A SPECTROPHOTOMETRIC ASSAY OF TRIBUTYL Tin-INDUCED CREATINE KINASE RELEASE FROM HERRING SPERMATOZOA

KATARZYNA GRZYB, MICHAŁ RYCHŁOWSKI
and EDWARD F. SKORKOWSKI
Gdańsk University Biological Station, 80-680 Gdańsk-Sobieszewo and
Department of Molecular Virology, 80-952 Gdańsk, Kładki 24, Poland

Tributyltin is the most common organotin derivative used in antifoulant paints. Despite the prohibition of its use, it is still used in many industrial processes. Tributyltin has been found to be toxic to the sperm cells of aquatic organisms, and even to mammalian species. Sperm cells react rapidly to environmental pollution in water ecosystems. Embryotoxicity is the more sensitive endpoint of toxicity assessment.

Creatine kinase is an enzyme participating in ATP regeneration, which is the primary source of energy in living organisms. It was shown that creatine kinase from herring spermatozoa has especially high activity (about 452 μmols/min per gram of fresh semen). Creatine kinase could be a biomarker of sperm cell membrane degradation.

The aim of the study was to demonstrate the toxic effect of tributyltin on herring spermatozoa using a specific sperm viability kit (L-7011, Molecular Probes) to show live and dead sperm cells. This is a fluorescence-based method, where the sperm cells with intact membranes fluoresce green, and cells with damaged cell membranes fluoresce red. The effects were observed using confocal microscope (Nikon PCM2000). It was found that even a very low concentration of tributyltin (5 μM) caused a fall of spermatozoa viability in 6 hours. It means that herring spermatozoa are sensitive to tributyltin treatments.

We also monitored creatine kinase release from damaged spermatozoa into the surrounding medium containing different concentrations of tributyltin. The higher the concentration of tributyltin used, the more creatine kinase was released from spermatozoa. This correlates well with the number of dead herring spermatozoa in the sample.

Creatine kinase in spermatozoa was characterized as a different isoenzyme from the isoenzymes in the skeletal muscle, heart and brain. Creatine kinase from herring spermatozoa was run on cellulose acetate native electrophoresis and stained for activity. It was found that it had a different electrophoretic mobility to other isoenzymes. Preliminary results indicate that in herring spermatozoa there are two forms of creatine kinase. A similar situation is observed in herring skeletal muscle, where one of the isoforms is a cytosolic dimer and the other one is a mitochondrial octamer.