Various Peptides as Substrates for Aminopeptidases from Salmon Eggs during Embryogenesis

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Seven p-nitroaniline-modified peptides were used as substrates for studies of aminopeptidase and peptidase activities in eggs of lake salmon *Salmo salar Sebago* at different stages of embryogenesis. The formation of p-nitroaniline during peptide hydrolysis was measured in the incubation medium and in maturing eggs. Activities of all studied aminopeptidases and peptidases increased during the development of fertilized eggs and sharply increased before hatching. L-alanin-p-NA, L-pro-p-Na, L-Arg-p-Na, and L-Phe-p-NA were hydrolyzed most of all, while N-Glt-L-Phe-p-NA and Z-Gly-Pro-p-NA least of all.

Key Words: *peptides; aminopeptidases; eggs; embryogenesis; salmon*

Study of the role of peptidases in the development of eggs in different fishes showed that enzymes cleaving Arg, Leu, Ala, Cys, and Phe p-nitroanilides exhibited the highest activity in tench and sheatfish eggs incubation medium, while enzymes cleaving Bzl-L-Arg-pNA, L-Pro-pNA, N-Glutaryl-L-Phe-pNA, and Z-Gly-Pro-pNA exhibited lower activity [2,4]. Activities of certain aminopeptidases (AP) gradually increase during maturation of salmon eggs and significantly increase before hatching [3]. The system of intracellular proteolysis includes proteinases and peptidases. We previously analyzed activities of intracellular proteolytic enzymes, such as lysosomal cathepsins B and D and cytosol Ca^{2+}-activated proteinases in salmon embryogenesis [1]. Here we studied changes in activities of peptidases and AP in developing eggs of *Salmo salar Sebago* (a representative of limnetic fishes of the Russian European North).

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MATERIALS AND METHODS

Lake salmon eggs before and after artificial fertilization were obtained at a fish breeding station on the Shuya river (the Onega lake basin, Republic of Karelia). The eggs were grown under laboratory conditions on lattices washed with river water in a chamber at constant temperature of 5°C.

Salmon eggs were collected directly before fertilization and at the following embryogenesis stages: 4 h after fertilization (early division), after 1 week (late blastula-early gastrula), 3 weeks (organogenesis), 6 weeks (eye pigmentation), 7, 10, 14, and 15 weeks after fertilization (before hatching).

The rate of embryogenesis and vital processes in cold-blooded animals depends on environmental temperature. The maturation of salmon eggs under natural conditions takes 100-230 days. In our experiment hatching was observed on day 108 after fertilization, because the temperature in the incubation chamber was higher than after spawning under natural conditions.

Peptides containing a chromogenic component p-nitroaniline (p-NA; Table 1) released under the effects of specific peptidases and AP were used as

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substrates for AP and peptidases. All peptides were a gracious gift from Department of Peptides, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic.

Enzyme activities were measured as described previously [3]. To this end 10 eggs at each developmental stage were incubated in 5 ml 0.05 M Tris-HCl buffer (pH 8.0) for 24 h, after which 0.2 ml incubation medium was added to 0.2 ml 0.5 mM peptide solution in 1 ml buffer.

For preparing intracellular enzyme preparation, the membranes of 10 eggs were cut with scissors in 5 ml of the above buffer and centrifuged at 8000 rpm. The reaction was carried out in a solution containing 0.05 ml 0.5 mM peptide solution, 0.25 ml resultant supernatant, and 1 ml buffer. The volumes of the reaction mixtures were brought to 1.5 ml with distilled water.

The absorption capacity of p-NA formed as a result of peptidase reaction was evaluated after 24 h by spectrophotometry at λ=405 on an SF-2000 device (Spektr Firm).

RESULTS

Activities of AP specific for L-Ala-p-NA, L-Arg-p-NA, L-Pro-p-NA, and L-Phe-p-NA during the development of fertilized eggs were higher than activities of peptidases cleaving Z-Gly-Pro-p-NA and N-Glt-L-Phe-p-NA. AP hydrolyzing L-Ala-p-NA was most active in salmon eggs and in the incubation medium. It was previously found for developing *Oncorhynchus mykiss* eggs that this AP is a dimer consisting of two subunits (molecular weights 33,000 and 16,000, respectively) connected via a disulfide bridge [3].

Changed activities of the studied peptidases correlated with stages of embryo development (Fig. 1). A burst of AP and peptidase activities at the stage of late blastula-early gastrula (week 1) can be due to activation of protein metabolism, because the stage of early gastrula is the "critical" point of embryonal development in many fishes. The rate of yolk protein degradation to peptides and free amino acids increases during this period and they migrate into embryonic cells of developing eggs.

