Amylases and its Isozymes in the Yolk of Egg and in the Pancreas and the Serum of Domestic Fowl

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We found amylase in the yolk of the ovary of a hen. Amylase was made in layers on the yolk and the total amount was in direct proportion to the weight of the yolk. The isoamylase pattern in the yolk of a fertilized egg was identical electrophoretically to that in the pancreas of the chick embryo during the incubation period. Polymorphism of isoamylases was seen in the yolk, the pancreas and the serum of a hen. The phenotype of isoamylases in these organs was one of three types (A, B or AB).

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Introduction

We previously reported a marked increase in the amount of amylase activity in the yolk of fertilized eggs corresponding to development of the embryo (IKENO *et al.*, 1991). A mechanism used to increase the amount of amylase activity in the yolk during incubation is partial accumulation of pancreatic amylase biosynthesized by the chick embryo. The source organ responsible for an increase of the amount of amylase in the yolk is the pancreas of the embryo (IKENO *et al.*, 1992). Amylase activity was found not only in the fertilized egg but also in the unfertilized egg. We, therefore, think that there are two types of amylases in the yolk. One is the amylase in the yolk of an unfertilized egg or in a fertilized egg incubated for less than two weeks. This amylase may be synthesized at the same time yolks are formed in the ovary. Another type is detected in the yolk of a fertilized egg incubated for more than two weeks. This amylase is identical to pancreatic amylase in a chick embryo electrophoretically.

Some papers reported that there is polymorphism in saliva, serum and pancreatic amylases of animals (HASHIGUCHI *et al.*, 1970 ; IKEMOTO *et al.*, 1985 ; MATUSHIMA *et al.*, 1990 ; SEARCY *et al.*, 1966 ; MORAN, 1982). In the previous report, authors *et al.* (1992) described the electrophoretic variations of amylase in the yolk and the pancreas. It is therefore necessary to analyze variations of amylase isozymes in the yolks of fertilized eggs, and relationships of amylases in the yolk of the ovary, the pancreas and the serum of a hen also should be examined.

In this paper, we describe contents of amylase in the yolk of the ovary and relationships among amylases in the yolk of fertilized eggs, and the pancreas and the serum of hens.

Materials and Methods

Eggs were obtained from hens of the Rhode Island Red breed. Fertilized eggs were incubated in a incubator described previously (IKENO *et al.*,1991). Ten to twelve eggs were withdrawn from the incubator and used for the experiment. In some eggs, the yolk and the albumen were separated, weighed and then homogenized in 10 ml of saline. The pancreas of the embryo was dissected out, weighed and the homogenized in 2 ml of saline. The homogenates were used for the assay of amylase activity and the separation of isoamylases by electrophoresis. Amylase activity in the yolk, albumen, pancreas and serum was assayed photometrically using blue-starch as substrate (Neo-Amylase Test, Daiichi Pure Chemical Co. Ltd.). The method is based on measurement of the released blue dye from the substrate by the enzyme reaction. One unit of amylase activity is defined as the amount of enzyme catalyzing the change in 1.0 of absorbance at 620 nm per 15 min. per 1.0 g of the yolk or albumen (1.0 ml of the serum or 1.0 mg of the pancreas) incubated at 37°C.

Separation of amylases in the yolk was done by horizontal acrylamide gel electrophoresis (IKENO *et al.*,1992). Amylases on the gel were detected by iodine-starch reaction. The distribution of amylase in an egg was visualized by laying a frozen egg slice on CELLOGEL (Chemotron, Italy) which was in contact with blue-starch then incubated at 37°C until the CELLOGEL was stained a blue color by the amylase of the egg. Ten hens (Rhode Island Red breed, 35 months old) were killed by bleeding from the carotid artery. Serum was obtained from the blood and yolks from the ovaries of the hens. These yolk and pancreas were dissected out, weighed and homogenized in saline to assay the amount of amylase activity. The serum was prepared by centrifugation.

Results

Amylase activity was detected in the yolks from the ovaries of the hens. And the total amount of amylase activity was in direct proportion to the weight of the yolk

Fig. 1. Total amount of amylase activity in the yolk of the ovary.

(Fig. 1). This result coincides with the distribution of amylase in the egg (Fig. 2). Most of the amylase activity was found in the yolk, not in the albumen, of the egg. The outside of the striped pattern was the part in contact with the albumen for the incubation period. Amylase activities were detected in a concentric configuration in both the central and peripheral portions of the yolk. Dark striped patterns show high amylase activities.

Figure 3 and Figure 4 show electrophoretic patterns of amylase in the yolk and embryo, respectively, during 16 to 22 days incubation. Each sample of the pancreas and the yolk (Fig. 3) was prepared from the same egg. Three phenotypes (A, B and AB) of amylases were observed in the pancreas, and isoamylase patterns in the

Fig. 2. Distribution of amylase activity in a cross-sectional egg.

Fig. 3. Acrylamide gel electrophoretic patterns of amylases in yolks of fertile egg (16 to 22 days incubation) (a) and the diagrammatic illustration (b).

Fig. 4. Acrylamide gel electrophoretic patterns of amylases in the pancreas of chick embryos (16 to 22 days-old) (a) and the diagrammatic illustration (b).

