EFFECT OF "DECREASING OF SENSITIVITY" TO HEPARIN OF CASEIN KINASE II TYPE (CK II) IN EARLY DEVELOPMENT OF LOACH *MISGURNUS FOSSILIS L.*

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CK II is an ubiquitous enzyme that phosphorylates *in vitro* and *in vivo* a large spectrum of proteins many of which play a key role in the regulation of cell growth and metabolizm. In particular CK II phosphorylates enzymes of glycogen and lipid changing, protein synthesis factors, topoisomerase II, RNA-polymerase II, some oncogene products, HMG-proteins, proteins of SV-40, nucleolin etc. A possible role of CK II in the regulation of cell activity suggests that the enzyme is activated by some mitogens, epidermal growth factor, insulin, insulin-like growth factor, dsDNA and is inhibited by mRNA (Hathaway and Traugh, 1982, Kapkov *et al.*, 1995). The present communication is to demonstrate our preliminary results obtained when investigating the CK II activity in early development of loach.

Loach embrios developed at +21,5°C were used. Age of the embrios - 2 hs (8 blastomers), 14 hs (epiboly 50%), 24 hs (7 pares of somites), 31 hs (19-20 pares of somites) and 45 hs (before hatching) post-fertilization.

Preparation of cell extracts: 500 embryos of each stage were homogenized in 3 ml of solution A (0.1 M Tris-HCl, pH8.0; 0.7 M NaCl; 1.5% NP-40; 15 mM $MgCl_2$; 2 mM DTT; 1.5 mM PMSF; 1.5 μM pepstatine; 1.5 μg/ml leupeptine) and were stirred on ice (1 h) with periodic sonicating (4x30"). Then homogenates were centrifugated (50,000 g, 1 h). Before DEAE-chromatography the extracts were dialysed (8 hs) against 10 V of solution B (20 mM Tris-HCl, pH8.0; 10 mM $MgCl_2$; 2 mM DTT; 10% glycerol). Proteins were eluted from DEAE-column by linear gradient of NaCl (0 - 0.5 M) in solution B. Before heparin-agarose chromatography the extracts were diluted by solution B (1:3). The extract was passed through heparin-agarose column, the column was washed by solution B with 0.1 M NaCl. Then fractions with CK-activity were eluted by solution B with 0.8 M NaCl. Determination of CK-activity: reaction mixture - 20 mM Tris-HCl, pH 8.0; 0.1 mM ATP; 1 μCi γ-[^{32}P]ATP; 5 mg/ml dephosphorylated casein; 10 mM $MgCl_2$; 2 mM DTT; 100 mM KCl (final concentrations). Probes (50 μl) were incubated at 37°C (30'), then the reaction was terminated by 0.5 M EDTA with saturated Na pyrophosphate (25 μl), dropped on Whatman 3MM filters, washed by cold TCA and ethanol, dried and counted.

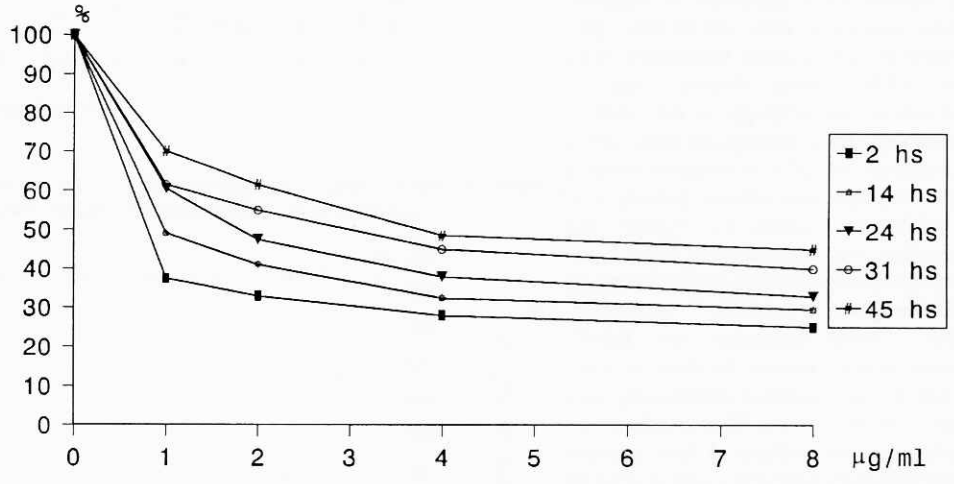

Figure 1. Inhibition of CK-activity of cell extracts by heparin.