The increase in activity of AP with some fluctuations after fertilization until hutching reflects intensification of protein metabolism, associated with the formation and growth of the embryo. Fertilization process is known to be associated with changes in structural, biochemical, and physiological characteristics of the oocyte. Embryo growth is paralleled by differentiation and shaping. The rate of these processes changes at various stages of development and is realized at the expense of substances stored in the yolk.

Activities of the studied AP increased significantly before hatching (Fig. 1), which can be explained from physiological and biochemical viewpoints. The process of hatching is associated with activity hatching glands releasing the so-called "hatching enzyme" destroying the oocyte membrane. The substances released by these glands belong to the group of proteolytic enzymes (trypsin and pepsin type) [6]. The peptidase cleaving N-Bzl-L-Arg-p-NA is a trypsin-like enzyme and presumably participates in the processes associated with

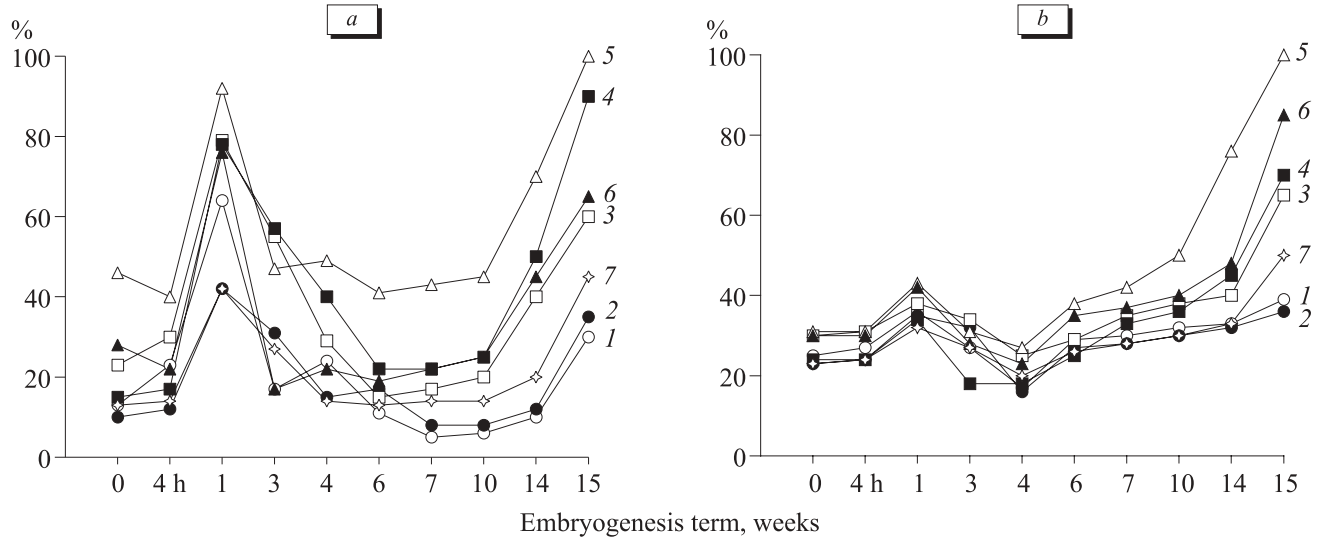

Fig. 1. Absorption (λ=405 nm) of p-NA formed during hydrolysis of various substrates (in percent of maximum absorption capacity A_{max} observed for L-Ala-p-NA) in salmon eggs incubation medium (*a*) and in salmon eggs (*b*). *1*) N-Bzl-L-Arg-p-NA; *2*) 2-Gly-Pro-p-NA; *3*) L-Phe-p-NA; *4*) L-Arg-p-NA; *5*) L-Ala-p-NA; *6*) L-Pro-p-NA; *7*) N-Glt-L-Phe-p-NA.

TABLE 1. Peptides Used as Substrates

Peptide	Molecular weights of substrates	Enzyme
N-Bzl-L-Arg-p-NA	498.31	Trypsin-like peptidase
Z-Gly-Pro-p-NA	425.00	Endoprolylpeptidase
L-Phe-p-NA	295.30	AP specific for aromatic amino acids
L-Arg-p-NA	375.10	B-AP
L-Ala-p-NA	209.30	Alanine AP
L-Pro-p-NA	235.12	Proline AP
N-Glt-L-Phe-p-NA	384.40	Chymotrypsin-like peptidase

hutchling release from the membranes. Peptidases are very stable proteins, the can present in incubation medium for a long time and accelerate destruction of membranes in immature eggs. The physiological role of peptidases and the cause of enzymes penetration through the membrane during egg maturation is unclear. Presumably, they protect the developing eggs from bacterial and fungal diseases [5]. Hence, AP and peptidases, along with proteases stored in eggs during oogenesis, are actively involved in the processes associated with embryo development in the studied salmon species.

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