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New Malate Dehydrogenase-Glutamate Oxaolacetate Aminotransferase Glutamate Oxaloacetate Aminotransferase Enzyme System from Cereals and its Bioengineering Application

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***Abstract*—**Malate dehydrogenase-glutamate oxaloacetate aminotransferase (MDh-GOAT) enzyme complex (the EC) was isolated and purified from wheat and rise, their some main physic-chemical properties were studied. Michael's constants of the EC MDh-GOAT to malate, glutamate and NAD were investigated. This kinetic results show a high relationship to glutamate. Taking into account important role of the the EC in catabolism of glutamate – the central amino acid of a nitric exchange, there is a sharp necessity of deeper studying of this enzyme complex. Therefore the basic purpose of the work is studying the basic physical and chemical properties of this enzyme complex discovered by us, which would be very important for understanding the mechanisms of reaction catalyzed by the EC.

***Keywords*—**Malate dehydrogenase-glutamate oxaloacetate aminotransferase, enzyme complex, glutamate.

I. Introduction

THE glutamate play central role in nitrogen metabolism [1]. Until now it was considered, that the basic way of catabolism of glutamate is carried out by NAD-dependent glutamatedehydrogenase. A question arises. Is there another way of glutamate catabolism? For solution of this question we carried out the investigation on search of other enzymes which participate in catabolism of glutamate. In this reason the main task of our investigation is to find new enzyme of glutamate catabolism, it's characterization and the development of the method of it's application in bioengineering.

II. Materials and Methods

Material for isolation of the enzyme complex MDH-GOAT were: dry seeds of spring wheat cultivar "Kazakhstanskaya - 10" (*Triticum aestivum*) and rice cultivar "Leader" (*Orizae sativa*).

The optical measurement was carried out by spectrophotometer Ultrospec-1100 pro, (Bioscience, Amersham, UK). Determination of protein quantity is carried out by microbiuret Bailey methods [2]. Activity of NAD-GDh was determined by spectrophotometric method by measurement adsorption at 340 nm during one minute. Reaction mixture contains 1,1mM NAD and 87mM of sodium glutamate [3]. The total volume of reaction mixture is equal 2 ml by adding of 0,05M tris-glycine buffer, pH 8,3. While we discovered the new enzyme system MDh-GOAT we developed the spectrophotometric method of its determination. The reaction mixture for determination of the EC activity contains 1,1mM NAD, 12mM malate and 87mM of sodium glutamate. The total volume of reaction mixture is equal 2 ml by adding of 0,05M tris-glycine buffer, pH 7,7. Procedure of determination of activity of the EC has some features. First of all it's necessary separately to determine the activity of NAD-GDh. For determination of activity of the EC MDh- GOAT, first of all it's necessary to determine the activity of MDh. In this case the reaction mixture doesn't contain glutamate. We determined base activity of malatedehydrogenase within 1-2 minutes. For determination of the EC activity MDh-GOAT it's necessary to add the solution of glutamate. This activity was determined during 1-2 minutes. For calculation of MDh-GOAT activity it's necessary to minus the activity of NAD - GDh and activity of MDh. The chromatography elution control was carried out by Uvicord SU, 2238, LRB (Pharmacia, Sweden).

III. Results and Discussions

The EC MDh - GOAT is isolated from dry seeds of wheat cultivar "Kazakhstanskaya – 10" and rice cultivar "Leader". For isolation of the EC MDh-GOAT the dry seeds of cereals were milled on laboratory mill then we obtained the floor by sieving. Then 10 grams of floor of wheat and rice were homogenized in 0,05M tris-HCl buffer, pH 7,5 taken in the ratio of 1:4, (weight to volume) in porcelain mortar. Then homogenate was centrifuged at 10 000 x g during 10 minutes. Obtained supernatant was purified from low-molecular substances and by gel-chromatography on a column with

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Sephadex G-50, the size (3,5x20cm). Then the fractions containing activity of the EC were put on a column with DEAE-cellulose type DE-52 size (5x2 cm), Vattman (England), the column was equilibrated by the 0,05M tris-HCl buffer, pH 7,4.

The results of separation of the EC MDh-GOAT by ion-exchange chromatography are represented at Fig. 1 and Fig. 2. The EC was eluted by 0,1M NaCl in the same buffer.

