A COMPARATIVE STUDY ON THE SOLUBLE MUSCULAR PROTEINS OF FOUR SPECIES OF BONY FISHES

NADIA-ALINA BALAN[1], ELVIRA TĂNASE[1], COSTICĂ MISĂILĂ[1], VLAD ARTENIE[1*]

Keywords: fish sarcoplasmic proteins, PAGE fish sarcoplasmic proteins, bony fishes, *Carassius auratus*, *Cyprinus carpio*, *Perca fluviatilis*, *Rutilus rutilus*

Abstract: The paper constitutes a quantitative and qualitative study of the sarcoplasmic proteins from the striated muscle of four species of bony fishes: *Carassius auratus*, *Cyprinus carpio*, *Perca fluviatilis* and *Rutilus rutilus*. The content of muscular proteins soluble in Na, K – phosphate buffer solution is lower than the amount of sarcoplasmic proteins, extracted with bidistilled water, from the muscles of the four fish species. Between the three cyprinid species, on one hand, and *Perca fluviatilis*, on the other, some significant differences are to be observed only in the case of muscular proteins soluble in bidistilled water. The electrophoretic spectrum of the sarcoplasmic proteins extracted with a phosphate buffer solution and, respectively, with bidistilled water, shows that the species under investigation differ only as to the number of protein fractions and to their mobility.

INTRODUCTION

Among the proteins forming muscular tissue fishes, a special place is occupied by soluble or sarcoplasmic proteins, actually a mixture of proteins with similar physical – chemical properties, soluble in water and in solutions of neutral salts with low ionic force (below 0.15 M). Such a mixture constitutes 25 – 30 % of the total content of proteins occurring in muscle fishes.

The group of sarcoplasmic proteins includes myoalbumin, globulins and various enzymes participating in the cell metabolism. Most of the sarcoplasmic proteins are formed by the enzymes of the glycolytic system involved in the anaerobic conversion of glycogen into ATP – necessary in muscular contraction. If the organelle muscular cells are broken, the number of sarcoplasmic proteins increases with the enzymes localized into endoplasmic reticulum, mitochondria and lysosomes.

Once known that the different fish species possess sarcoplasmic proteins with a characteristic electrophoretic pattern [Jurca, Matei, 1975], the special importance of proteins in domains such as taxonomy and evolution [Tsuyuki, 1965, Lundstrom, 1980], biotechnology [Rehbein, 1990] etc. is evidenced beyond any doubt.

This communication reports the quantitative and qualitative study of the soluble proteins from the striated muscle of four fish species, namely: *Carassius auratus*, *Cyprinus carpio*, *Perca fluviatilis* and *Rutilus rutilus*.

MATERIAL AND METHODS

The fishes taken into study, coming from the Iaşi Research Station for Aquaculture and Aquatic Ecology of the "Alexandru Ioan Cuza" University of Iaşi, have been taken over in the middle of November 2002. All fishes were habituated to laboratory conditions for two weeks. After this period, fishes were sacrificed for taking over the white muscle localized near the dorsal fin. Samples of muscles were immediately stored at – 30°C until biochemical assays were done.

Tissue extracts were prepared by homogenizing the samples in a Na, K – phosphate buffer solution 0.1 M (pH = 7.4) and in bidistilled water, in a 1: 2.5 (w/v) ratio.

For each of the four fish species under study, three experiments have been performed, both for the extraction with phosphate buffer solution and for the utilization of bidistilled water. The samples were mentioned on ice during and between periods of homogenization.

Protein concentration in extract muscle was measured using the method of Bradford [Bradford.1976]. Bovine serum albumin was used as standard. Statistical analysis on the basis of Student's test included calculation of mean values and standard deviation. Differences were considered significant at $p<0.05$.

Electrophoretic separation of the soluble muscular proteins of the four fish species was performed in polyacrilamide gel, in a discontinuous buffer system, with vertical migration [Popescu, 1990]. Gel migration had a concentration of 9 %, while that of the concentration gel was of 5 %.

Gels were stained with a 0.07 % Coomassie Brilliant Blue R 250 dye solution and calibrated with molecular weight standards (Pharmacy Biotech): 53 – 212 kDa.

RESULTS AND DISCUSSIONS

One of the most important aspects followed in our investigations refers to the content of sarcoplasmic proteins in the four fish species considered in the study. The date listed in Table 1 evidence certain difference between the amounts of proteins extracted with a 0.1 M phosphate buffer solution (pH = 7.4) or only with bidistilled water. Thus, it was observed, for all the four fish species that the level of proteins extracted with bidistilled water exceeds by 29 – 37.11 % that of the proteins soluble in phosphate buffer solution (Table 1).

