproteins and lipoproteins was accomplished.

RESULTS AND CONCLUSIONS

Electrophoretic separations of hemolymph proteins of three crayfish species were performed.

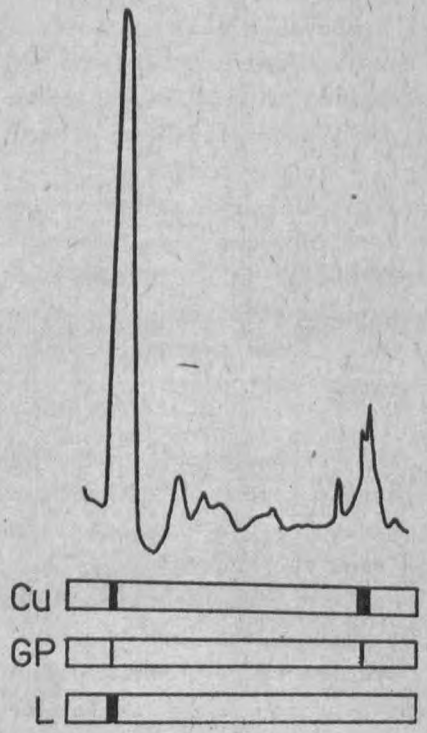

Fig. 1. Electrophoretic separation of hemolymph proteins of *Astacus leptodactylus*. *Cu* - copper proteins, *GP* - glycoproteins, *L* - lipoproteins

Rozdział elektroforetyczny białek hemolimfy z *Astacus leptodactylus*. *Cu* - miedzioproteidy, *GP* - glikoproteidy, *L* - lipoproteidy

Enregistrement d'électrophorégramme des protéines de l'hémolymphe *d'Astacus leptodactylus*. *Cu* - fractions protétiques comportant du cuivre, *GP* - glycoprotéines, *L* - *lipoproteines*

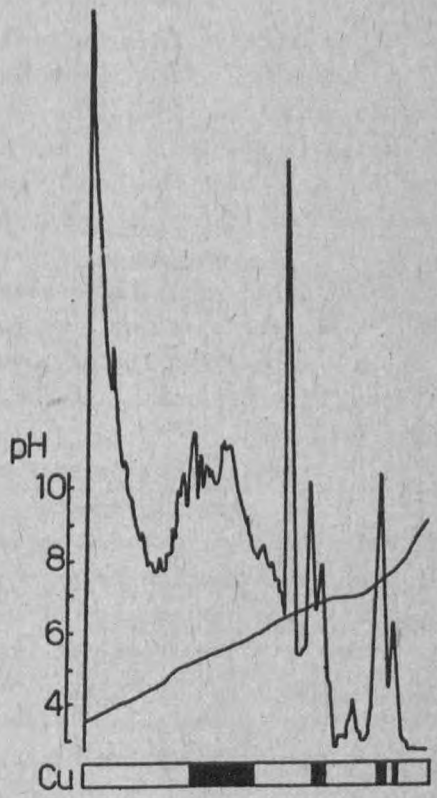

Fig. 2. Isoelectric focusing of hemolymph proteins of *Astacus leptodactylus*. *Cu* - copper proteins

Ogniskowanie izoelektryczne białek hemolimfy z *Astacus leptodactylus*. *Cu* - miedzioproteidy

Electrofocalisation des protéines de l'hémolymphe *d'Astacus leptodactylus*. *Cu* - fractions protétiques comportant de cuivre

Figure 1 shows a typical electropherogram of *A. leptodactylus* hemolymph. From among 5 protein fractions obtained, fract-

tions I and V contained copper. Moreover, fraction I contained both glycoproteins and lipids.

A similar electropherogram pattern was obtained for *O. limosus* hemolymph proteins. In the case of *A. astacus* five fractions were obtained, too, but three major fractions contained copper.

Since fraction I, representing a hemocyanin component contains always sugars and lipids in all the studied species, one may propose this fraction to be a glycolipoprotein complex. In order to exclude a possibility that sugars or lipids found in this fraction are of a non-hemolymph origin, it was necessary to check whether these compounds are present in pure hemocyanin preparations. The presence of carbohydrates in those preparations was established previously using the method of gas chromatography. Parallel staining, of pure hemocyanin electropherograms for protein, copper and lipids revealed that lipids are also present in those preparations, thereby confirming the validity of the above proposal.

On the basis of isoelectric focusing of hemolymph proteins of the three crayfish species, isoelectric points of subunits of their hemocyanins were estimated. A typical separation of *A. leptodactylus* hemolymph is presented in Fig. 2.

Apart from estimation of isoelectric points of hemocyanin subunits after selective staining for copper, per cent content of this protein in hemolymph of the studied species was determined. This determination made use of a proportional relationship between the numbers of integrator impulses and the areas below protein peaks. Relevant results are presented in Table 1.

T a b l e 1

Isoelectric points of hemocyanin subunits of three crayfish species and per cent content of hemocyanin in hemolymph

Species	pI	Hc(%)
Orconectes limosus	5.1-7.0	91
Astacus astacus	6.4-8.0	94
Astacus leptodactylus	5.0-7.5	93

In this Table, the values from 7 separations are given. The hemocyanin content exceeds 90% of the total protein content of the hemolymph in all the three crayfish species.

Studies of the effect of a prolonged storage of hemolymph at a low temperature were performed on two species only: *A. leptodactylus* and *A. astacus*, due to difficulties in the supply of *O. limosus*.