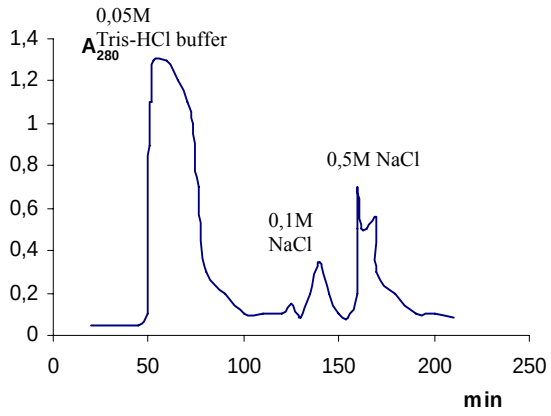

Fig. 1 Chromatography of stage of the EC purification on column with Sephadex G-50. Ion – exchange chromatography of protein of cell free extracts from seeds of rice "Leader" cultivar

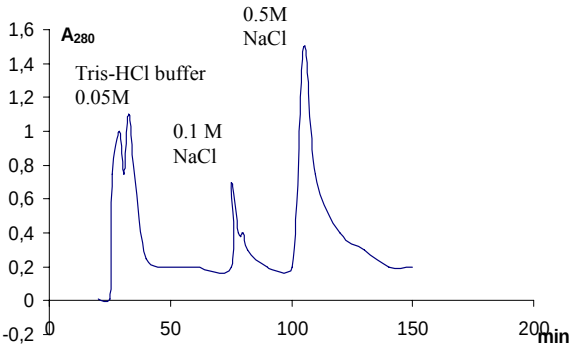

Fig. 2 Chromatography of cell-free extract from wheat "Kazakhstanskaya-10" cultivar of stage of the EC purification on column with DEAE-cellulose

Fraction containing the EC MDh- GOAT activity then was separated by gel- chromatography on a column with Sephacryl S-300, the size of a column (2x95cm). Results of chromatographic isolation are presented in Fig. 3 and Fig. 4.

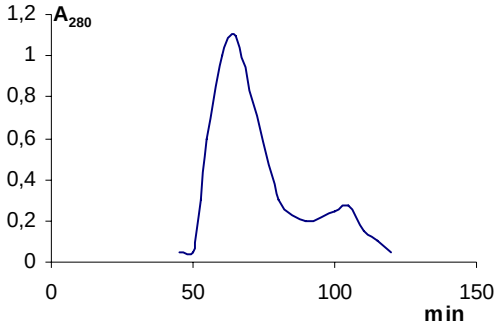

Fig. 3 Gel-chromatography of the EC MDh- GOAT from wheat seeds of "Kazakhstanskaya-10" cultivar on column with Sephacryl S-300

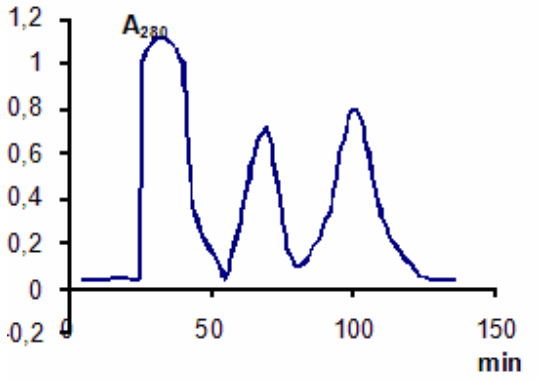

Fig. 4 Gel-chromatography of EC MDh- GOAT from rice seeds of "Leader" cultivar on column with Sephacryl S-300

The fraction of the EC MDh- GOAT was eluted in the second peak. The purification of the EC speaks about that the EC is single, stable and strong protein complex which not dissociates under the separation conditions. The purified enzyme hasn't any activity of NAD- GDh and MDh.

Basic physical and chemical properties of the EC from wheat and rice were investigated. First of all was carried out SDS electrophoresis by Laemmly (1970). Electrophoresis was carried out with presence of proteins-taps with known molecular masses. Determination of molecular masse has shown that the EC consists of two heterologic subunits with molecular masses of 50 kDa and 60 kDa accordingly. Results are presented in Fig. 5.