The interspecific analysis of the content of sarcoplasmatic proteins shows that the *Rutilus rutilus* and *Carassius auratus* species posses the same level of soluble proteins in phosphate buffer solution as the *Perca fluviatilis* one, which is situated on a higher evolutive stage than the cyprinides under investigation. Instead, in the case of *Cyprinus carpio*, the amount of proteins soluble in phosphate buffer solution takes the lowest value which is nevertheless statistically insignificant.

Table 1. Content of soluble proteins (mg/g) extracted with phosphate buffer solution and bidistilled water in the striated muscle of four species of bony fishes

SPECIE	n	Proteins soluble in phosphate buffer solution (mg/g fresh tissue) M ± ES	Proteins soluble in bidistilled water (mg/g fresh tissue) M ± ES
Rutilus rutilus	3	0.77233 ± 0.00192	1.13100 ± 0.00233 $p < 0.001$
Cyprinus carpio	3	0.70000 ± 0.03122	1.11367 ± 0.00135 $p < 0.001$
Carassius auratus	3	0.78033 ± 0.00703	1.09933 ± 0.00150 $p < 0.001$
Perca fluviatilis	3	0.76133 ± 0.00483	1.07700 ± 0.00371 $p < 0.001$

The content of muscular proteins extracted in bidistilled water, from the three cyprinide species – *Rutilus rutilus*, *Cyprinus carpio* and *Carassius auratus*- is higher comparatively with the level of the proteins soluble in bidistilled water observed in the case of *Perca fluviatilis*. However, such an increase is statistically assured only for the first two species under investigation: *Rutilus rutilus* and *Carassius auratus*.

Another objective of the study followed the electrophoretic separation of the sarcoplasmic proteins extracted with phosphate buffer solution or bidistilled water from the skeletal muscle of the four fish species.

As seen from the electrophoregrams plotted in Fig. 1 and 2, the saline and aqueous extracts from the striated muscle of all the four fish species considered in the study contain proteins with both low, respectively average and high molecular weight

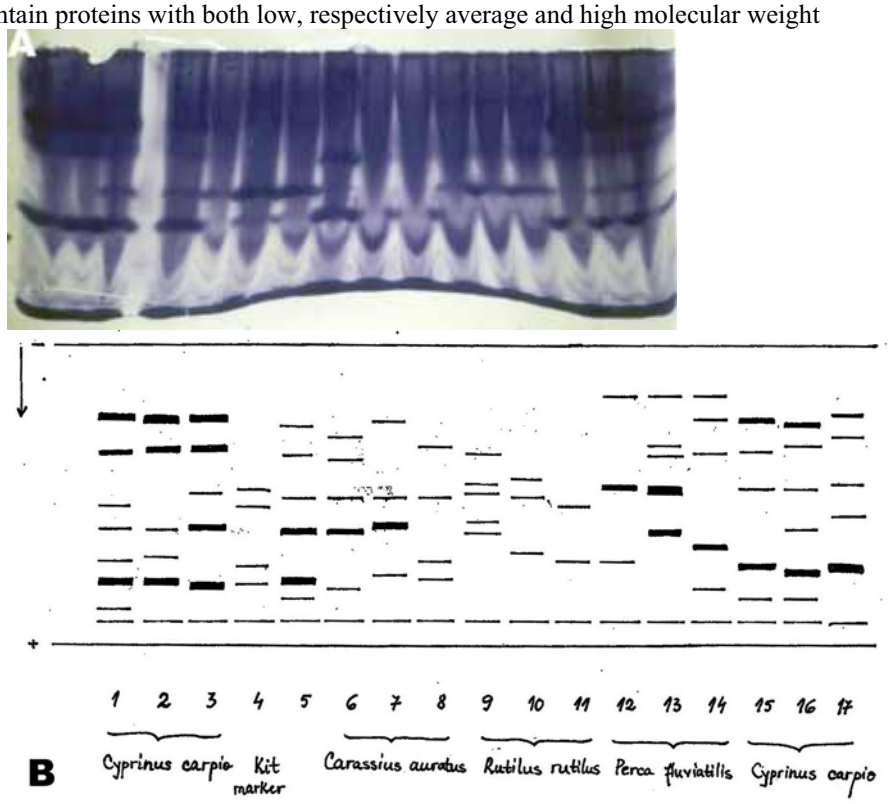
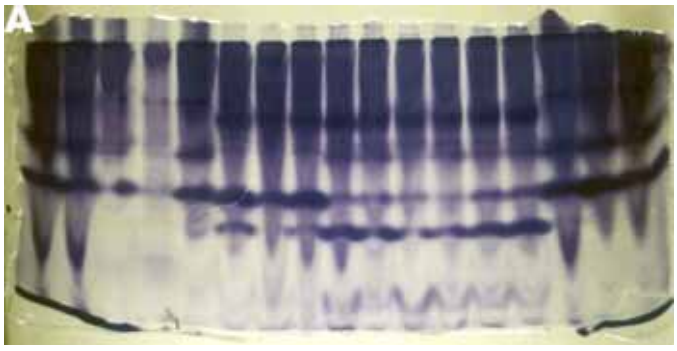


Fig.1.The electrophoregram (A) and the electrophoretic spectrum diagram (B) of the muscular soluble proteins in phosphate buffer solution 0.1 M (pH=7.4) of the four fish species.



