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Mechanisms of the Plurality of *Scorpaena porcus* L. Serum Albumin

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Abstract

The proteins, which bind albuminspecific dye Evans blue, are revealed in the low-molecular protein fraction of the blood serum from *Scorpaena porcus* L. and identified as serum albumin. They were represented by three bands in 2D-SDS-PAAG. MALDI-TOF-analysis revealed the fundamental similarity of the mass spectrum of the fragments of tryptic cleavage of proteins with molecular weight 73 and 76 kDa. The role of duplications and intragenic reconstructions in the creation of the plurality of scorpaena albumins is discussed.

Keywords: Scorpaena, Blood, Tissue Fluids, Low-Molecular Proteins, Albumin, Mass Spectrum, Duplications

1. Introduction

Serum albumins accomplish important functions in the organism of vertebrates, participating in the filtration of tissue fluid, in the transport of biomolecules and in the plastic metabolism. Mammalian albumins are simple monomeric proteins with the molecular weight about 67 kDa; they are represented, as a rule, by one component on the electrophoregram [1]. Fish albumins differ from mammalian ones in the diversity of organization ways, physical and chemical properties: there are simple proteins and glycoproteins, monomers, oligomers and aggregates among them. Usually their electrophoretic mobility does not coincide with that of mammalian albumin, and they are often represented by the plural forms in the electrophoresis [2-20]. The ability of fish albumins to bind albuminspecific dyes, palmitic acid, incapacity to bind nickel, molecular weight and other characteristics are used for the fish albumin identification [9,11,14, 17,21,22]. We identified serum albumin of scorpaena *Scorpaena porcus* by the molecular weight, the ability to bind albuminspecific dyes and by means of MALDI-TOF-analysis; the results obtained were used to reveal the mechanisms of albumin plurality.

2. Material and Methods

2.1. Objects of Study

The objects of this study were Scorpaenas *Scorpaena porcus* L. from the Black Sea. For comparison we used *Mullus barbatus* L., *Uranoscopus scaber* L., *Symphodus tinca* L., *Gaidropsarus mediterraneus* L., *Neogobius melanostomus* P. and *Mesogobius batrachocephalus* P. from the Black Sea and also roach *Rutilus rutilus* L. and perch *Perca fluviatilis* L. from the Rybinsk Reservoir. For the work we used proteins from blood serum and plasma and tissue fluids from the peritoneum, brain and white muscles.

2.2. Methods of Analysis

The biological fluids obtaining. The blood was obtained from the caudal artery, tissue fluids were taken by pipetting or by the impregnation of the strip (0.5 × 4.0 mm) of the chromatographic paper Watmann 3 MM [23].

Protein concentration measurement. We used microbiuret method to estimate the concentration of total protein [24].

Electrophoresis methods. We analysed the albumins by disk- and 2D-electrophoresis (in gradient of PAGE concentration 5% - 40%, in PAGE with 8 M urea [17] and SDS [25]. For calculation of the molecular weights (MM) of proteins we used: myoglobin and the polymeric forms of human serum albumin HSA and ovalbumin OVA; the markers Fermentas PageRuler^{TM} Prestained Protein Ladder Plus (11, 17, 28, 36, 55, 72, 95, 130, 250 kDa). Results were processed statistically with program package OneDscan.

The binding of proteins by albuminspecific dyes. We studied the binding of proteins by Evans blue and brom-

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cresol purple BCP by recording the formation of the protein-dye complexes in PAGE and, in the case BCP, spectrophotometrically. The formation of specific complex is accompanied by λ max shift from 590 to 603 nm [26].

MALDI-TOF-analysis. This method was used for the precise determination of protein MM and for comparative analysis of the mass-spectrum (MS) of the fragments of tryptic cleavage of proteins, which bind Evans blue under the native conditions. Data obtained data were used to determine homology of the scorpaena proteins and for scorpaena albumin identification. Analysis was performed on the base of Scientific Research Institute of Physical-Chemical Medicine in the laboratory of proteomic analysis. Proteins for the MALDI-analysis were obtained from 2D-SDS-PAGE. Mass-spectra MS and the fragmentation spectra MS/MS were obtained by the mass-spectrometer Ultraflex II BRUKER (Germany), equipped by UV laser (Nd). The accuracy of the measured masses of fragments was 1Da.

Albumin identification. The binding of proteins by the albuminspecific dyes, the value of MM and MALDI-TOF-analysis data were used for scorpaena albumin identification. The proteins were identified by means of "peptide fingerprint" and the fragmentation spectra MS/MS by means of the Mascot program (www.matrixscience.com). The search was carried out in the NCBI database among the proteins of all organisms with prescribed accuracy, the possible oxidation of methionine by atmospheric oxygen and possible modification of cysteines by acrylamide were taken into consideration. The cumulative search on the basis of MS + MS/MS was carried out by means of a program BioTools of v.3 (Bruker, Germany). Only those proteins with the test of significance *score* > 85 ($r < 0.05$) were considered as the reliable candidates.

