THE MECHANISMS INHIBITING PYRUVATE- AND OXOGLUTARATE DEHYDROGENASE COMPLEXES IN MUSSELS

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Abstract

Activity of purified pyruvate dehydrogenase and 2 - oksoglutarate dehydrogenase multienzyme complexes was studied and a quantity of free SH-groups in them being influenced by chlorides of cadmium, copper and lead was determined. The conducted research proved a considerable activity impairment of dehydrogenases of oxoacids and of the free SH – groups level.

Keywords: Heavy metals, activity of multienzyme complexes, SH-groups level

Introduction

The world scientific literature contains a considerable bulk of data describing a study of the impact which heavy metals produce on marine aquatic organisms, particularly on mussels, and accumulation of certain substances in their body tissues, survival of marine aquatic organisms, impact on their physiological functions and activity of various enzymes, etc. (Bren' 1999; Soldatov et al., 2005; Gostyukhina, 2005). However, the influence of heavy metal compounds upon the activity of the purified dehydrogenase of the Krebs cycle was studied in the individual works only. Therefore, the purpose of our work was to study the activity of purified pyruvate dehydrogenase (PDC) and 2 - oksoglutarate dehydrogenase (OGDC) multi-enzyme complexes isolated from the hepatopancreas of mussels, and the level of free SH - groups in them being influenced by chlorides of such heavy metals as cadmium, copper and lead (Cu, Cd, Pb).

Materials and methods

We selected the Black Sea mussels Mytilus galloprovincialis Lam. Black morphs of 4-5 cm size. The adaptation period equalled 5 days and afterwards the mussels were used for experiments. Effects of various concentrations (0.1, 1.0 and 10.0 mg/l) of Cu, Cd and Pb chlorides upon the activity of purified mussel hepatopancreas according to (Roche & Cate, 1977) dehydrogenase oxoacids of Krebs cycle (the activity was determined by Potassium ferricyanide method (Kiessling & Lunquist, 1962), and the concentration of SH - groups - by the method using the Ellman's reagent (Orekhovych, 1977). The obtained data was statistically processed according to S. Glantz (Glantz, 1998).

Results

The results of the research are presented in Table 1.

As follows from Table 1, the activity of purified PDC and OGDC reduced pro rata when exposed to the increasing concentrations of $CdCl_2$ and PbCl2; as regards the action of

$CuCl_2$ in the incubated environment, the activity of both enzyme complexes was strongly inhibited at all concentrations, especially of the OGDC at 1 mg/l concentration.

Having obtained the data that confirmed a considerable inhibition of the PDC and OGDC activity by heavy metal chlorides, we decided to clarify a possible mechanism of this phenomenon. It is well known that many heavy metals found in tissues of humans and mammals can inhibit activity of a diversity of enzymes due to blocking their SH-groups that participate in the catalytic process either directly or indirectly.

Table 1. Activity of purified pyruvate dehydrogenase complex (PDC) and 2-oxoglutarate dehydrogenase (OGDC) multienzyme complexes and concentration of SH-groups in hepatopancreas of the Black Sea mussels Mytilus galloprovincialis Lam. being influenced by Cu, Cd and Pb chlorides in 0.1, 1.0 and 10 0 mg / l concentrations

		PDC		OGDC	
		Activity, nM $K_3[Fe(CN)_6]$/mg protein/min	SH-groups, nM/mg protein	Activity, nM $K_3[Fe(CN)_6]$/mg protein/min	SH-groups, nM/mg protein
Control	-	263.19±5.72	67.76±3.54	266.46±1.39	75.46±3.77
Cd mg/ml	0.1	210.55±8.55*	47.37±1.09*	243.26±10.83	52.69±0.40*
	1	124.73±8.86*	21.05±0.96*	168.59±6.28*	33.50±0.40*
	10	88.11±7.24*	13.82±0.40*	101.83±7.66*	14.31±0.61*
Cu mg/ml	0,1	35.47±6.35*	8.68±0.32*	202.53±7.80*	44.50±0.64*
	1	26.32±7.60*	7.10±0.34*	16.97±6.83*	1.76±0.24*
	10	32.04±6.85*	7.90±0.32*	43.00±3.72*	11.38±0.52*
Pb mg/ml	0,1	127.02±7.53*	31.25±0.52*	168.59±7.80*	31.44±0.84*
	1	107.57±5.91*	24.67±0.74*	118.80±10.83*	26.67±0.40*
	10	34.33±9.34*	8.88±0.40*	61.10±6.28*	17.24±0.40*

Note: * significant difference from control group equals $p \leq 0.05$.

Therefore it was necessary to investigate whether a similar phenomenon takes place in case of enzymes of the mussel tissue. To this end, we determined the number of SH-groups in the purified enzymes of the control group and in the presence of the cadmium, copper and lead salts (Table 1). According to the obtained data, the salts of all investigated heavy metals reduced the content of SH-groups in the enzymes in a dose-dependent manner, following the almost similar regularities that govern the changes in their activity.

Conclusion

The conducted study has proved that at the level of the purified enzymes of PDC and OGDC copper exhibited the highest inhibitory activity as compared to other studied metals – cadmium and lead. Inhibition of the PDC and OGDC activity occurs due to blocking SH-groups of enzymes.

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