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ROLE OF LOW-WEIGHT PROTEIN FRACTIONS IN THE PROCESS OF REVERSIBLE INACTIVATION OF WHITE RAT LIVER ARGINASE

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The reversible rat liver arginase at low pH conditions was investigated. It is shown that after 12 *h* pH 3.6 enzyme inactivation is able to recover its activity at the presence of different factors. Due to hypoxia 2 protein fractions (molecular weight of 55650–71200 *Da*) were indused, which then were added to the reactivation medium. It is reported that the addition of these fractions leads to reactivation up to 72%. Therefore, it is possible that during our experiments low weight proteins have chaperon-like properties, which assist with folding process.

***Keywords*:** coprinoid mushrooms, mycelium, fatty acids, GC analysis.

Introduction. It is known, that there are 2 different types of arginases, ureotelic which occurs only in the liver of ureotelic organisms and participates in ammonia neutralization and non-ureotelic arginase, which is found in all organisms, regardless the type of nitrogen and ammonia excretion of cell metabolism and has other functions. It is already proved that these two forms of arginases differ from the type of induction, immunological properties, suppression of substrate excess, electorophoretic mobility output, isoelectric point and other properties [1].

Nowadays it is proved that the formation of mechanisms of ureotelism, induction of ureotelic arginase is a significant issue, thus in the chromosomes of all organisms information of different forms of arginase exists [2]. It can be said that this opportunity during ontogenesis provides organisms optimum adaptation to the environment condition changes, in terms of isofunctional proteins (isoenzymes).

Ureotelic and nonureotelic isoensymes of arginases of different origin, their physico-chemical properties, including some of the kinetic and structural features have been studied in the Biochemistry chair of YSU for many years. For optimal enzymatic activity the conformation of enzyme is significant. Even slight changes in conformation, which occur due to temperature, hydrogen ion concentration changes and the effect of chemical reagents can affect enzymatic reaction, speed, K_m value, thermostability and other properties of enzyme. Revealing the conditions, which lead to stable native conformation and functional activity of particular enzyme, are important not only in practice, but also for understanding its mechanisms of regulation.

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Inactivation and further reactivation of enzyme by different methods are considered as one of the prevailing methods of investigations of proteins and also isofunctional protein structural characteristics. It has been shown, that enzymatic activity of the arginases of different origin decrease under the low pH conditions. Inactivation of purified rat liver arginase under the pH 3–5 occurs slowly, but below the pH 3 the inactivation intensity rises sharply [3]. Bovine arginase below the pH 4 is inactivated also very fast [4]. Purified rat liver arginase is totally inactivated in case of pH 2 within 10 *min* [5]. Under the low pH conditions initially inactivated arginases are reactivated while incubating under the pH 7–9 in presence of Mn^{2+} ions [6]. Supposedly the reactivation of the enzyme is due to the recovering of its oligomeric structure [7].

During the acid-based inactivation the dissociation of enzyme molecule and probably also degradation of disulfide bonds occur when tertiary structure is changed [8]. It is notable that, according to the authors, during the acid-based inactivation the recovery of native structure and consequently the activity of enzyme doesn't always occur, as it has been shown for amphibians, when an octamer is formed instead of tetramer. In authors opinion during the inactivation the right folding of enzyme subunits is distorted, because of the removal or dilution of molecular chaperons. It has been shown that chaperons do not change the output of folding, but they prevent the aggregation of protein by the formation of nonfunctional products. They increase the speed of folding thus preventing the number of these products [9, 10].

In this investigation during the reversible inactivation by pH 3.6 we studied the role of protein chaperons in the reactivation and stabilization process of rat liver ureothelic arginase. The synthesis of chaperons (heat-shock proteins) signify-cantly increase stressful conditions in cell's. It is known that each cell contains certain amount of chaperons in physiological conditions, which are essential for its metabolism [11]. Due to the fact that chaperons are considered as stress proteins, the amount of considerably rises during the stress, we studied the reactivation process in hypoxia treated animals also.

