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ACTIVITY OF BETA-N-ACETYLHEXOSAMINIDASE IN MEMBRANES AND CHICKEN EMBRYONIC FLUIDS

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The enzymatic activity of β-N-acetylhexosaminidase during the chick embryonic development of Leghorn H-22 obtained from the flock in Chorzelów measured in shell membranes and chorioallantoic membrane is high (20–60 mU/mg of protein), lower in allantoic fluid (0,2–14,2 mU/mg of protein) and amniotic fluid (0,3–3,2 mU/mg of protein) and the lowest in albumen (0,01–0,15 mU/mg of protein). The measured activity of the enzyme grows along with the age of the embryo, excluding shell membranes. It is assumed, that the activity of the enzyme connected with the protective function may be also valid during the embryonic development.

Key words: HEXOSAMINIDASE, SHELL MEMBRANE, EXTRAEMBRYONIC MEMBRANE, DEVELOPMENT, CHICK EMBRYO

Beta-N-acetylhexosaminidase (β-HEX) is one of the most active lysosomal glycosidases (EC 3.2.1.). Due to its common and high activity, it has been widely investigated in human and different animals [1–3]. It exists in many molecular forms, abundantly in the tissues encompassing fagocitosing cells. In human, the greatest activity of β-HEX has been determined in adrenal glands and the liver, lower in the placenta and kidneys and the lowest in the cerebral tissue and muscles [4]. In an egg, particularly in egg albumen, this enzyme was discovered early, in 1966 by Lush and Conchie [5]. Later, β-HEX has been purified and described in more detail [6–7]. In our laboratory, this enzyme was also investigated many times in the male reproductive system of birds [8–14] and in egg albumen and hen oviduct [15].

The aim of this study is to define the activity level of β-HEX in membranes and embryonic fluids during the prenatal development of a hen, what is the preliminary stage of the studies on biochemical characteristics of the enzyme in the chick embryo.

Materials and methods

Animals. The experimental material constituted 200 fertilized chick eggs of the breed Leghorn H-22 (*Gallus Gallus*) coming from the flock kept at the Experimental Station of National Research Institute of Animal Production in Chorzelów. The eggs were collected during the spring peak of egg laying and directly after the collection, they were brought to the University Laboratory in Rzeszów and stored one day on a table top in room temperature. The experiment was conducted twice, each time transporting fresh eggs and covering two day eggs, though, in each of the performed experiments, all assays were doubled. In the 6, 9, 11, 15 and 18-th day of incubation, the embryos were anesthetized in the temperature of 5 ºC, and then frozen and stored in the temperature of -20 °C.

Preparation of embryo. After defrosting, the content of the egg was poured out and allantoic fluid was sampled with a syringe from the allantoic sac. The next stage relied on dissecting free, by means of classical dissection tools such as scalpels and tweezers, the chorioallantoic membrane from which, after draining the excess of fluids on the tissue-paper, the samples were weighed on analytical scales. Next, the amniotic fluid was sampled with a syringe. In order to collect albumen and yolk a syringe without a needle was applied. Dense yolk and the liver have been excised. Solid tissues after dissecting were draining on the tissue-paper and weighed on analytical scales, then homogenized. Glass, manual, conical-shaped homogenisators with volume of 2 mL were applied. The proportions were used: one part of wet tissue to four parts of 1 % NaCl. The gained homogenates were diluted to further assaying as required. In order to prepare all dilutions the NaCl solution was used with concentration of 1 percent w/v. After breaking an egg, the inside of the shell was washed out with 1% NaCl and dissected shell membranes with tweezers, which were dried on the table top, cut with scalpels and then weighed on analytical scales directly in Eppendorf tubes which were used for performing the enzymatic reaction.

