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ROLE OF FUNCTIONAL SH GROUPS ON THE ACTIVITY EXPRESSION AND CONFORMATION MAINTENANCE OF *RANA RIDIBUNDA* FROG LIVER ARGINASE

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After inactivation by **e**thylenediaminetetraacetic acid (EDTA) the subunits (dimer, monomer) of *Rana ridibunda* frog's liver arginase differs by sensitivity towards thiol reagents. Moreover, the particular activation of subunits is also observed at the presence of p-chloromercuribenzoate (p-CMB). It is notable, that p-CMB significantly decreases the activity of reactivated enzyme in the presence of Mn^{2+} ions, despite the fact that in native extracts the enzyme is not inhibited by the thiol reagent. Here we discussed the effect of p-CMB in the inactivation process not only as a result of SH groups' modification, but also because of structural changes in native conformation by EDTA and non specific reaction of p-CMB with other functional groups.

***Keywords*:** thiol reagent, dimer, monomer.

Introduction. Investigation of the effect of different reagents on the enzyme can illustrate the nature of the enzyme active site's functional groups and the participation of them in the enzyme specific conformation maintenance. The organic compounds of mercury (Hg), especially p-chloromercuribenzoate (p-CMB) that react with SH groups and produce mercaptides are considered as specific and sensitive reagents for SH groups. According to some results, pCMB does not effect on rat liver and human erythrocyte's arginase, as well as on the ox liver [1–3] and sea mollusc *C. concholepas* arginases [4]. It has been clarified that birds, crayfish and peas arginases are inhibited by mentioned reagents [5–7]. According to the authors, depending on the studied object, arginase is either inhibited, or does not react or even is activated by different sulfhydryl reagents. For example, mammalian liver's ureotelic arginase doesn't contain activity responsible free SH groups, which means that it is not inhibited by thiol reagents [3]. Thus, to clarify the question of how p-CMB effect on the activity of amphibians (*R. ridibunda*) liver ureotelic arginase, also on the inactivated subunits by ethylenediaminetetra-acetic acid (EDTA) and further reactivated enzyme's activity herein, we illustrate the effect of functional SH groups on the conformation maintenance of enzyme during reversible inactivation by chelating agents at different periods of time.

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Materials and Methods. *Rana ridibunda* Pallas adult frogs are served as a research object (150–200 *g*), and was homogenized in 0.05 *M* Tris-HCL buffer, pH 7.4 at 20^0C. Liver extracts final concentration is 5%, which were prepared by centrifugation at 20 000 *g* for 30 *min*. As previously described in our study enzyme activity was measured by Ratner and Pappas method, subsequently the urea, which emerges by substrate dissection, was measured according to the method of Archibald, modified by Moore and Kauffman [8]. The absorbance of protein was measured in a spectrophotometer Genesys 10S UV-VIS at 280 *nm*. Enzyme activity is expressed by emerged urea ($\mu M/g$ tissue). The inactivation is done by EDTA, which concentration is $5 \cdot 10^{-2}$ *M* per 1 *mL* of studied sample. The reactivation of inactivated enzyme is done by 0.005 *M* glycine–NaOH buffer, pH 9.5 at 25^0C in the presence of Mn^{2+} ions (25 μM/ 1 *mL*) during 22 *h*.

Results and Discussion. In the current study the effect of p-CMB in different concentrations on the activity of *R. ridibunda* frog's liver arginase was investigated. In order to study this issue enzyme was preincubated at 25^0C, pH 9.5 with 1×10^{-4}, 3×10^{-4} and 5×10^{-4} *M* concentration of p-CMB, respectively. Subsequently, the preincubated enzyme was further incubated for 1 *h* at 37^0C with appropriate concentration of p-CMB at the pH 9.5. The results are presented in Tab. 1.

