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Enzymes, proteins, and soluble multi-protein complex from eggs of sea urchin *Strongylocentrotus intermedius*

The results of the studying of enzymes, proteins, and soluble multi-protein complex from eggs of sea urchin Strongylocentrotus intermedius are discussed. The extracts of sea urchin eggs contain a very stable protein complex consisting of a large number of different proteins and peptides. Progress in the study of embryos protein complexes can help to understand their biological functions.

Key words: enzymes, proteins, multi-protein complex, eggs of sea urchin Strongylocentrotus intermedius

Eggs of the sea urchin are an exceptionally convenient model for studying the patterns of development from embryo to the body.

Genome of all living beings exists in a dynamic equilibrium between ongoing DNA damage and reversal of the damage, a process known as DNA repair [2]. Decrease in DNA repair capacity ultimately manifests itself in the form of mutagenesis, carcinogenesis, or cell death, and is implicated in a number of human diseases. DNA repair is crucial both for rapidly proliferating cells, in which lesions in DNA interfere with replication fork progress and may be converted into mutations upon replication, and for terminally differentiated cells, which sometimes have to maintain their genome integrity for the entire lifespan of the organism and have cell division-dependent checkpoints downregulated or turned off [2]. Several pathways have been defined in most organisms, including direct reversal, base excision repair (BER), nucleotide excision repair, mismatch repair, non-homologous end-joining, and recombination repair. Of those, BER, which removes small non-bulky lesions, the most abundant type of spontaneous and induced DNA lesions, seems to be of the greatest importance in multicellular animals, judging from the embryonic lethality of knockouts inactivating the whole pathway. In the course of BER, one of several enzymes belonging to the class of DNA glycosylases excises a damaged base from DNA, leaving an apurinic/apyrimidinic (AP) site; then an AP endonuclease cleaves DNA at the AP site providing a free 3′-OH terminus, which is further used by a DNA polymerase to incorporate a normal dNMP [2]. Finally, DNA ligase restores the integrity of the formerly damaged strand.

In actively proliferating cells, such as cells of the developing embryo, DNA repair is crucial for preventing accumulation of mutations and synchronizing cell division. Sea urchin embryo

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growth was analyzed and extracts were prepared. The relative activity of DNA polymerase, apurinuc/apyrimidinic (AP) endonuclease, uracil–DNA glycosylase, 8-oxoguanine–DNA glycosylase, and other glycosylases were analyzed using specific oligonucleotide substrates of these enzymes; the reaction products were resolved by denaturing 20% polyacrylamide gel electrophoresis [2]. We have characterized the dynamic of relative change of the activity of several key base excision repair a in the developing embryos during 26 stages (2 blastomers to mid-pluteus) of the grey sea urchin, *Strongylocentrotus intermedius*.

The uracil–DNA glycosylase specific activity sharply increased after blastula hatching, whereas the specific activity of 8-oxoguanine–DNA glycosylase steadily decreased over the course of the development (Fig. 1).

The AP-endonuclease activity gradually increased but dropped at the last sampled stage (mid-pluteus 2) (Fig. 1). The DNA polymerase activity was high at the first cleavage divisions and then quickly decreased, showing a transient peak at blastula hatching. It seems that the developing sea urchin embryo encounters different DNA-damaging factors early in development within the protective envelope and later as a free-floating larva, with hatching necessitating adaptation to the shift in genotoxic stress conditions. No correlation was observed between the dynamics of the enzyme activities and published gene expression data from developing congeneric species, *S. purpuratus* [2]. The results suggest that base excision repair enzymes may be regulated in the sea urchin embryos at the level of covalent modification or protein stability.

It was proposed that most biological processes are performed by different protein complexes. In contrast to individual proteins and enzymes, their complexes usually have other biological functions, and their formation may be important system process for the expansion of diversity and biological functions of different molecules. Identification and characterization of embryonic components including proteins and their multi-protein complexes seem to be very important for an understanding of embryo function. We have isolated and analyzed for the first time a very stable multi-protein complex (SPC, ~1100 ± 100 kDa) from the soluble fraction of extracts of