Table 1. Amylase activity in the yolk and the albumen of the egg and in the pancreas and the serum of the hen

	Amylase activity (units*)
Unfertilized egg	
(1 day incubation)	
Yolk	8.0±0.6
Albumen	1.0±0.1
(20 days incubation)	
Yolk	11.2±1.2
Albumen	1.1±0.1
Fertilized egg	
(1 day incubation)	
Yolk	7.9±0.8
Albumen	0.9±0.1
(20 days incubation)	
Yolk	193.4±20.2
Albumen	1.3±0.1
Hen	
Pancreas	291.5±18.3
Serum	15.5±2.0

*A value of 1.0 for the absorbance at 620 nm per 1.0 g of the yolk or albumen (1.0 ml of the serum or 1.0 mg of the pancreas) incubated for 15 min at 37°C was defined as 1 unit. Values represent the means±SEM of 10-12 samples.

pancreas of the embryo were the same as those in the respective yolk.

Table 1 indicates the amylase activity in the yolk and albumen of eggs and in the pancreas and the serum of hens. Amylase is distributed mainly in the pancreas, although, some amylase activity was detected in the serum.

Electrophoretic patterns of amylases in the serum, in the yolk of the ovary and in the pancreas of four hens are shown in Fig. 5 (a, b). Amylase patterns of these three samples (serum, pancreas and yolk) are quite identical in same individual.

Discussion

We found amylase in the yolk of the ovary of domestic fowl. Amylase is made in layers coinciding with the formation of the yolk in the ovary. We speculated previously that the yolk of the egg was a kind of storehouse of amylase synthesized in the pancreas of the chick embryo (IKENO *et al.*, 1991) and amylase was not synthesized in the yolk (IKENO *et al.*, 1992). In a hen, we think that the yolk in the ovary may also be an accumulator of amylase. Amylase activity in the serum of a hen was detectable (Table 1 and Fig. 5). Amylase synthesized in the pancreas (about 292 units of amylase activity/mg pancreas) of a hen is, probably, transported into the yolk (about 8 units

Fig. 5. Acrylamide gel electrophoretic patterns of amylases in the serum, the yolk and the pancreas of four hens (1, 2, 3, 4) (a) and the diagrammatic illustration (b). SE ; serum, YK ; yolk, PC ; pancreas

amylase activity/g yolk) via the blood (about 16 units amylase activity/ml serum).

Three phenotypes of amylase in the yolks were identical to those in the pancreas and the serum in same hen (Fig. 5). These amylases are biosynthesized in a hen. One of the highest organs of amylase activity is the pancreas (Table 1). In some animals, amylases in the parotid gland or the pancreas are easily released into the blood (IKENO *et al.*, 1982 ; SAIKATSU *et al.*, 1989), and the released amylase is taken into the sub-mandibular gland (IKENO *et al.*, 1982).

Polymorphisms of egg proteins are reported using the yolk and the albumen in avian eggs. Polymorphisms of the albumen, prealbumen and transferrin are observed in the serum of a chicken. A previous paper showed that the amount of amylase activity in the yolk of fertilized egg increases markedly after 13 days of incubation (IKENO *et al.*, 1991). Figure 3 indicated that increases in the amount of amylase activity in the yolk corresponding to the development of the chick were caused by the increase in amylase protein not by appearance of new isoamylases. There are three kinds of variations of isoamylases in the yolk of fertilized egg. We recognized three phenotypes of amylase (A, B and AB) in fertilized eggs (yolk and pancreas of embryo) and in hens (yolk, pancreas and serum). These variations depend simply upon individual variations. Variations of the yolk isoamylase were quite similar to those of the pancreatic isoamylase in respective samples (Fig. 3, 4 and 5). These results support the theory that pancreatic amylase of a chick embryo is accumulated partially in the yolk during development in fertilized egg, and in the case of a hen, pancreatic amylase of a hen is accumulated partially in the yolk during formation of the yolk in the ovary. These results suggest that some interactions exist between the embryo and the yolk during developmental periods.

In the serum of Guinea fowl, five phenotypes (A, B, C, AB and BC) of amylase were distinguished and these are controlled by three autosomal codominant alleles ; AmyA, AmyB and AmyC (SHIRAISHI *et al.*, 1978). In the Japanese quail, there are three phenotypes (AA, BB and AB) of serum amylase which are controlled by autosomal codominant alleles ; Amy-1A and Amy-1B (WATANABE *et al.*, 1975). Same phenotypes of amylase were also observed in sera of some Japanese native fowl breeds (TANABE *et al.*, 1977). But there was no report on amylase in the yolk of fertilized eggs. Figure 5 indicates that three phenotypes of amylase are recognized not only in the serum of domestic fowl but also in the yolk and the pancreas.

We hope to observe the continuous changes of isoamylase patterns in the yolk throughout the development period using a single fertilized egg.

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ニワトリの受精卵, 卵黄, 臍臓, 血清中のアミラーゼと そのアイソザイムについて

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ニワトリの受精卵, 卵巣内卵胞, 臍臓, 血清を材料にしてアミラーゼとそのアイソザイムを観察した。卵巣内卵胞中のアミラーゼ活性は卵胞重量と比例して増加し層状に分布していた。アイソアミラーゼの多型 (A, B, AB) は胎仔発生にともなう卵黄ならびに胎仔臍臓の他,

ニワトリの卵巣内卵胞, 臍臓, 血清中のアミラーゼにも認められた。同一個体のそれぞれの臓器のアミラーゼの多型は一致していた。

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