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STUDY OF PROTEIN MEDIATORS "SELF-FORMATION" PROCESSES IN RABBIT LIVER ARGINASE AS EXAMPLE

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The aim of the work was to study changes in the structure of oligomer during reverse inactivation on the example of a hepatic arginase of a rabbit to understand the unique aspects of type I mammalian hepatic arginase. It was shown that by presence of Ni^{2+} cation the "self-formed" oligomer is 1.3-1.8 times more active than the reactivated oligomer by presence of Mn^{2+} cation. It was also shown that the enzyme's physiological stimulant Mn^{2+} almost 20% inhibits the catalytic activity of Ni^{2+} oligomer.

Reverse inactivation – "self-organized" – "molten globule" – hepatic arginase I – Ni^{2+} oligomer

Աշխատանքի նպատակն է եղել ճագարի լյարդի արգինազ I օրինակով ուսումնասիրել դարձելի ապաակտիվացման գործընթացում օլիգոմերային կառուցվածքի կրած փոփոխությունները, պարզաբանելու համար կաթնասունների լյարդի I տիպի արգինազի յուրահատուկ կողմերը: Պարզվել է, որ Ni^{2+} կատիոնի առկայությամբ «ինքնահավաքված» օլիգոմերը 1,3-1,8 անգամ ակտիվ է ի համեմատ Mn^{2+} կատիոնի առկայությամբ ռեակտիվացված օլիգոմերի: Ցույց է տրվել, որ ֆերմենտի ֆիզիոլոգիական խթանիչ Mn^{2+} մոտ 20 %-ով արգելակում է §ինքնահավաքված¦ Ni^{2+} օլիգոմերի կատալիտիկ ակտիվությունը:

Դարձելի ապաակտիվացում – «ինքնահավաքում» – «հալված գլոբուլ» – լյարդային արգինազ I – Ni^{2+} օլիգոմեր

Целью работы являлось изучение изменений структуры олигомеров в процессе обратимой инактивации на примере печеночной аргиназы I кролика, с целью выяснения уникальных сторон печеночной аргиназы I типа млекопитающих. Было показано, что в присутствии катиона Ni^{2+} "самоорганизующийся" олигомер в 1,3-1,8 раза активнее, чем реактивированный олигомер в присутствии катиона Mn^{2+}. Также было показано, что физиологический активатор фермента Mn^{2+} почти на 20 % ингибирует каталитическую активность Ni^{2+} олигомера.

Обратимая инактивация – "самоорганизация" – "расплавленная глобула" – печёночное аргиназа I – Ni^{2+} олигомер

Formation or "self-formation" of the spatial structure of a globular protein is a complex and phase process.

Not all proteins retaining their primary amino acids sequence may be renatured *in vitro*, as protein aggregation is a barrier to renaturation. A big complexes of fibrillar

proteins and many globular oligomeric proteins aren't usually renatured. Membrane proteins, which can reach to their functional state only with lipids' participation, are difficult to renature [1].

The basic experimental facts and other simple physical models have been observed by several authors, who explain the processes occurring during "self-formation". This allows us to evaluate the rate of proteins' "self-formation" and identify the most important part of "self-formation" - "core packing". The process of forming a three-dimensional structure from a polypeptide chain is one type of denaturation study and subsequent renaturation [3].

Loss of ability to restore the native conformation of individual proteins in vivo leads to the formation of several pathological conditions of the organism. Studying the mechanisms of regulation of nitrogen metabolism is one of the important problems of modern biochemistry. And the study of this problem will allow us to develop targeted resources, those will regulate the vital functions of organisms.

The task of the presented work based on modern approaches to the "self-formation" of proteins' structure in order to study reverse inactivation of the globular-hydrophilic- oligomeric type I arginase protein and oligomer's structure changes during that time. The purpose is to understand the unique aspects of type I mammalian hepatic arginase.

Materials and methods. The main experimental facts were discovered by us: an example of a model of reverse inactivation of type I hepatic arginase and experiments were carried out for evaluating the rate of denatured proteins' "self-formation" and for underlining main factors influence on this process.