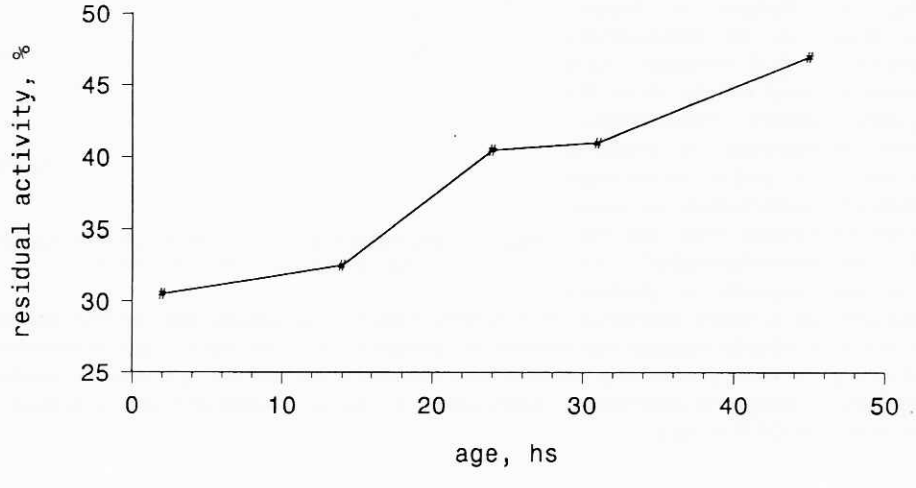

Figure 2. Residual CK-activities of heparin-agarose fractions (0.8M NaCl) at heparin (8μg/ml) inhibition.

Determination of CK-activity in the cell extracts of loach embryos revealed that the level of CK-activity increased approx. 1.5 time during embryogenesis. It is known that most part of CK II in cells is inactive, and true level of CK II can be determinated after partial enzyme purification only. We used partial CK II purification by column chromatography on heparin-agarose. It was shown that CK II activity increases more than 2 times during the period from 2 hs to 45 hs of embryogenesis. To differentiate CK I and CK II activities heparin was used. Heparin is a strong inhibitor for CK II but does not influence CK I activity at the same

concentrations. Dependence of residual CK-activity of cell extracts on heparin concentrations is presented in the fig. 1. Inhibition curves of all preparates reaches plateu at heparin concentration about 8 μg/ml. Literature data show that residual CK II-activity in the presence of the same heparin concentration is not more than 5% (Hathaway and Traugh, 1983). Our results demonstrate higher residual CK-activity, increasing from 25 to 45% during embryogenesis. The same results were obtained in heparin-agarose fractions with CK-activity (fig. 2): residual CK-activity increased from 31 to 47%. These results may be explained by the change of CK I/CK II-ratio during loach embryogenesis, since the presence of CK I in heparin-binding protein fraction was shown (Vasilyev *et al.*, 1988). To confirm (or reject) this explanation the cell extracts from 2 hs- and 45 hs-embryos were fractionated by DEAE-Toyopearl column chromatography. Chromatography on DEAE-sorbents is well known method of CK I and CK II fractionation (Hathaway and Traugh, 1979). Using this method we obtained typical picture of two caseine kinases fractionating: CK I was eluted by 0.1 M NaCl, and CK II - by 0.16-0.17 M NaCl (fig. 3). Analysis of heparin inhibitory effect (fig. 4) demonstrates characteristic of CK II inhibition curve with residual enzyme activity about 5% at 8 μg/ml heparin concentration. Quantitative comparison of enzymes obtained from 2 hs- and 45 hs-embryos showed that CK I-activity does not exeed 15% of total CK-activity. Thus, the most part of "heparin-independent" CK-activity in cell extracts is probably associated with CK II. When inhibiting CK II activity heparin competees with protein substrate - casein (Kapkov *et al.*, 1995). However, our experiments suggest thatinteractions between CK II and some macromolecules, presenting in the cell extracts and heparin-binding fractions, protect the enzyme from heparin effect but fail to influence catalytical activity *in vitro*. "Decreasing" of CK II-sensitivity to heparin *in vitro* during embryogenesis may be a result of complex protein-protein interactions and may have a regulatory effect on CK II *in vivo*.