Figure 3 displays an electrophoretic separation of fresh and stored hemolymph of *A. leptodactylus*. In fresh hemolymph, two main

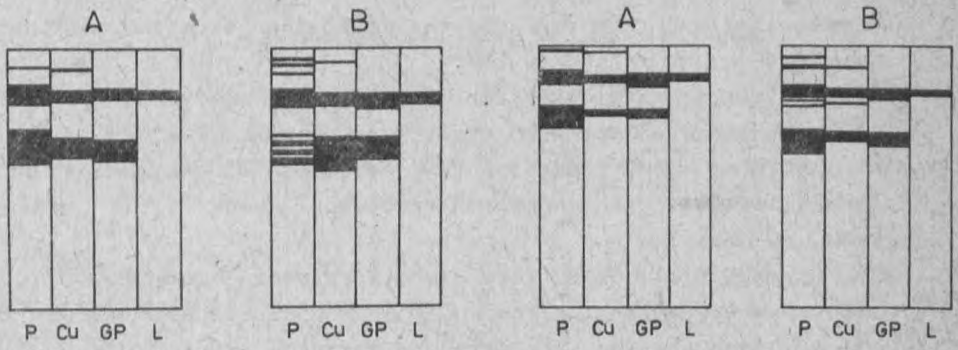

Fig. 3. Electrophoretic separation of proteins of fresh and stored hemolymph of *Astacus leptodactylus*. *P* - proteins, *Cu* - copper proteins, *GP* - glycoproteins, *L* - lipoproteins, *A)* fresh hemolymph, *B)* stored hemolymph

Rozdział elektroforetyczny białek hemolimfy świeżej i przechowywanej z *Astacus leptodactylus*. *P* - białka, *Cu* - miedzioproteidy, *GP* - glikoproteidy, *L* - lipoproteidy, *A)* hemolimfa świeża, *B)* hemolimfa przechowywana

Electrophorégramme schématique des protéines de l'hémolymphe fraiche et conservée *d'Astacus leptodactylus*. *P* - protéines, *Cu* - fractions protétiques comportant du cuivre, *GP* - glycoprotéines, *L* - lipoprotéines, *A)* l'hémolymphe fraiche, *B)* l'hémolymphe conservée

Fig. 4. Electrophoretic separation of protein of fresh and stored hemolymph of *Astacus astacus*. *P* - proteins, *Cu* - copper proteins, *GP* - glycoproteins, *L* - lipoproteins, *A)* fresh hemolymph, *B)* stored hemolymph

Rozdział elektroforetyczny białek hemolimfy świeżej i przechowywanej z *Astacus astacus*. *P* - białka, *Cu* - miedzioproteidy, *GP* - glikoproteidy, *L* - lipoproteidy, *A)* hemolimfa świeża, *B)* hemolimfa przechowywana

Electrophorégramme schématique des protéines de l'hémolymphe fraiche et conservée *d'Astacus astacus*. *P* - protéines, *Cu* - fractions protétiques comportant du cuivre, *GP* - glycoprotéines, *L* - lipoprotéines, *A)* l'hémolymphe fraiche, *B)* l'hémolymphe conservée

hemocyanin fractions were present, while in the stored hemolymph four fractions occur instead of second, each containing copper. An electrophoretic comparison of proteins of fresh and stored hemolymph of *A. astacus* is shown in Fig. 4.

In this case, too, two main hemocyanin fractions are present in fresh hemolymph, and additional copper-containing fractions close to the slower electrophoretically hemocyanin subunit appear upon storage.

One may inter that in the both studied species, several-month storage of hemolymph at a low temperature results in a degradation of one hemocyanin subunit into smaller ones.

Summarizing one can conclude the following from the obtained results:

1. Electrophoretic separation patterns of hemolymph proteins of the three crayfish species studied are similar but 2 hemocyanin fractions are present in the hemolymph of *O. limosus* and *A. leptodactylus* while 3 hemocyanin fractions occur in the hemolymph of *A. astacus*.

2. Hemocyanin molecules are glycolipoproteins complexes.

3. Per cent contents of hemocyanin in hemolymph of the three studies crayfish species are very similar and exceed 90% of the total protein.

4. Isoelectric points of hemocyanin subunits of *A. leptodactylus* and *O. limosus* are like and are in the pH range of 5-7.5. Isoelectric points of hemocyanin subunits of *A. astacus* are in the pH range of 6-8.

5. Several-month storage of hemolymph of two crayfish species at a low temperature results in a degradation of one from two main hemocyanin fractions into smaller subunits, probably retaining their functional properties since still containing copper.

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BIAŁKA HEMOLIMFY NIEKTÓRYCH *DECAPODA*

Stwierdzono obecność dwóch frakcji hemocyjaninowych w hemolimfie raka amerykańskiego i błotnego oraz trzech w hemolimfie raka szlachetnego. Zawartość procentowa hemocyjaniny w hemolimfie trzech gatunków raków wynosi ponad 90%.

Hemocyjanina jest kompleksem glikolipoproteidowym, a punkty izoelektryczne jej podjednostek mieszczą się w granicach pH 5-8. Długi okres przechowywania hemolimfy w temperaturze -20°C powoduje degradacje hemocyjaniny na mniejsze podjednostki, które zachowują jednak najprawdopodobniej swoje własności funkcjonalne.

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PROTEINES DE l'HEMOLYMPHE CHEZ CERTAINS *DECAPODA*

On a mis en évidence la présence de deux fractions d'hémocyanine dans l'hémolypmhe *d'Orconectus limosus* et *Astacus leptodactylus* mais trois fractions dans l'hémolymphe *d'Astacus astacus*. La teneur en hémocyanine dans l'hémolymphe est plus que 90% chez tous les trois éspeces des ecrevisses.

l'Hémocyanine est le complexes glycolipoproteique et les points isoélectriques de ses sous-unités se trouvent à pH 5-8. La conservation à -20°C cause la degradation de cette protéine mais les sous-unités gardent toujours ses proprietés fonctionelles.