SALT OF HEAVY METALS AS EFFECTORS OF PEPTIDEHYDROLASE FROM *DROSOPHILA MELANOGASTER*

I. L. RYZHKO, A. M. ANDRIYEVSKYI, S. A. PETROV

Odessa I. I. Mechnykov National University, Ukraine;
e-mail: i.l.ryzhko@onu.edu.ua

The purpose of the presented work was to investigate the biochemical properties and functional activity of the alkaline peptidehydrolase in the intestine of Drosophila in the ontogenesis of the flies, with keeping them under standard conditions and in the presence of heavy metal salts (Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}) in the environment.

The anilidase activity of alkaline peptidehydrolase was determined by the hydrolysis of 1.0 mM chromogenic substrate N, α-benzoyl-L-arginine-p-nitroanilide (BAPNA), in 0.1 M glycine-NaOH buffer pH 9.0 at 382.5 nm. The esterase activity of the alkaline peptidehydrolase was determined by the hydrolysis of 1.0 mM N, α-benzoyl-L-arginine-ethyl ether (BAEE) in 0.1 M glycine-NaOH buffer pH 9.0 at 253 nm. Specific activity of alkaline peptidehydrolase was determined in mU related to 1 mg of total protein of the investigated tissue extract or enzyme preparation. The influence of metal cations on the activity of alkaline peptidehydrolase was investigated using solutions of chlorides of Co^{2+}, Cu^{2+}, Zn^{2+} и Cd^{2+} at the final concentration: 0.2, 0.4, 1.0 and 2.0 mM.

Pre-incubation with 0.2-0.4 mM $CoCl_2$ and $CuCl_2$ increased the activity of the alkaline peptidehydrolase of the original extract of tissues of the Drosophila larvae of the Normal line, respectively, by 21.6 and 11.2% and led to a decrease in purified enzyme activity by 10.8 and 27.5%. In the presence of 1.0 and 2.0 mM $CoCl_2$, the activity of both purified and non-purified enzyme was suppressed by more than 50%. Reduced purity of enzyme activity by 88-95% and complete inhibition of the activity of the crude enzyme were established by pre-incubation with 2.0 mM $CuCl_2$ and $ZnCl_2$ solutions. The activity of the alkaline peptidehydrolase of the extract of the Muller-5 line increased by 15.0-25.7% in the presence of 0.2 mM $CoCl_2$ and $CuCl_2$. In the presence of 1.0-2.0 mM $CoCl_2$, the purified and untreated enzyme activity was suppressed by 20 and 67%, respectively. Complete inhibition of the activity of crude and purified enzyme was determined by pre-incubation with 2.0 mM of $ZnCl_2$ solution and 2.0 mM of $CuCl_2$ solution. The obtained data indicate inhibition of activity of alkaline peptidehydrolase of Drosophila larvae of both lines in vitro with high concentrations of chlorides Co, Cu, Zn and Cd.

The influence of metal cations on the activity of alkaline peptide glycosylates of larvae of both lines of Drosophila can be explained by non-specific irreversible inhibition associated with the adsorption of metal ions on the surface of the protein molecule and their interaction with functional active groups, which leads to conformational changes in the enzyme molecule or their effect on the active center.