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Biochemistry

The Role of Mitochondrial Lectin in the Work of Creatine Kinase System

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(Presented by Academy Member N. Aleksidze)

ABSTRACT. Influence of glucose specific lectin separated from the mitochondrial membranes of bovine brain on creatine kinase activity has been studied. It has been observed that inhibition of enzyme activity starts with the lectin action. The inhibition process starts in the conditions of a certain concentration of the components of enzymatic reaction, which presumably facilitates regulation of the reaction. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words*: creatine kinase, lectin, mitochondria, enzyme.*

Cells require energy and its main source is ATP. Maintaining ATP homeostasis is especially important. The ATP received through ordinary mitochondrial metabolism usually meets the levels required by the cell. However, during increased consumption the energy reserves are fully consumed and the cell dies. Main role in maintaining ATP homeostasis is attached to creatine kinase (CK) system that is catalyzed by creatine kinase [1, 2].

Existence of isoforms of creatine kinase is an example of how cell energy is stored and carried to other places [3]. Phosphocreatine originating from mitochondrial creatine kinase (Mi-CK) acts as an energy carrier system between mitocondrion and cytosol, thus connecting the energy generation place with the place where it is consumed. Despite the fact that many aspects of functioning of this enzyme have been established, here are still many unclear issues connected with its functionality and regulation process.

The goal of the research has been to define the influence of glucose-specific lectin BML-Glu released from mitochondrial membrane on the creatine kinase activity of the brain.

Materials and Methods

We have chosen bovine brain as the object of research. Mitochondrial fraction has been obtained by De-Robertis [4] lectin activity has been determined by 2% suspension of rabbit's trypsin erythrocytes [5] on titer plates. Lectin activity has been expressed by specific lectin activity (titer $^{-1}$*Mg/Ml protein^{-1}) [6]. Creatine kinase activity has been determined by creatinephosphate ion freed as a result of creatine kinase hydrolysis measured on a 400 nm length wave.

Results and Discussion

At the first stage of experiments influence of BML-Glu lectin on the activity of Mi-Ck was studied. It has been found that increase in the lectin concentration is accompanied by inhibition of Mi-CK activity and in the conditions of concentration of 30 μg/ml virtually full inhibition of enzyme activity takes place. The character of lectin activity on Mi-CK changes in the presence of lectin hapten, 5mM glucose in the incubating medium. In such cases enzymatic activity almost returns to the initial indices. In order to determine the character of the influence lectins have on Mi-CK it has been considered

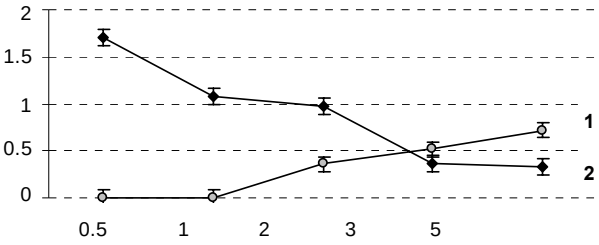

Fig. 1. Change in Mi-CK enzymatic activity with varying ATP concentrations
1. Mi-CK; 2. Mi-CK + BML-Glu
X-axis – ATP concentration (mM); Y-axis – Enzymatic Activity CK (μkat / L)

expedient to find out the character of interdependence of BML-Glu lectin with the components necessary for enzymatic activity, namely we have studied hemagglutination activity of creatine and ATP BML-Glu lectin. It has been discovered that both compounds necessary for enzymatic activity reduce the lectin's hemagglutination activity. Due to the fact that creatine kinase is involved in the process of maintaining of ATP homeostasis the character of ATP influence on the activity of Mi-CK has been established (Fig.1). It has been found out that enzyme activity decreased the ATP concentration increases. In the presence of the lectin character of the reaction changes and in the increase of ATP growing enzymatic activity is revealed.