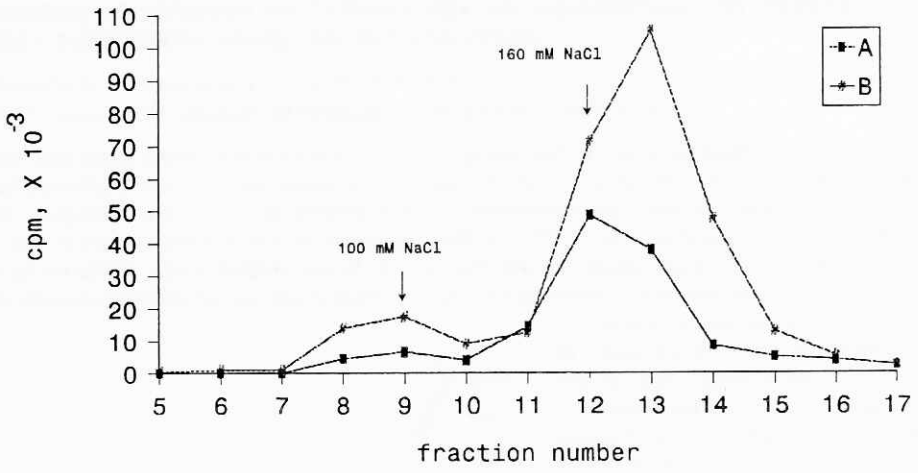

Figure 3. Fractionating of CK I and CK II of 2 hs- and 45 hs- embryos cell extracts (A and B respectively) by DEAE-Toyopearl column chromatography.

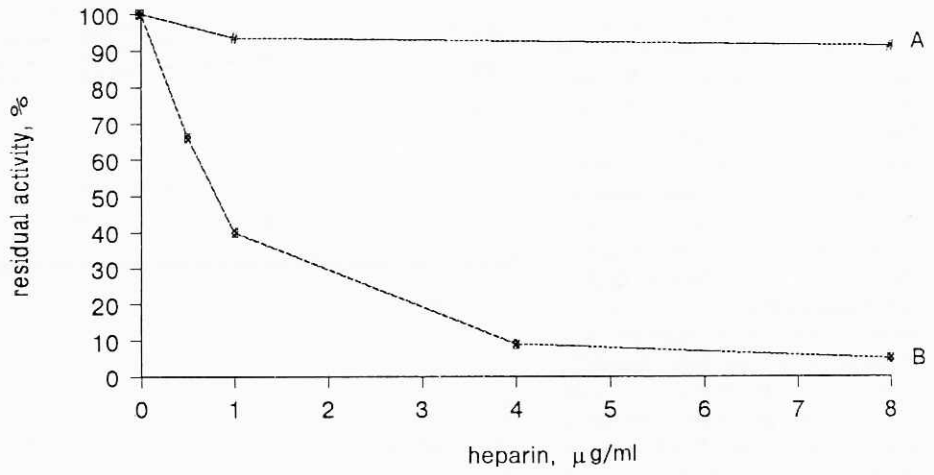

Figure 4. Dependence of CK I- and CK II- activities fractionated by DEAE-Toyopearl from heparin concentrations (A - CK I , B - CK II).

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