The experimental object is a rabbit ***(****ORYCTOLAGUS CUNICULUS DOMESTICUS)* with the mass of 1600-2400 grams. The animals were kept a temperature of 18-23°C, a correlation 12/12 hour night/morning with a standard diet.

Animals liver were used for experiments. 2.5 and 10 % homogeneities of the test tissue were prepared using distilled water. The homogeneity was centrifugate 20.000 G 30min at 0-4°C in cold conditions (ЦЛР-1, Russia). The extracted extract served as a source of enzyme. The enzyme hydrolyzes L-arginine to L-ornithine and urea. The optical density of the final product (urea) is determined of spectrophotometric method (Genesys 10S UV-Vis, Thermo scientific, USA) at a light length of 478 nm.

The acid inactivation of enzyme was carried out by affect to 0.05 M glycine-HCl buffer (pH 9,5), under conditions of +20°C and +4°C, in the presence of divalent cations: Mn^{2+} (physiological stimulant) and Ni^{2+} chlorides (25 μmol in 1 ml of test tube). During the experiments were also studied those variants of the enzyme preparation I arginase, where the concentration of Ni^{2+} 12.5μmol in 1 ml of test tube, taking into consideration that fact, that high concentration of metal ions in some cases have an inhibitory effect.

Results and Discussion. Such as an experimental object was used partially pure 2.5 and 10 % enzyme preparations of rabbit liver's I arginase. We studied the role of Mn^{2+} and Ni^{2+} cations in acid inactivation and reactivation of rabbit liver's I arginase.

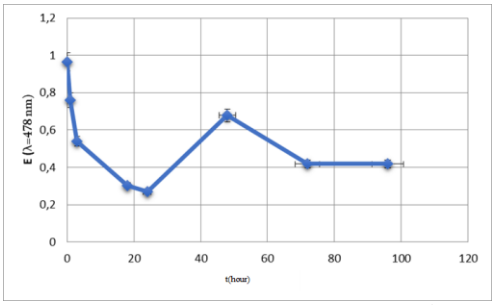

Fig. 1. Reverse inactivation (pH 4) and reactivation (pH 9.5) by presence of 25 μmol/ml Mn^{2+} (2.5 % enzyme preparation, n=5, $p<0.05$)

The results are presented in figures 1 and 2. The first figure we shows as a result of reverse inactivation (pH 4), the enzyme activity decreased by 68,5 % (18 h), and as a result of reactivation of the arginase activity by presence of 25 μmol/ml Mn^{2+} ion pH 9.5, after 24 h reactivation 70 % (fig.1).

In the presence of Ni^{2+} ion, enzyme activity after reactivation is 19 % higher, then by presence of Mn^{2+}(fig. 2).

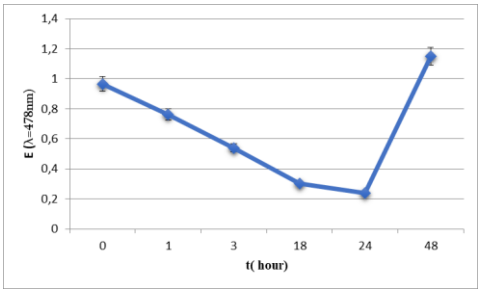

Fig. 2. Reverse inactivation (pH 4) and reactivation (pH 9.5) by presence of 25 μmol/ml Ni^{2+} (2.5% enzyme preparation, n=5, p<0.05)

The aim of the next phase of the experiments were to identify the difference in activity of the formed oligomers in the case of a difference in the concentration of Ni^{2+} cations. And study the effect of Mn^{2+} and Ni^{2+} cations on "self-formed" oligomers. The results of studies are presented in 3-5 figures. After 18 h inactivation (pH 4) enzyme's activity decreased by 59.5 %, and after 72 h reactivation arginase's activity, by presence of Mn^{2+} 25 μmol/ml and at pH 9.5 was 81 % (fig. 4). By presence of Ni^{2+} ion 12.5 μmol/ml, the activity of the "self-formed" oligomer was equivalent to the activity of reactivated Mn^{2+} oligomer (fig. 3). And in the case of 25 μmol/ml concentration of Ni^{2+} ion , the resulting oligomer was active by 10%, than starting point (fig. 5).