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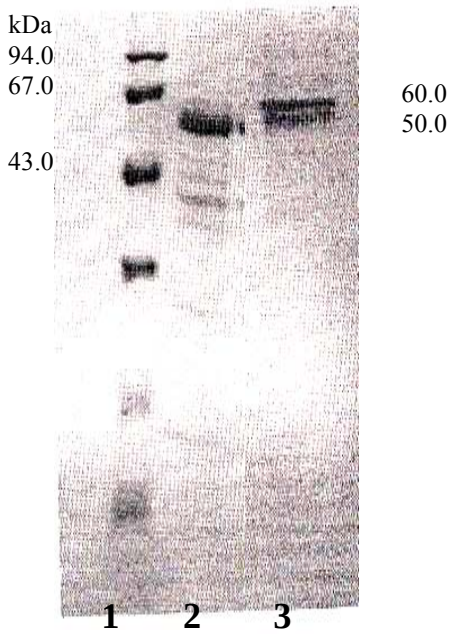

Fig. 5 SDS - electrophoresis of the enzyme complex (EC). 1-protein-tap; 2 -the EC up to FPLC; 3 - EC after FPLC Zones of proteins markers: phosphorylase 94 kDa; bovine serum albumin 67 kDa; egg albumin 43 kDa

Then we study the kinetic properties of the EC. Basic kinetic characteristics of the EC - The Mikhaelis constant (Km) for substrates of the EC from wheat and rice were determined. The following values of Km for EC of wheat -Km for glutamate 2,29·10-3 M, for malate 1,47·10-3, and for NAD 4,33·10-4, for Km of rice for glutamate 2,88·10-3, for malate 4,81·10-4, for NAD 1,76·10-3 were established. Kinetic characteristics of glutamate speak, that enzymes, both from wheat, and from rice has high affinity to glutamate.

One of the perspective directions of using of EC MDh-GOAT is its application as enzyme test for determining of tolerance of genotypes to salt stress.

It's well known that during abiotic stress there is the intensive accumulation of ammonia in plant cells. This process is due to increasing of activity of NAD-GDh during the stress. The accumulating ammonia destroys the cell membranes. That led to damaging of the plant cells. As it was established by us EC MDh- GOAT play key role in catabolism of glutamate. EC carries out the catabolism of glutamate without ammonia production. Thus, by our hypothesis the genotypes with high activity of the EC in less degree are damaged by stress than the genotypes with low activity of the EC. To check of our hypothesis we tested of activity of the EC of the forty six genotypes of wheat and barley differing by tolerance to salt stress. It was established that the genotypes sensitive to salt stress have a low activity of the EC. Whereas, genotypes with high tolerance to salt stress have the high activity of the EC. Also they have one surprising feature. They have the ability to increase activity of the EC, and then plants were transferred from normal to stress condition. By other words they have the specific mechanism of adaptation to salt stress. Whereas this mechanism is absent of the genotypes sensitive to salt stress. Thus the activity of the EC is serving as objective enzyme test for determining of cereals genotypes with high tolerance to salt stress.

The investigation of kinetic properties of the EC allows using it as a very effective biosensor for determination of glutamate. It's well known that glutamate has high neurotoxic properties [4]. Especially it's very dangerous to use by children 1-5 years. It's using as food stuff for children lead to damaging of their brain. In this reason the European Commissions had forbidden the content of glutamate in children food stuff. In the same time glutamate is often added to many food stuffs for giving of meet tastes. In this reason it's very important to determine the exact quantity of glutamate in food stuffs and in biological liquid in clinic biochemistry. We developed the biosensor for determination a quantity of glutamate by using our purified EC MDh - GOAT.

IV. Conclusion

Thus, we for the first time have studied some basic physical and chemical properties EC MDH-GOAT discovered by us. The most unusual property of given EC is that it carries out reactions unidirectionally. All these speak that we discovered new enzyme way of glutamate catabolism without allocation of toxic ammonia. As on share EC it is necessary more than 90 % catabolism of glutamate it speaks that by us the new basic way of catabolism glutamate wheat is open and fig. It is necessary to note that reactions catalyzed by EC are irreversible, that is exception for usual enzyme reactions.

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