Enzyme assay. The activity of β-HEX was assayed spectrophotometrically according to Barret and Heath [16] with slight modifications. The source for the enzyme was the tissue in form of homogenate, mostly 20 % or 4 %, or diluted and undiluted embryonic fluids. To 50 μL of homogenate (of proper dilution) 100 μL of citrate buffer was added with concentration of 0,2 mol/dm^3 and pH 3,75 (optimal for the enzyme) including nitrophenylglycoside as a substrate (p-nitrophenyl β-N-glucosaminide manufactured by Sigma) with concentration of 4,4 mmol/dm^3. The solution was incubated in Eppendorf tubes in the temperature of 37ºC for 5 to 30 minutes depending on the material and dilution. The reaction was inhibited by precipitating protein and adding 250 μL 3,3 % TCA to the incubated solution. Then the sample was centrifuged and there was collected 250 μL of supernatant and carbonate buffer 250 μL was added with the concentration of 0,5 mol/dm^3 in order to dye the yellow p-nitrophenol colour developer by the separated enzyme of nitrophenol. Absorbance of samples

was measured with the wave length of 400 nm. Blind reagent trials were performed separately and the value of absorbance was subtracted from the absorbance of factual trials.

The other conduct was in case of shell membranes which were not homogenized. To the weighed amount of shell membranes with weight of 1,5 ±0,1 mg, 50 μL of buffer was added and incubated in the water bath with the temperature of 37 ºC for 5 minutes. The reaction was inhibited by adding 500 μL of carbonate buffer with concentration of 0,5 mol/dm^3, after which absorbance was directly measured with the wave length of 400 nm. The protein concentration was assayed by the Bradford method [17] using bovine serum albumin as a standard.

All gained values of absorbance were calculated regarding the activity of the enzyme and considering the value of mol absorbance of the measured component (p-NP), level of concentration of the material and the incubation time. It was assumed that the unit of enzymatic activity (U) shall be regarded as the amount of the enzyme which transfers 1 micromole of the substrate within the time of 1 minute in the temperature of 37 ºC. The activity of the enzyme was expressed as the number of units (U) per 1 mL of tissue or 1 mg of protein included in the assayed tissue.

Results and discussion

There were gathered in total more than 400 analytical assays of the activity of β-HEX in various materials coming from chick embryos.

The specific activity assayed in the tested material and the content of protein was presented in Table 1. Analysing the gathered results it may be claimed that activity of β-HEX in shell membranes, chorioallantoic membrane, yolk and liver of the embryo has the same value oscillating between 30 and 40 mU/mg of protein.

In egg shells, the specific activity of β-HEX oscillates at a balanced level (Tab. 1.) from 32.8 ± 4.0 mU/mg of protein in the 6th day of the experiment to 32,5±3,7 mU/mg of protein in the 18th day of incubation of an egg excluding the 15th day, when it was lower (though not with such a great scatter) and amounted to 15.,9±5,5 mU/mg of the protein. For the shell membranes, this level differs from the one given by Winn and Ball [6] 6 mU/mg of protein in unfertilised eggs, however, it is well adjusted to the results gained by Droba et al. [15] for the breed Polish Partridge Green-legged hen Ż-33 (28 ± 5 mU/mg of protein) and Rhode Island Red (19±3mU/mg of protein) It is confirmed with a high level of activity of β-HEX not only in shell membranes, just laid eggs but also in the eggs incubated with embryos.

The activity of β-HEX in chorioallantoic membrane is a little bit lower than in shell membranes and it grows along with the development of the embryo from the level of 18,6±2,8 mU/mg of protein in the 9th day of incubation to 34.0 ± 2.2 mU/mg of protein in the 18the day of incubation, with the peak of 58,1±13,7 mU/mg of the protein in the 15-th day, when the chorioallantoic membrane is developed most and its functions are the greatest. The growing tendency can be observed during the comparison of the results presented as the activity coming from the tissue volume unit (Fig. 1.). The growth of activity in the chorioallantoic membrane in the 15-th day of incubation agrees with the fall in the activity of egg shells.

In yolk and liver of the embryo, the activity of β-HEX was assayed in the 18th day of life. It amounts to 40.9±38 mU/mg of protein in yolk and 47,0±12,6 mU/mg of protein in the liver and that is why it is at the similar level (Tab. 1.). It is worth paying attention to the fact that the level of activity of the enzyme in the chorioallantoic membrane and yolk is the same as in the liver, in the organ of great biochemical activity.

Table 1.

Specific activity of β-N-acethylhexosaminidase and protein concentration from chick embryo during prenatal development. Values are given as mean ±SE for n=5.