In separate phases of the "self-formation" process, a cation Mn^{2+}- physiological stimulant for the enzyme was added to the incubation environment of Ni^{2+} variants to understand its effect on the formed oligomers.

By presence of Mn^{2+} cation in incubation variants was recorded lower activity, which were 78 % and 98 % (comparative figures 3 and 5).

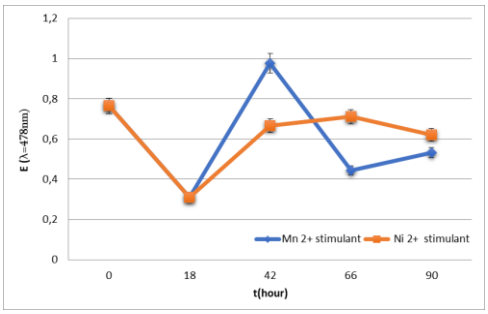

Fig. 3. Reverse inactivation (pH 4) and reactivation (pH 9.5) by presence of 12.5μmol/ml Ni^{2+} (10 % enzyme preparation, n=5, p<0.05)

At the same time, "self-formed" oligomers showed higher activity at the 24th and 48th hours of renaturation, then at the 72nd hour of renaturation.

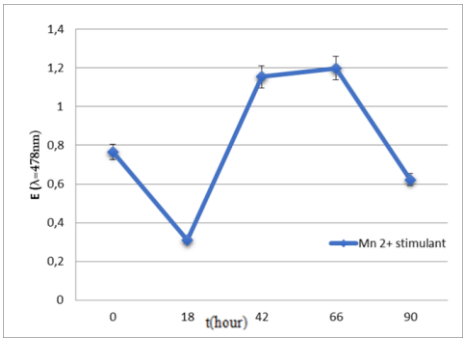

Fig. 4. Reverse inactivation (pH 4) and reactivation (pH 9.5) by presence of 25 μmol/ml Mn^{2+} (10% enzyme preparation, n=5, $p<0.05$)

According to Finkelstein[4] low temperature contribute to stabilization of native conformation and increase the packing rate. For this reason, in some experiments, the last phase of protein renaturation was carried out under +4ºC condition, and after 72 h of reactivation, the studied variants had 60-80 % higher activity, then starting point.

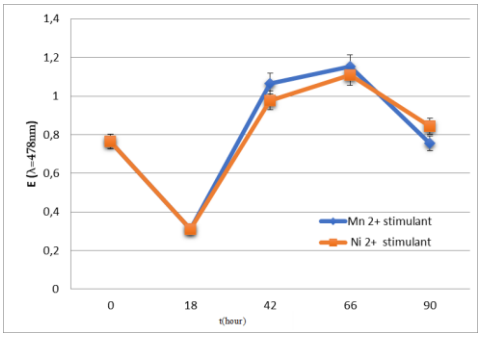

Fig. 5. Reverse inactivation (pH 4) and reactivation (pH 9.5) by presence of 25 μmol/ml Ni^{2+} (10% enzyme preparation, n=5, $p<0.05$)

Aggregation of deactivated subunits is a disruptive factor in the process of "self-formation", and the probability of aggregation increases with increasing temperature and protein concentration. Therefore, spontaneous packing of the polypeptide chain is more productive in dilute solutions and at low temperature [2].

By presence of Ni^{2+} ion (12.5 μmol/ml), the activity of the "self-formed" oligomer was equivalent to the activity of reactivated Mn^{2+}(25 μmol/ml) oligomer. In cause of equivalent concentrations Ni^{2+} and Mn^{2+} cations, by presence of Ni^{2+} cation "self-formed" oligomer is 1.3-1.8 times more active than the reactivated oligomer by presence of Mn^{2+} cation. But physiological stimulant Mn^{2+} almost 20 % inhibits the catalytic activity of Ni^{2+} oligomer.

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