3. Results and Discussion

Differentiation of low-molecular proteins from fishes extracellular fluids in electrophoresis. The low-molecular fraction of the scorpaena plasma contained 6-10 proteins with MM from 20 to 90 kDa, the relative content of this fraction was 28% (**Figure 1**).

The same proteins were also presented also in the tissue fluids of scorpaena, however, their relative content in the peritoneal fluid was above (39.9%), and in brain tissue fluid it was lower (22.3%), than in the plasma (28%). The subunit repertoire of the proteins from tissue fluids coincides with that of plasma proteins, this fact confirms the identical composition of the proteins in all extracellular fluids of organism (**Figure 2**).

Scorpaena had 15 low-molecular serum and tissue fluid proteins in the 2D-electrophoresis in the PAGE concentration gradient, 24 LM-protein in PAGE with 8M urea and 34 LM-proteins in SDS-PAGE (**Figure 3**). And we detected only 3 macrocomponents with MM about 60 - 70 kDa under the denaturing conditions (**Figure 3**).

The binding of low-molecular proteins by albumin-specific dyes. The low-molecular fraction in the disk-electrophoresis of scorpaena and fresh-water perch plasma contains 1-2 proteins, which bind the Evans blue, (**Figure 4**).

Unlike Evans blue, the BCP dye did not bind scorpaena proteins, but it bound all roach serum proteins unspecifically, shifting λ_{max} from 590 to 593 nm [27,28]. BCP did not bind scorpaena proteins and binds all roach blood proteins in PAGE as well.

Scorpaena proteins, which bind Evans blue in the disk-electrophoresis, were represented in the 2D-electrophoresis by the large number of protein spots, among which there were only three macrocomponents with MM

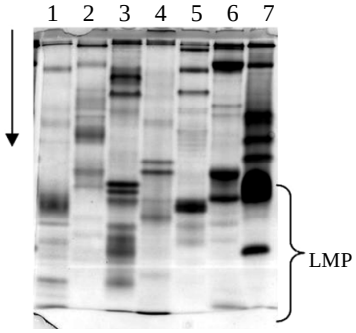

Figure 1. Disk- electrophoresis of the blood plasma proteins of *Mullus barbatus* L. 1. *Gaidropsarus mediterraneus* L.; 2. *Mesogobius batrachocephalus* P.; 3. and *Neogobius melanostomus* P.; 4. *Uranoscopus scaber* L.; 5. scorpaena; 6. and roach; 7. LMP—low-molecular proteins. Vertical arrow shows the electrophoresis direction.

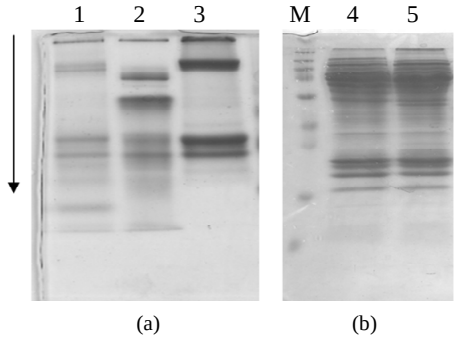

Figure 2. Electrophoresis of blood and tissue fluid proteins of scorpaena and *Mesogobius batrachocephalus* P.: (a) Disk-electrophoresis of peritoneal fluid 1, brain tissue fluid 2 and plasma 3 from scorpaena; (b) SDS—electrophoresis of brain tissue fluid (4) and plasma (5) from *Mesogobius batrachocephalus* P.; M—the marker Fermentas. Vertical arrow shows the electrophoresis direction.

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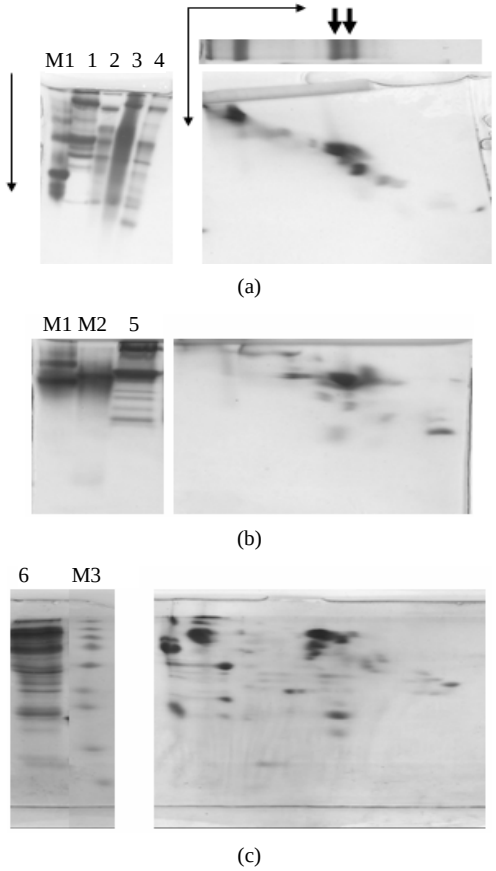

Figure 3. 2D-electrophoresis of plasma and tissue fluid proteins from scorpaena: in the PAGE concentration gradient (a), in PAGE with 8M urea (b) and SDS-PAGE (c). (1—scorpaena plasma; 2, 3, 4—tissue fluids from peritoneal, white muscles and the brain. Marker proteins: M1—HSA and OVA; M2—myoglobin, M3—the Fermentas marker. Horizontal arrow shows the disk-electrophoresis direction, vertical—gradient-electrophoresis direction, electrophoresis with urea and SDS-electrophoresis directions respectively. Two small vertical arrows show the paths of proteins with MM 60—70 kDa.)