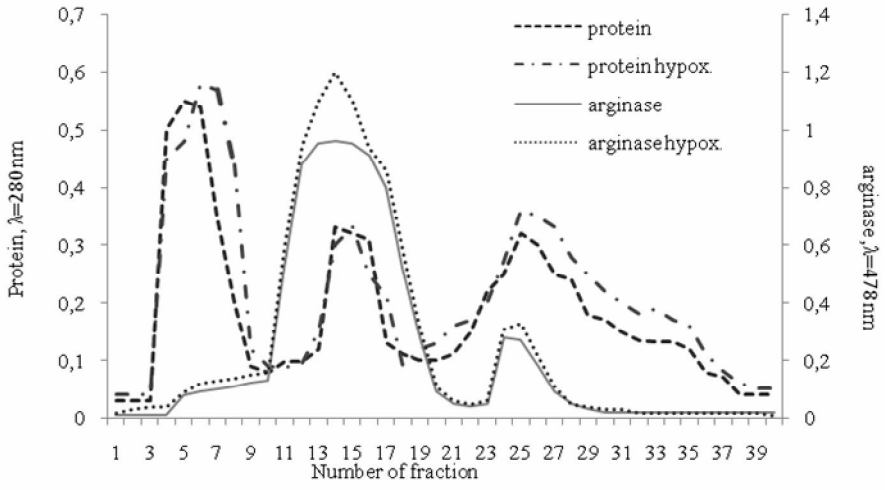
As it is already known that biological oxidation is suppressed in hypoxia, and as a result the level of ATP and creatine tri-phospate decreases and increases the amount of ADP and inorganic phosphate. In this conditions the lack of energy causes metabolic disorders, which leads to the disturbance of cell's specific and non-specific functions up to irreversible disorders.

Materials and Methods. White laboratory rats are served as a research object. The rat liver arginase homogenate concentration is 10%, which was prepared by 0.005 *M* Tris-HCl buffer (pH 7.4). The supernatant is obtained by centrifugation in 25000 *g*, 30 *min*. The 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 [12]. The absorbance of protein was measured in spectrophotometer (Genesys 10S UV-VIS) at 280 *nm*. The enzyme was purified by Gel-filtration method (Sephadex G-200 Uppsala, Sweden). The equilibration and elution was done with buffer solution containing 0.005 *mol* Tris-HCl (pH 7.4) at 22°*C*, elution volume is 4 *ml* and the velocity is 20 *mL* per hour.

Enzyme was treated with 0.05 *M* glycine-HCl buffer (pH 3.6) and the reactivation was done by 0.01 *M* Tris-HCl buffer (pH 7.4) In the reactivation environment Mn^{2+} bivalent cation, 25 *μmol* per 1 *mL* to the studied sample were used. Low-weight proteins were obtained from the liver of normal and hypoxia treated rats, which initially were kept in the special camera for 15 *min*, where the atmosphere pressure corresponds to the altitude of 6000 *m* a. s. l.

Results and Discussion. As one can notice from literature, a protein molecule spontaneously accept thermodynamically certain conformation under the hydrophobic and hydrophilic interactions, and the partial or complete recovery is possible, when the native conformation is changed. Any globulin protein *in vitro* is able to refold spontaneously. But this process differs from *in vivo* refolding, as in this case functionally active structure should be formed, which is unique for a particular protein.

In the current study we investigated the reactivation properties of initially inacativated (at the pH 3.6) partially purified enzyme by adding low-weight protein derived from the liver of normal and hipoxia treated rats. The reactivation was done in pH 7.4, 0.01 *M* Tris-HCl buffer, in the presence and absence of Mn^{2+} ions. Low-weight protein, which was derived from partially purified samples of rat liver homogenates and do not have arginase activity were added to the reactivation environment. This proteins (molecular weight of 55650–71200 *Da*) were purified by Sephadex G-200. In this study we attempt to investigate their chaperon-like activity during the reactivation process. The results are presented in the tables and figures.



