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THE EFFECT OF SEVERAL L- AMINO ACIDS ON THE INACTIVATION PROCESS OF *RANA RIDIBUNDA* ADULT FROG LIVER ARGINASE

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The effect of 8 L-amino acids (alanine, valine, leucine, isoleucine, γ-aminobutyric acid, lysine, proline, ornithine) on the *Rana ridibunda* adult frog liver arginase limited trypsinolysis process was studied. The results revealed that under the influence of trypsine at 20^0C after 20 hours L-alanine has appeared as one of the most potent protector of liver arginase. Accordingly, the other 5 amino acids are considered as competitive protectors. It is notable, that L-lysine and L-ornithine increase the sensitivity of studied enzyme towards proteolytic inactivation by trypsine.

Limited trypsinolysis– proteolytic sensitivity – ureotelic arginase

Ուսումնասիրված է 8 L-ամինաթթուների (ալանին, վալին, լեյցին, իզոլեյցին, γ-ամինակարագաթթու, լիզին, պրոլին և օրնիտին) ազդեցությունը *Rana ridibunda* հասուն գորտերի լյարդի սահմանափակ տրիպսինոլիզի ընթացքի վրա: Ցույց է տրված, որ տրիպսինի 20 ժ ազդեցության դեպքում 20^0C-ում հետազոտվող ֆերմենտի համար լավագույն պաշտպանիչ է հանդիսացել L-ալանինը իսկ մասնակի պաշտպանիչներ են՝ L-վալինը, L-պրոլինը, L-լեյցինը, L-իզոլեյցինը և γ-ամինակարագաթթուն: Հատկանշական է, որ L-լիզին և L-օրնիտինը փորձի նույն պայմաններում բարձրացնում են ֆերմենտի զգայնությունը տրիպսինով պրոտեոլիզի նկատմամբ:

Սահմանափակ տրիպսինոլիզ – պրոտեոլիտիկ զգայնություն – ուրեոթելիկ արգինազ

Изучено влияние 8 L-аминокислот (аланин, валин, лейцин, изолейцин, γ-аминомасляная кислота, лизин, пролин, орнитин) на процесс ограниченного трипсинолиза аргиназы печени взрослых лягушек *Rana ridibunda*. Показано, что после 20 ч воздействия трипсина при 20^0C для изучаемого фермента лучшим протектором являлся L-аланин, частичными протекторами были лейцин, пролин, изолейцин, валин и γ-аминомасляная кислота, а лизин и орнитин повышали чувствительность фермента к протеолизу трипсином.

Ограниченный трипсинолиз – чувствительность к протеолизу – уреотелическая аргиназа

The inhibition of arginase activation by several amino acids has been investigated in detail long ago. Subsequently it has been approved that almost all L-amino acids have minor inhibitory effect on several mammals purified liver arginase [5, 6]. The investigation of bovine liver arginase inhibition by different amino acids revealed, that ornithine, lysine, leucine, isoleucine, valine and proline have significant inhibitory effect, in the case when alanine and histidine cause a slight impulsive effect on studied enzyme activity. According to the authors, the inhibitory effect of amino acids depends on carbon chain length and only L-isomers of amino acids have this influence [4]. Likewise, authors reached the similar results about *Rana ridibunda* frog liver arginase [1, 2]. The studied amino acids inhibitory effect is due to the changing in the α-amino groups' protonation constant values or in α-carboxyl groups and enzyme active site binding [8].

In this study, we attempt to investigate the effect of several amino acids (alanine, valine, leucine, isoleucine, γ-aminobutyric acid, lysine, proline, ornithine) on the sensitivity of *Rana ridibunda* frog liver ureotelic arginase towards proteolytic inactivation.

