JJBS
Jordan Journal of Biological Sciences

Volume 5, Number 1, March 2012
ISSN 1995-6673
Pages 55 - 64

Lysozyme Turnover during the Development of Chicken Oocyte

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Received on October 3, 2011, Accepted on November 22, 2011

Abstract

Lysozyme, the major oocyte enzyme was studied using the lysoplate assay, the turbidimetric assay, SDS-PAGE assay and native gel electrophoresis coupled with activity gel overlay system. Using the lysoplate assay, lysozyme activity was shown to exist in all developmental stages of oocytes although to various degrees. Both eggwhite and yolk, had lysozyme activity. Using the less sensitive quantitative turbidimetric lytic assay, lysozyme activity started to appear in the yolk fractions collected from the caudal region of the infundibulum. For the egg white, lysozyme activity started to appear in samples collected from the anterior portion of the magnum. It increased sharply when the ovum was found in the posterior portion of the magnum. Using the SDS-PAGE, a lysozyme band was not detectable in all the follicular stages of oogenesis. A very faint band of lysozyme was detected in all the yolk fractions collected from the different segments of the oviduct. For the egg white lysozyme started to appear in the fractions collected from the anterior portion of the magnum; this continued in all fractions until egg maturation. In native gel electrophoresis, coupled with the activity gel overlay, a distinct lysozyme activity band appeared in all fractions including egg yolk and egg white collected during the process of oogenesis .To our knowledge, this is the first report of the existence of lysozyme in both egg white and yolk, in all the developmental stages of the oocyte.

keywords: Lysozyme, egg white, egg yolk, oogenesis, developmental regulation.

1. Introduction

Hen oogenesis provides an ideal model system for studying protein turnover for obvious reasons: chicken ova are visible and easy to obtain and manipulate during essentially all stages of their development. Their size is rather convenient to fractionate and determine the various molecular components at every stage *in vitro*.

Lysozyme is one of the most extensively studied enzymes (Jolles and Jolles, 1984). Its ubiquitous distribution, ease of isolation, high stability and relatively small size made it an ideal model system in a diverse number of investigations (Prager and Jolles, 1996).

In pharmaceutical investigations, hen egg white lysozyme was found to protect the body against bacterial, viral and inflammatory diseases (Sugahara *et al*., 2000). It has been used in aerosols for the treatment of bronchopulmonary disease, as a prophylactic treatment for dental caries, for nasal tissue protection and was incorporated in various therapeutic creams for the protection and topical reparation of certain dystrophic and inflammatory lesions of the skin and soft tissue (Lacono *et al*., 1980). Oral administration of lysozyme has been shown to have immunostimulatory effect and an antihistaminic effect (Namba *et al*., 1981). Most recently, lysozyme has been found to have an anti HIV effect (Lee-Huang *et al*., 1999); this effect was attributed to a nonapeptide isolated from a digest of lysozyme (Lee-Huang *et al*., 2005).

Furthermore, lysozyme has been used as a marker in the course of several diseases. Serum lysozyme levels have been determined and used to assist in the diagnosis of several diseases including different types of leukemias (Osserman, 1973) as well as sarcoidosis (Canfield *et al*., 1974). Urinary lysozyme measurement is useful in evaluating patients with renal diseases, especially tubular dysfunctions (Hayslett *et al*., 1968). Lysozyme level determination has also been performed in several secretions including tears and cerebrospinal fluid (Sen and Sarin, 1982). The degeneration of tear glands in keratoconjunctivits sicca reduces the tear fluid production as well as the tear lysozyme concentration (Sen and Sarin, 1982). In the cereribrospinal fluid, the concentration of lysozyme is used as a sensitive index of inflammatory and neoplastic disease of the central nervous system (Harrison *et al*., 1973). Point mutations in human lysozyme gene were found to cause hereditary systemic amyliodosis, a disease in which amyloid deposition of the lysozyme variant in the viscera occurs (Pepys *et al*., 1993).

Previously, we have investigated lysozyme turnover during the process of embryo development (Eshbailat *et al*., 2004). However, to our knowledge,

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lysozyme activity has not been described in the early developmental stages of chicken oocytes. Therefore the present study was conducted to investigate the turnover of lysozyme during chicken ova development.

The significance of this study stems from the fact that lysozyme in the developing Oocyte could act as a prototype innate immune system for the protection of the Oocyte from infection.

2. Materials and methods

2.1. Materials

All chemicals were purchased from BDH Chemicals Ltd. or Sigma Chemical Company unless otherwise indicated. Chicken egg white lysozyme (three times recrystalized) and *Micrococcus Luteus* (freeze-dried bacteria) were from Sigma Chemical Company.