Enzyme source	Protein concentration means [mg/mL]	Incubation age of chick embryo				
		6th day	9thday	11thday	15thday	18thday
Shell membrane	132	32.8±4.0	26.8±1.7	32.4±2.1	15.9±5.5	32.5±3.7
Chorioallantoic membrane	48	–	18.6±2.8	21.8±6.7	58.1±13.7	34.0±2.2
Allantoic fluid	27	0.25±0.07	2.5±0.7	4.2±0.6	5.3±1.2	14.2±3.3
Amniotic fluid	20	0.31±0.16	1.21±0.56	3.23±1.70	–	–
Albumen	224	0.01±0.003	–	0.09±0.05	0.15±0.02	–
Yolk	195	–	–	–	–	40.9±17.2
Liver	142	–	–	–	–	47.0±5.6

Embryonic fluids (allantoic and amniotic) had the specific activity of β-HEX at the lower level than the chorioallantoic membrane however greater than albumen. In the allantoic fluid this activity depicts a growing tendency from the level of 0,25±0,07 mU/mg of protein in the 6-th day of development to 14,2±3,3 mU/mg of protein in the 18-th day of embryo development (Tab. 1). The activity of β-HEX per 1mL of tissue volume in the 6th day of incubation is greater than in the 9-th day, differently than after having considering the protein (Fig. 1.)

The amniotic fluid was tested three times, in the chick embryo aged 6 days, with the results of 0,31±0,16 mU/mg of protein and greater at the age of 11 days at the level 3,23±1,70 mU/mg of protein (Tab. 1.).

The specific activity of β-HEX in albumen was lower than among the tested material (Tab. 1.) and its level amounting to 0,01±0,003 mU/mg of protein in the 6th day of egg incubation approached to the separation boundaries of assay methods. Activity in the later period of development of the embryo was great, though, accompanied with a great dispersion. In the 11th day of embryo development it amounted to 0,09±0,05 mU/mg of protein and in the 15-th day 0,15 mU/mg protein, with SE±0,02, observing 10 time increase. It shall be remembered that even β-HEX in albumen of a fresh laid eggs has great activity of 8.5 mU/mg of protein [6] and 2,6±0,65 mU/mg of protein [15], though it does not decrease hour by hour (when stored in ambient temperature) and on the third day it amounts to 1,1 mU/mg of protein and in the ninth day only 0,06 mU/mg of protein. As seen from the gained results, during incubation of the embryo the activity of β-HEX «starts» from a much lower level, as to gain the similar to the one several days after laying eggs. Embryos at the age of 18 days practically used the whole albumen, so assays were not performed. For the albumen, there is the greatest difference between the activity of coming from the tissue volume and the activity per the amount of protein in this tissue (Fig. 2.). Because the activity of β-HEX in albumen and in shell membranes connected with lytic properties of enzyme towards gram negative bacteria, though, supposedly this protective role towards the egg is still valid during the embryonic development.

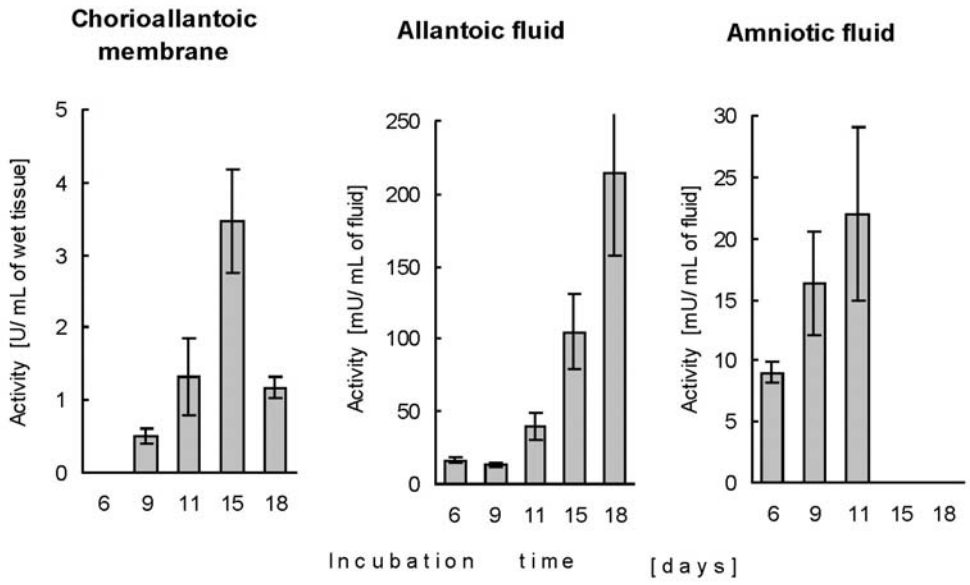

Fig. 1. Activity of β-N-acetylhexosaminidase per 1 mL volume of chorioallantoic membrane, allantoic fluid and amniotic fluid of the chicken embryo. Values are given as mean ±SE for n=5