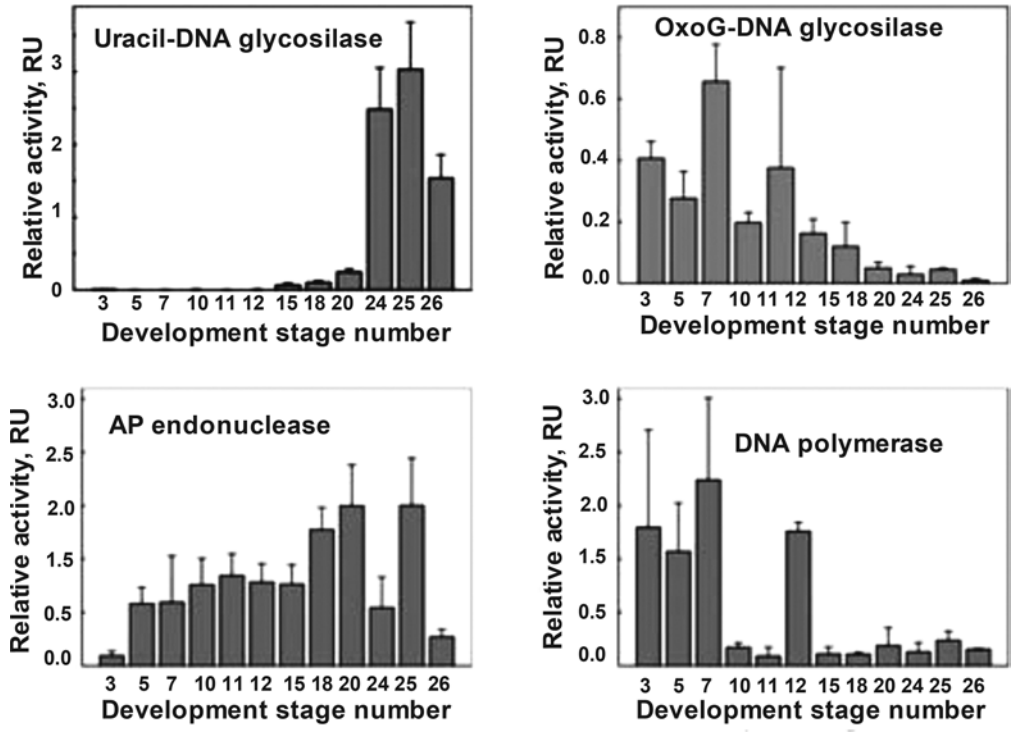

Fig. 1. Dynamics of changes in the relative activity of repair enzymes and DNA polymerase during the 26 stages of the development of sea urchin embryos

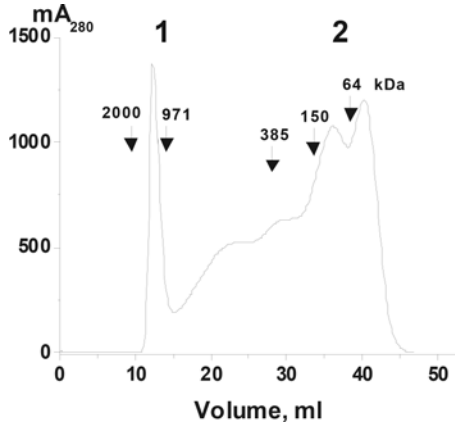

Fig. 2. Separation of very stable protein complex (SPC, peak 1) from other proteins (peak 2) by gel filtration on a Sepharose 4B columnthe extract of urchin's embryos

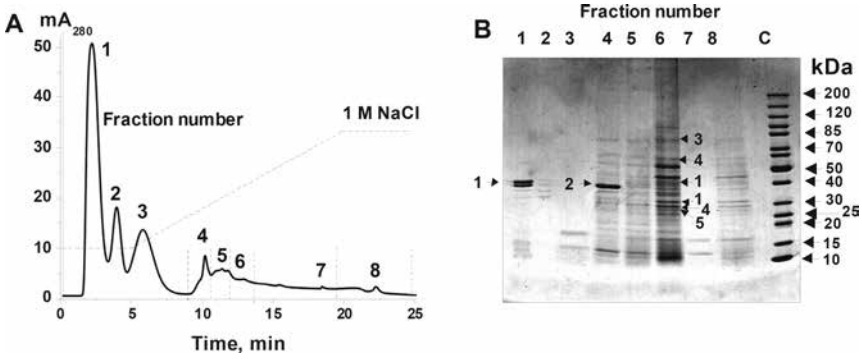

Fig. 3. Proteins of partially destroyed SPC by its incubation with buffer containing 8 M urea and 1 M NaCl were separated by ion exchange chromatography on Poros HQ (A); (—), absorbance at 280 nm (A_{280}). The SPC proteins corresponding to fractions 1-8 (A) were separated by SDS-PAGE and their molecular masses were estimated (B)

the sea urchin embryos [1]. By FPLC gel filtration the SPC was well separated from other extract proteins (Fig. 2).