Molecular weight standards were obtained from Pharmacia Fine Chemicals. Poly-L-lysine Hydrochloride was also from Sigma Chemical Company. Single-comb white leghorn hens (*Gallus Domesticus*) 38 to 50 weeks of age, laying regular sequences of four or more eggs were brought from the university research farm, college of agriculture, University of Jordan.

2.2. Methods

2.2.1Rearing of experimental animals

A total of 25 hens were used in this study. Hens were individually housed in laying batteries with free access to feed and water under a controlled photoperiod of 17 hours light, 7 hours dark (lights on at midnight). Approximate time of oviposition was monitored daily and visually at 30 minute intervals so that it became possible to determine the prospective ovulation time. Each hen was sacrificed by cervical dislocation to collect eggs of both preovulatory and postovulatory stages of the hen ovulatory cycle.

2.2.2Preparation of oocyte extracts

Extracts from ovarian follicles at different stages of the follicular hierarchy of hen ovulatory cycle were prepared. One hen was dissected for that purpose at 20 minutes after oviposition. After dissection, the ovary was removed and immediately immersed in a beaker containing 0.9% physiological saline solution cooled on ice. Preovulatory follicles (9-32mm diameter) and prehierarchal follicles (2-8.9mm diameter) were removed from the ovary and their diameter was measured under the dissecting microscope by using a caliper. Follicles from 2- 10 mm diameter were sorted into 2 - 2.9 mm (10 follicles), 3 - 3.9mm (9 follicles), 4 - 4.9mm (6 follicles), 5 - 5.9mm (4 follicles), 6 - 6.9mm (2 follicles), 8 - 8.9mm (1 follicle) and 9 - 9.9mm (1 follicle) diameter groups. The follicles in each group were immersed according to their sizes in 0.006M PBS, pooled as one follicle pool and homogenized at low speed using the sorvall omni-mixer. The homogenate was then centrifuged twice at 6000 x g for 20 minutes at 4 °C to get a clear supernatant which was aliquoted and stored at -20°C to be used in electrophoresis and enzyme assays. Follicles above that diameter range; including the largest preovulatory follicle (32mm diameter) were cut down their stigma with a scalpel blade and the yolk was extruded from the membrane and transferred into falcon tube. Each yolk sample was then diluted with two volumes of 0.006M PBS and homogenized at low speed on ice. The homogenate was centrifuged twice at 6000 x g for 20 minutes at 4°C. The clear supernatant was aliquoted and finally stored at -20°C.

The oviducts and the oviductal eggs which represent the sequential stages of the postovulatory cycle were removed from adult hens several hours after prospective ovulation time. The character of the egg varies according to the time of collection from hens after prospective ovulation. At these stages of development, it was possible to separate egg yolk from egg white except for the egg collected from the posterior (caudal) portion of the infundibulum.

Each time, the eggs were collected on ice and each egg yolk was manually separated from the egg white and carefully rolled with a paper towel to remove all albumins from the vitelline membrane.

The membrane was then cut with a scalpel blade and the content of the yolk collected, diluted with two volumes of 0.066M PBS, 0.3M NaCl pH 6.2 and gently homogenized at low speed on ice. The homogenate was then centrifuged at 6000 x g for 30 minutes at 4°C. The supernatant was stored at -20 °C and recentrifuged next day to get a clear supernatant. The clear supernatant was collected, aliquoted and kept at -20°C. Egg white samples were collected after their separation from egg yolk, diluted with two volumes of PBS and gently homogenized at low speed on ice. The homogenate of the samples that contain mucin fibers was then centrifuged at 6000x g for 20 minutes at 4°C. The supernatant was aliquoted and stored frozen at -20 °C to be used in electrophoresis and enzyme assays.

2.2.3 Lysozyme activity assays

Lysozyme activity was detected in non-denaturing gels before staining by use of the substrate *Micrococcus Leutus* incorporated into an overlaying polyacrylamide gel as previously described (Reisfeld *et al*., 1962). To optimize sensitivity and suitability for photography a 4 step procedure was used as previously described (Saleh and Ibrahimi 1995). The overlaying gel was fixed in a solution containing 1% SDS, 0.1M NaCl and 0.05M ethylenediamine tetraacetic acid (EDTA) for 20 minutes.

Lysozyme activity in solution was determined by the lysis of bacterial cells of *Micrococcus Leutus* (Dobson *et al*., 1984). The mixture contained 0.75 mg of freeze-dried bacteria in 1.5ml of 0.066M phosphate buffer pH 6.2 plus 0.5ml of 0.3M NaCl. The decrease in turbidity at 540 nm every 0.5minute for a total of 12 minutes in 1ml assay at room temperature was recorded. One unit of lysozyme activity was defined as 1% increase in transmittance per minute, and was estimated from the initial slope of the line which relates turbidity to time. The bacterial suspension and lysozyme solution were always prepared fresh with Sigma chicken lysozyme used as a standard to correct for the variations in the assay.