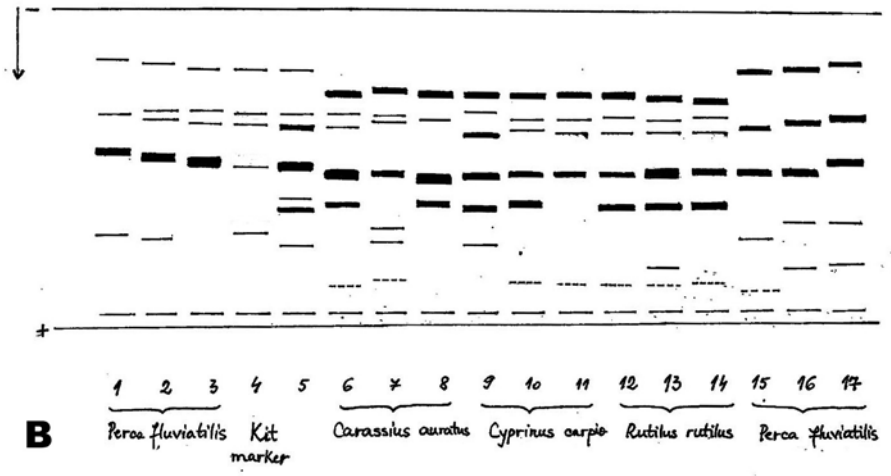


Fig.2.The electrophoregram(A) and the electrophoretic spectrum diagram (B) of the muscular soluble proteins in bidistilled water of the four fish species.

The electrophoretic spectrum of the proteins extracted with phosphate buffer solution (Fig.1) differs from the electrophoretic pattern of those extracted with bidistilled water by the number of protein fractions and by their mobility. In the case of sarcoplasmic proteins extracted with bidistilled water (Fig. 2), the number of separated fractions is even higher and, besides, in all species taken into study, very fine fractions, corresponding to the proteins with low molecular weight and rapid electrophoretic migration, do appear. This aspect may be explained by the ability of the bidistilled water of extracting, in a non – selective manner, muscular proteins, especially the albumin fractions with small molecule, involved in the transport of calcium ions in the muscle [Paaver, 1983].

Analysis of the two electrophoregrams (Fig. 1 and 2) evidences an interspecific differentiation of the sarcoplasmic proteins in the four fish species, regardless of the extract employed. This difference is weaker among the crucian, carp and roach, and more pronounced between the three cyprinids and *Perca fluviatilis*.

CONCLUSIONS

The content of the muscular soluble proteins in Na, K – phosphate buffer solution is lower than the amount of sarcoplasmic proteins, extracted with bidistilled water, from the muscles of the four fish species.

The content of the sarcoplasmic proteins in the four fish species, regardless of the extract employed, evidences an interspecific differentiation.

The electrophoretic spectrum of the muscular soluble proteins extracted with bidistilled water in all the four fish species evidences a higher number of separated fractions, comparatively with the sarcoplasmic proteins extracted with the phosphate buffer solution 0.1 M (pH = 7.4).

BIBLIOGRAPHY

Bradford, M., 1976 - A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal Biochemistry 72: 248 –254

Jurca Valentina, Matei C., 1975 – Proteinele din muschii scheletici la unele specii şi linii de ciprinide. Stud. cerc. biochim., Vol.18, No.2, 115

Lundstrom, R.C., 1980 - Fish species identification by thin larger polyacrilamide gel isoelectric focusing collaborative study. J. Pissoc. Off. Anal. Chem., 66, 1158

Popescu, O., 1990 - Electroforeza proteinelor in geluri de poliacrilamida. Editura Tehnica, Bucureşti, p. 83.

Paaver T. K., 1983 – Biohimiceskaja genetika karpa *Cyprinus carpio* L.. „ Valgus ”, Tallin, p. 85

Rehbein, H., 1990 - Electrophoretic techniques for species identification of fishery products. Z. Lebensm. Unters. – Forsch., 111, 1- 10

Tsuyuki H., Roberts E. and Vanstone W. E., 1965 – Comparative zone electrophoregrams of muscle myogenes and blood hemoglobins of marine and freshwater vertebrates and their application to biochemical systematics. Fish. Res. Board. Canada, Vol. 22, No.1, 203

[1]„ Alexandru Ioan Cuza ” University, B-dul Carol I, 20A, 700506 Iaşi, România
[*] corresponding author: vartenie@uaic.ro