Materials and methods. Rana ridibunda Pallas adult frogs are served as a research object. The study was approved by the ethics committee of the Biochemistry Department of YSU and implemented in accordance with the Helsinki convention related to animal studies. The *Rana ridibunda* frog liver arginase preparations final concentration is 5 %, which were prepared by distilled water. The absorbance of protein was measured in a spectrophotometer (Genesys 10S UV-VIS) at 280 nm. The molecular weights of fragments were determined by Gel-filtration method (Sephadex G-200, Uppsala, Sweden). The equilibration and elution was done with buffer solution containing 0.005 mol glycine-NaoH (pH=7.4) at 25^0C, elution velocity is 20 ml per hour.

Marker proteins (urease, alcohol dehydrogenase, human serum albumin, pepsin, trypsin and ribonuclease) are used for determining the molecular masses. During the experiments we used purified, chymotrypsin free trypsin preparations, which was added by 1 mg/ml concentration to 5% enzyme preparation. After 20 hours the reaction stopped by soybean antitrypsin, with 0.2% concentration in the solution. We used L-ornithine, L-lysine HCL and γ-aminobutyric acid, leucine, isoleucine, valine, proline and alanine amino acids, respectively, 0.38 mM of amino acids per 1 ml of studied sample.

Results and Discussion. In this study, we have investigated the inhibitory effects of the studied amino acids on liver arginase proteolytic inactivation. Prior to data collection, all samples were incubated for 30 min in 20^0C in the presence of corresponding amino acids. The results are presented in tab. 1 and fig. 1-8.

Tab. 1. The effect of several amino acids on the proteolytic inactivation process of *Rana ridibunda* adult frog liver arginase, n=5

Amino acid	Molecular weights of fragments					№ of fragments
Limited tripsinolysis without amino acid	62000	40000	33000	13700		4
L-alanine	86600	37500				2
L- isoleucine	53000	48800	33700			3
L- leucine	30800	21700	16700			3
L- valine	86600	48800	19900			3
γ-aminobutyric acid	53000	33700	16700			3
L- proline	48800	16700	12300			3
L- ornithine	86600	62600	48800	33700	16700	5
L- lysine	86600	62600	48700	33700	19900	5

According to the results obtained we can assume, that several L-amino acids protect the liver arginase from inactivation by trypsine. During the limited proteolysis a number of amino acids, including L-valine,L-leucine, L-isoleucine, γ-aminobutyric acid and L-proline consider the beneficial inhibitors of liver arginase (fig.1-5). Thus, enzyme pre-incubation by these amino acids before trypsinolysis decrease the number of derived fragments. Comparing the number of fragments in limited trypsinolysis without and by studied amino acids can be observed, that in preparation without amino acids after trypsinolysis appeared 4 fragments, while quantity of fragments decreases to 3 in preincubated samples by mentioned amino acids, which is considered as the result of inhibitory activity of L-amino acids.

Intriguingly, L-alanine (fig. 6) appears as one of the most potent protectors of liver arginase during the limited trypsinolysis. Therefore, after enzyme pre-incubation by alanine appeared only 2 fragments with 86000 and 37500 Da molecular weights. It is notable that, the stabilizing effect of alanine is reflected not only in the number of derived fragments likewise in the molecular weights of them. Presumably, alanine is considered a protector or surrender during the limited trypsinolysis by protecting trypsine sensitive bound on the surface of arginase.