Fractionation of rat liver homogenate by Sephadex G-200.

Due to our experiments the rat liver ureotelic arginase is eluted from 12 to 18th fractions. We choose the low-weight proteins, according to literature data [10], that the molecular weight of chaperones and chaperone-like proteins which are between 60000–70000 *Da*. In the reactivation environment in one case the proteins with 66100–71200 molecular weight (29, 30 fractions) were added and in the other case the proteins with 55270–60850 molecular weights (31, 32 fractions).

Table 1

The reactivation of initially inactivated (12 h, pH 3.6) partially purified rat liver arginase in pH 7.4 by adding 29, 30 and 31, 32 low-weight protein fractions (n=6, M±m, p<0,05)

Extent of reactivation	Partially purified arginase	Activity of arginase sample, *μmol/mL*		
		–	React. % compared with native	React./inact.
	Native	3.680+/–0.454	–	
	Inactivated 12 *h*	0.739+/–0.120	–	
12 *h*	– Mn^{2+}	1.656+/–0.331	45	
	+ Mn^{2+}	2.208+/–0.452	60	
	29, 30 protein fraction – Mn^{2+}	1.729+/–0.378	47	
	29, 30 protein fraction + Mn^{2+}	2.318+/–0.245	63	
	31, 32 protein fraction – Mn^{2+}	1.840+/–0.156	50	
	31, 32 protein fraction + Mn^{2+}	2.428+/–0.245	66	

As one can notice from Tab. 1, the reactivation in the absence of Mn^{2+} ions is 45%, and in the presence of Mn^{2+} ions it is 60%. By adding low-weight protein fractions (29, 30) to the reactivation environment the reactivation increases slightly, by 2–3%. In the presence of manganese ions the reactivation is 59% and in the absence of Mn^{2+} is 65%. The addition of 31, 32 fractions reactivation increases by 5–6%.

According to the results of Tab. 1, we can conclude that during the reactivation of initially inactivated enzyme in the presence of low-weight protein fractions occur conformation changes, which reflect on monomer/oligomer proportion and probably leads to the stabilization of oligomeric structure of enzyme.

Table 2

The reactivation of initially inactivated (12 h, pH 3.6) partially purified rat liver arginase in pH 7.4 by adding 29, 30 and 31, 32 low-weight protein fractions derived from hypoxia treated rats (n=6, M±m, p<0,05)

Extent of reactivation	Partially purified arginase	Activity of arginase sample, *μmol/mL*		
		–	React. % compared with native	React./inact.
	Native	3.680+/–0.454	–	–
	Inactivated 12 *h*	0.739+/–0.120	–	–
12 *h*	– Mn^{2+}	1.656+/–0.331	45	2.240
	+ Mn^{2+}	2.208+–0.452	60	2.987
	29, 30 protein fraction – Mn^{2+}	2.318+/–0.115	63	3.136
	29, 30 protein fraction + Mn^{2+}	2.649+/–0.251	72	3.485
	31, 32 protein fraction – Mn^{2+}	1.913+/–0.475	52	3.584
	31, 32 protein fraction + Mn^{2+}	2.502+/–0.290	68	3.385

In order to investigate the effect of low-weight proteins obtained from hypoxia treated rats livers during the reactivation (Tab. 2) we added 29, 30 and 31, 32 protein fractions to the reactivation environment, the amount of which significantly increases in case of hypoxia (see Figure).

Concluding the results of Tab. 2, it is notable that the reactivation significantly increase by adding 31, 32 protein fractions to the reactivation environment, which leads to 63% reactivation in the absence of manganese ions and 72% reactivation in the presence of ions. It should be also mentioned that reactivation maximal results obtained in the presence of manganese and the addition of low-weight proteins from the liver of hypoxia treated rats leads to higher percentage of reactivation.

In closing, our results showed that the addition of low-weight proteins increase the reactivation up to 72%. Evidently mentioned proteins have chaperon-like properties, which assist the folding process. Therefore, the additional studies may further illuminate the function and properties of this protein.

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