64, 69 and 70 kDa, which bind the dye (**Figure 4**). These very proteins are supposed to be albumins, because they bind albuminspecific dye and have MM most similar to HSA. The results obtained revealed the plurality of scorpaena albumins.

Scorpaena albumins mass-spectra. We obtained the mass-spectra for those albumins, which have MM 64 and 69 kDa in SDS-electrophoresis. Calculation of MM for these albumins by means of MALDI-TOF gave higher values—73.2 and 76.1 kDa. The MM comparison for the tryptic cleavage products of these two proteins revealed their almost perfect match (**Table 1**). These proteins differed only in three fragments (**Table 1**).

4. Conclusions

The results obtained show the plurality of scorpaena albumin and make it possible to assume that these proteins are the products of the different genes, which are united by the same origin. It is possible to explain the set of the identical amino-acid fragments in these proteins by the fact that one gene appeared as a result of the duplication of another initial (ancestral) gene. The presence of the amino-acid fragments in one protein, while they are absent in other protein, can arise from subsequent intra-genetic reconstructions—deletions or insertions. The search for the homologues of these scorpaena proteins in the NCBI database gave no results. However, data obtained made it possible to conclude that scorpaena has serum albumin, which differ from mammalian albumin.

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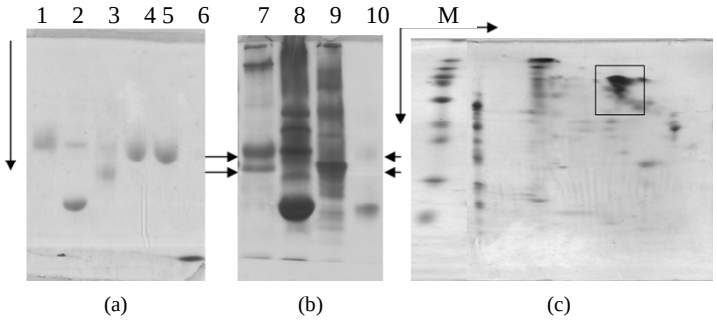

Figure 4. The binding of Evans blue by the blood proteins from: (a) Scorpaena 1, human 2, perch 3, HSA 4; controls: Evans blue 5, bromphenol blue 6 in the disk-electrophoresis; (b) The staining of proteins from scorpaena 7, human 8, perch 9 and HSA 10 by Coomassie R-250 in the disk-electrophoresis; small horizontal arrows show the areas of Evans blue binding; Vertical arrow shows the disk-electrophoresis direction; (c) 2D-SDS-electrophoresis of scorpaena plasma proteins; the proteins, which bind Evans blue, are outlined by the frame. M—the Fermentas marker. Vertical arrow shows SDS-electrophoresis direction, horizontal—disk-electrophoresis direction.

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Table 1. MM of scorpaena albumins and their tryptic cleavage products.

MM of albumin Da	MM of products after albumin tryptic cleavage Da
73214	723.38; 733.32; 778.40; 851.49; 901.43; 927.41; 949.57; 1011.51; 1050.53; 1083.52; 1125.62; 1197.59; 1214.65; 1243.6; 1248.57; 1256.64; 1309.63; 1310.60; 1317.73; 1386.78; 1420.69; 1466.74; 1509.80; 1636.81; 1658.83; 1660.69; 1680.86; 1682.79; 1698.78; 1704.88; 1717.86; 1739.85; 1785.89; 1808.95; 1866.83; 1908.86; 1954.88; 2165.02; 2540.06; 2629.26; 2805.20; 2933.31; 3054.37; 3121.7*; 3399.54
76128	723.38; 733.32; 778.40; 851.49; 901.43; 927.41; 949.57; 1011.51; 1050.53; 1083.52; 1125.62; 1197.59; 1214.65; 1243.6; 1248.57; 1256.64; 1309.63; 1310.60; 1317.73; 1386.78; 1420.69; 1466.74; 1509.80; 1636.81; 1658.83; 1660.69; 1680.86; 1682.79; 1698.78; 1704.88; 1717.86; 1739.85; 1785.89; 1808.95; 1866.83; 1908.86; 1954.88; 2165.02; 2540.06; 2629.26; 2805.20; 2933.31; 3005.54*; 3054.37; 3070.37*; 3399.54

*albumin tryptic cleavage products, which MM doesn, t match.

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