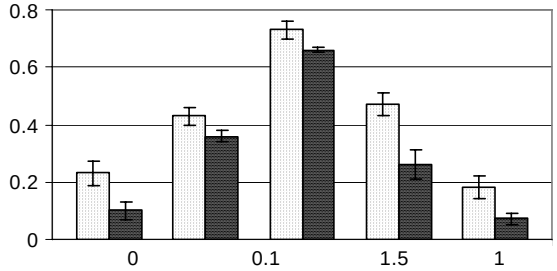

Fig. 2. Change in Mi-CK enzymatic activity with varying concentrations of Ca^{2+} ions

- Mi-CK + Ca^{2+} - Mi-CK + BML-Glu + Ca^{2+}

X-axis - Concentration of Ca^{2+} ions (mM)
Y-axis - Enzymatic activity CK ((μkat / L)

It has been interesting to investigate the influence of calcium ions on the activity of mitochondrial creatine kinase in the presence of BML-Glu lectin. To this end we have initially studied influence of Ca^{2+} on lectin hemagglutination activity. It has been found that Ca^{2+} ion causes change in the activity and that the ion acts as activator for Mi-CK. Highest enzymatic activity is discovered in case of 0.5 mM ion when the enzymatic activity increases almost 3 times; in case of a further increase in the quantity of ions (1-2 mM) enzymatic activity decreases (Fig. 2). In case of concentration of calcium and 15μg/ml lectin in the environment the effect of lectin on Mi-CK weakens. We assume that calcium influences Mi-CK system and when necessary, activates the enzymatic system. The same experiments have been carried out with variable concentrations of lectin (5-30μg/ml) and constant concentration of calcium (0,5mM), during which the ion eliminated the inhibition effect of the lectin in the conditions of its growing concentration (Fig. 3).

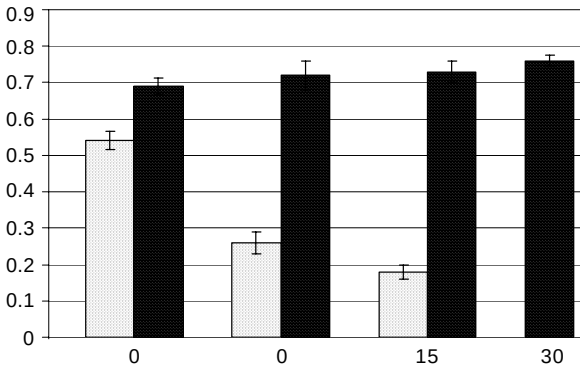

Fig. 3. Change in Mi-CK enzymatic activity with stable concentration of Ca^{2+} ions and varying concentrations of lectin.

- Mi-CK + BML-Glu - Mi-CK + BML-Glu + Ca^{2+}

X-axis – Concentration of BML-Glu lectin (mkg/ml)
Y-axis – Enzymatic activity CK (mkat/L)

Thus, it has been shown that under the influence of BML-glu-lectin activity of mitochondrial creatine kinase decreases and creatine that is a substratum of Mi-CK inhibits the lectin. In parallel, influence of ATP on both enzymatic and lectin activity has been noted. Presumably, BML-Glu lectin is a component taking part in the functioning of enzyme system and provides for optimal performance of the system.

ბიოქიმია

მიტოქონდრიული ლექტინის როლი კრეატინკინაზური სისტემის მუშაობაში

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სტატიაში შესწავლილია ხარის თავის ტვინის მიტოქონდრიების მემბრანებიდან გამოყოფილი გლუკოზოსპეციფიკური ლექტინის გავლენა კრეატინკინაზულ აქტივობაზე. დადგინდა, რომ ლექტინის მოქმედებით იწყება ფერმენტული აქტივობის ინჰიბირება. ლექტინის მოქმედების ხასიათი Mi-CK-ზე იცვლება საინკუბაციო არეში ლექტინის ჰაპტენის, გლუკოზის არსებობისას. ინჰიბირების პროცესზე გავლენას ახდენს ასევე ფერმენტულ რეაქციაში მონაწილე სხვა კომპონენტების (ატფ, კრეატინი, კალციუმის იონი) არსებობა, რაც, საბარაუდოდ, უჯრედში მიმდინარე რეაქციის რეგულირებაში მონაწილეობას მიუთითებს და უზრუნველყოფს ამ სისტემის ოპტიმალურ მუშაობას.

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