Table 1

The effect of p-CMB in different concentrations on the activity of R. ridibunda frog's liver arginase (n = 6, p = 0.005)

[p-CMB], *M*	Activity, $\mu M/g$
Control (–p-CMB)	29300 ± 293
1×10^{-4}	29300 ± 183
3×10^{-4}	29262 ± 205
5×10^{-4}	29383 ± 265

According to the results obtained, we can conclude that the aforementioned concentrations of p-CMB have no effect on the enzyme activity. It is interesting that similar results were obtained for *Rana terrestris* frog's [9], rat's [2] and human's liver [10] arginases, which shows that these arginases do not contain activity responsible SH groups.

The effect of p-CMB on the activity of inactivated enzyme by EDTA (1 and 2 *h*) and subsequently reactivated arginase has also been studied. Reactivation was done in the presence and absence of Mn^{2+} ions. The results are presented in Tab. 2. It should be noted that *R. ridibunda* frog's liver inactivated arginase dimers (1 *h*) as well as monomers (2 *h*) by EDTA, display the same properties as chicken brain, kidney and liver [11], rats mammary glands [12] and polychaete annelid's [13] tissues arginases, which are stimulated by p-CMB.

As one can notice from the results (Tab. 2) at subunits level (dimmers and monomers) in the presence of p-CMB observed slight stimulation of activity, 6 and 5% respectively. It can be assumed, that active SH groups are not detected in emerged subunits (dimer, monomer), when Mn^{2+} ions removed by EDTA.

Table 2

The effect of p-CMB on the R. ridibunda frog's liver Inactivated (by EDTA) and reactivated arginase (n = 6, p = 0.005)

Sample	Activity			
	(–p-CMB), *μM/g*	Inactiv. and reactive. %	(+p-CMB),*μM/g*	Inactiv. and reactive. %
Native	29300 ± 293	–	29383 ± 265	–
inactivated by EDTA, 1 *h*	7325 ± 153	75	9669 ± 184	67
inactivated by EDTA, 2 *h*	5049 ± 231	82.7	6525 ± 225	77.7
reactivated for 22 *h*, pH 9.5 + Mn^{2+}	21096 ± 304	72	11048 ± 286	37.7

The effect of p-CMB on the reactivated enzyme has been shown, that in this conditions the activity of enzyme decrease, in the case when in native extracts enzyme does not inhibited by p-CMB. In order to explain this fact we also been investigated the effect of p-CMB on the reactivation process of enzyme. The results are presented in Tab. 3.

Table 3

The effect of p-CMB on the reactivated process of R. ridibunda frog's liver arginase (n = 6, p = 0.005)

Sample	Activity			
	(–p-CMB), *μM/g*	reactivation, %	(+p-CMB), *μM/g*	inhibition of reactivation process, %
Native	29300 ± 293	–	29383 ± 265	–
reactivated for 22 *h*, pH 9.5 – Mn^{2+}	15580± 184	53	9790± 286	37
reactivated for 22 *h*, pH 9.5 + Mn^{2+}	21096± 304	72	12657± 286	40

It is notable that p-CMB suppresses the reactivation process and the reactivation inhibited by 37 and 40% respectively. According to the authors, such effect of p-CMB on the reactivated enzyme supposedly is due to the fact that p-CMB leads to enzyme inactivation not always as a result of SH groups' modification, but also because of structural changes in native enzyme while the thiol reagent reacts with other functional groups [16]. Besides, it has been shown that in some cases p-CMB can repress the activity of the enzymes, which generally do not contain sulfhydryl groups and/or is due to the not specific interactions between this reagent and protein [17].

Previously it has been shown that in rats mammary gland's arginase the SH groups are detected only after dialysis during iodoacetamide reaction [14]. It is likely that Mn^{2+} ions bind to the enzyme active site's SH groups and since it is known that the metal ions can cause chelating structure with cysteine and histidine, which lead to the metal-protein complex creation [15], therefore, the removal of Mn^{2+} ions by EDTA or dialysis leads to the emerge of free SH groups, which are reacted with p-CMB or iodoacetamide.

In closing, based on the obtained facts, it is important to note that at this stage of investigation it is truly difficult to conclude the availability of free SH groups in the structure of reactivated enzyme. Therefore, the additional studies may further illuminate these aspects of coordination chemistry and catalysis.

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