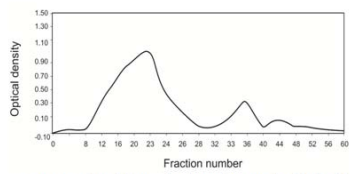

Fig.1. The effect of L-leucine on the limited trypsinolysis process

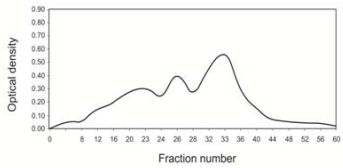

Fig.2. The effect of L-isoleucine on the limited trypsinolysis process

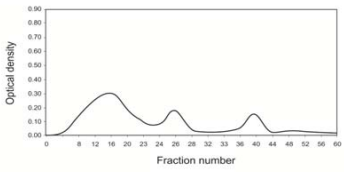

Fig.3. The effect of L-valine on the limited trypsinolysis process

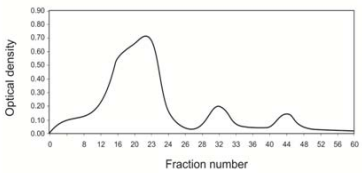

Fig.4. The effect of γ-aminobutyric acid on the limited trypsinolysis process

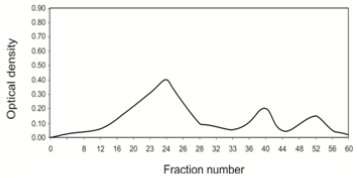

Fig.5. The effect of L-Proline on the limited trypsinolysis process

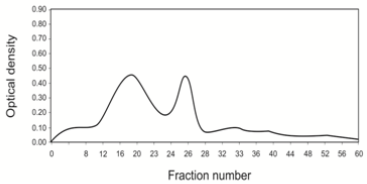

Fig.6. The effect of L-alanine on the limited trypsinolysis process

The other amino acids are either without effect or much weaker inhibitors of arginase, such as, frog liver arginase pre-incubation by L-ornithine and L-lysine (fig. 7, 8) which are considered as competitive inhibitors of frog liver arginase and as our attempts revealed simultaneously increase the sensitivity of enzyme towards trypsinolysis, producing minor changes in the proteolysis results. Therefore, the number of derived fragments has increased to 5.

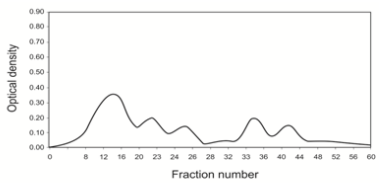

Fig.7. The effect of L-lysine on the limited trypsinolysis process

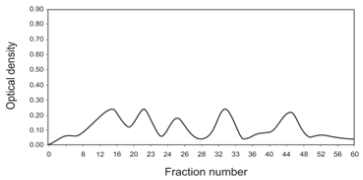

Fig.8. The effect of L-ornithine on the limited trypsinolysis process

Thus, we conclude that our results correspond with the studies relating to rat liver arginase, where the inhibitory effect of the studied amino acids toward arginase has previously been investigated by electron paramagnetic resonance method. According to the results obtained by EPR studies [7], arginase inhibitors divide into two classes which are differ by their interactions with enzyme active site. A number of inhibitors, including ornithine, citrulline and isoleucine which belong to the same class do not bind directly to the manganese cluster of arginase.

These amino acids producing insignificant change in arginase EPR intensity and presumably interact with the recognition regions of active site for the alpha-aminocarboxylate groups of the substrate, which are located near the protein surface. The second class of inhibitors, including L-lysine, Nω-hydroxy-L-arginine (NOHA) and L-arginine (substrate) induce extensive changes in the EPR intensity and probably directly interact with dimanganese cluster of arginase. Nω-hydroxy-L-arginine (NOHA) is an intermediate in the nitric oxide synthase-catalyzed oxidation of L-arginine to L-citrulline and nitric oxide, and it is also a competitive inhibitor of arginase [3]. According to suggested model, occurred deprotonation of the side-chain ε amino group of L-lysine or N amine atom transfer from arginine to the His141 residue, afterwards nitrogen neutral atom bind to the manganese ion in order to replace one of its ligands [7].

In conclusion, we note that almost all the studied 6 L-amino acids have protective effect and more or less protect the frog liver arginase from proteolytic inactivation, Exemptions are lysine and ornithine, which according to the authors are classified in different class of inhibitors and have distinct effect mechanisms [7]. However, they have the same effect on the *Rana ridibunda* frog liver arginase proteolytic inactivation process, which probably depends on the adult frog liver arginase structural characteristics.

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