SPC is stable in different drastic conditions but dissociates moderately in the presence of 8 M urea+1.0 M NaCl. According to SDS-PAGE data, this complex contains many major, moderate and minor proteins with molecular masses (MMs) from 10 to 95 kDa (Fig. 3).

The SPC was destroyed by 8 M urea or SDS, and its components were separated using thin layer chromatography (TLC), ion-exchange chromatography, gel filtration, and reverse phase chromatography (RPC). Using MALDI mass spectrometry of partially dissociated SPC, it was shown, that the complex contains not only proteins (10-95 kDa), but also few dozens of peptides with MMs from 2 to 9.5 kDa (Fig. 4).

Short peptides form very strong complexes, which at the treatment of SPC with urea or SDS can be partially break down into smaller protein-peptide complexes having different peptide compositions. Reverse phase chromatography of these complexes after all type of above-mentioned chromatographies led to detection from 6 to 11 distinct peaks corresponding to new complexes containing up to a few dozens of peptides (Fig. 4). The SPCs possess alkaline phosphatase activity.

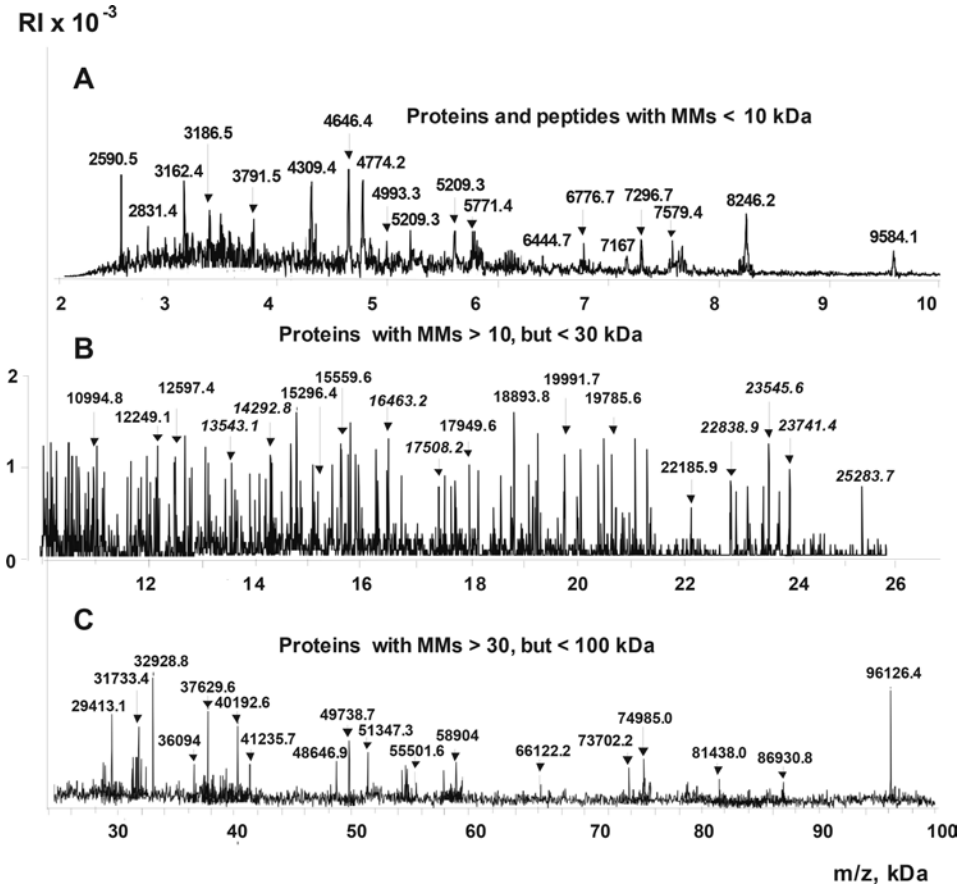

Fig. 4. MALDI mass spectra of three fractions of SPC proteins and peptides separated by sequential filtration using membranes with filters skipping proteins with MMs lower 100, then < 30, and finally <10 kDa. Before the filtration the intact SPC was boiled for 5 min with buffer containing 1 % SDS

Thus, it was shown that the extracts of sea urchin eggs contain a very stable protein complex consisting of a large number of different proteins and peptides. Progress in the study of embryos protein complexes can help to understand their biological functions.

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