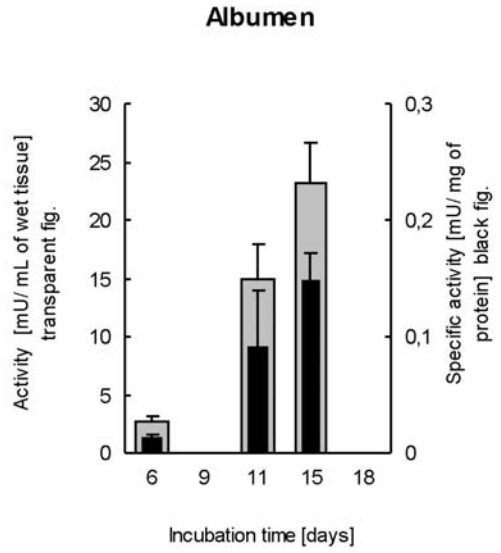

Fig. 2. Activity of β-N-acetylhexosaminidase per 1 mL volume of albumen (bright columns) and compared with the content of protein – specific activity (black columns). Values are given as mean ±SE for n=5

Conclusions

The gained results allowed for defining the level of activity of β-HEX in shell membranes, chorioallantoic membrane, allantoic fluid, amniotic fluid, albumen and the changes of that activity during the chick embryonic development. They constitute the basis for further studies on the role of β-HEX in the embryonic development of birds. It is assumed that the most abundant β-HEX in egg albumen has antibacterial activity. In light of the recent studies concerning the structure and changes of shell membranes that take place during embryonic development of Japanese quails [18, 19], it is possible that this enzyme may have a role in modifying membrane glycoproteins during embryo development.

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АКТИВНІСТЬ БЕТА-N-АЦЕТИЛ- ГЕКСОЗОАМІНІДАЗИ В ОБОЛОНКАХ І РІДИНАХ ЗАРОДКА КУРЧАТИ

Р е з ю м е

Ензиматична активність N-ацетил-бета-гексозоамінідази в процесі розвитку зародка курей породи Легорн Н-22 з племінного стада в Хожельові, визначена в скорлупових оболонках і в хоріоаллантоісній оболонці, висока (20–60 mU/мг білка), більш низька в аллантоісній рідині (0,2–14,2 mU/мг білка) і амніотичній рідині (0,3–3, 2 mU/мг білка), але найнижча в яєчному білку (0,01–0,15 mU/мг білка). Вимірювана активність ензиму зростала по мірі розвитку зародка за винятком скорлупових оболонок. Передбачається, що активність ензиму пов'язана із захисною функцією яйця і, відповідно, курячого ембріона.

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АКТИВНОСТЬ БЕТА-N-АЦЕТИЛ-ГЕКСОЗОАМИНИДАЗЫ В ОБОЛОЧКАХ И ЖИДКОСТЯХ ЗАРОДЫША ЦЫПЛЕНКА

А н н о т а ц и а

Энзиматическая активность бета-N-ацетило-гексозоаминазы в процессе развития зародыша кур породы Легорн Н-22 из племенного стада в Хожелёве, определяемая в скорлуповых оболочках и в хориоаллантоисной оболочке, высокая (20–60 mU/мг белка), более низкая в аллантоисной жидкости (0,2–14,2 mU/мг белка) и амниотической жидкости (0,3–3,2 mU/мг белка), но самая низкая в яичном белке (0,01–0,15 mU/мг белка). Измеряемая активность энзима возрастала по мере развития зародыша за исключением скорлуповых оболочек. Предполагается, что активность энзима связана с защитной функцией яйца и, соответственно, куриного эмбриона.

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