The lysoplate method was also adapted to measure the lysozyme activity in agar plate, which was proven to be more sensitive to low lysozyme concentrations (Eshbailat *et al*., 2004). The solution mixture contained 0.05M sodium phosphate buffer pH 6.2, 0.02M NaCl, 0.02% (w/v) *Micrococcus Leutus*, 0.02% (w/v) sodium azide and

1.5% (w/v) agar. It was heated at 100°C in a water bath for 1 hour. Then, 14ml of the suspension were poured in disposable petri dishes. After solidification of the mixture, the plates were transferred to a drying cabinet for 15min at 60°C to remove the condensed steam from the surface of the plates. Wells of 0.5 cm in diameter were made in the plates and 17 µl of lysozyme solution were applied in each well. Finally the plates were incubated for 6 hours at 37°C to allow diffusion of lysozyme and formation of clearance zones around the well. Lysozyme activity was measured with chicken egg white lysozyme as a standard.

2.2.4 Electrophoresis

Denaturing polyacrylamide gel electrophoresis with SDS was carried out based on the method of Laemmli (Laemmli, 1970) and was generally done through 12% gels at pH 8.3 as outlined before (Guilmineau *et al*., 2005). The samples were prepared for electrophoresis after precipitation with trichloroacetic acid followed by solubilization and reduction with dithiothreitol (DTT) at 70°C for 5 minutes and alkylation with iodoacetamide at 37°C for one hour. Electrophoresis was carried out at a constant current of 20 mA for 6-7 hours. The gels were then stained with Coomassie Brilliant Blue G-250 and destained with a solution of 35% methanol, 19% acetic acid in distilled water.

For non-denaturing gel electrophoresis the procedure was compiled and kindly provided by Dr. Ellen Prager from the University of California, CA. Electrophoresis was performed at 4°C in vertical polyacrylamide gel slabs 0.1cm thick, 15cm long and 13cm wide, using the pH 4.3 β-alanine acetate buffer system (Reisfeld *et al*., 1962). No stacking gel was used. The sample buffer, which made up one third of the total volume, contained 0.1 (w/v) methylgreen in 50% (v/v) glycerol and water. For electrophoresis in the cathodal direction the buffer contained in addition 5mg/ml of poly-L-lysine to minimize smearing of low lysozyme concentration samples (Jolles *et al*., 1990).

3. Results

The determination of the volumes of the yolk and diameter of follicles during different stages of follicular hierarchy as well as the volumes of egg yolk and egg white during postovulatory (oviductal) stages of chicken oogenesis have been previously reported (Salman, 2009).

3.1. Lysozyme activity and protein assays

3.1.1Lysoplate assay

The activity of lysozyme was determined during different sequential stages of chicken oogenesis by the lysoplate assay which was proven to be more sensitive than the turbidimetric assay. A wide variation in the levels of lysozyme activity was detected in the different stages. This ranged from very low, barely detectable, levels of activity in the follicular stages to a very high level of activity in the postovulatory stages. For the yolk samples which were collected from the ovarian follicles in the early stages of oogenesis, low detectable levels of lysozyme activity were noticed in the yolk samples collected from the prehierarchal follicles (2-8.9mm in diameter). The activity was maintained all over the samples collected from preovulatory follicles (9-29.1mm in diameter) until the largest preovulatory follicle (32mm in diameter) prepared for ovulation (Fig.1).

In samples of egg white collected from the oviductal stages, high levels of activity were detected in samples collected from the anterior portion of the magnum, approximately the same activity of the chicken standard lysozyme. A significant increase in the activity was noticed in the egg white samples from the sequential stages of the oviduct compared to the activity obtained from the chicken standard lysozyme, 1.0mg/ml. (Fig. 2). The yolk samples from these stages had a consistently higher activity compared to that of the yolk from previous follicular stages, and lower activity compared to the egg white from oviductal stages (Fig. 2).

3.1.2 Turbidemetric assay:

The activity of lysozyme in egg yolk and egg white of various sequential stages of chicken oogenesis was also determined by the turbidimetric lytic assay. Lysozyme activity per ml and lysozyme activity per fraction as well as the total activity per oocyte at different stages was calculated (Table 1). During the hierarchal and preovulatory stages of oogenesis, it was not possible to detect the activity of lysozyme in the yolk samples collected from all ovarian follicles regardless of their diameter.

During the postovulatory stages, lysozyme activity was detected in both egg yolk and egg white. For the egg yolk,the activity started to appear in the yolk fraction collected from the caudal region of the infundibulum; were the highest level of activity was recorded. As the egg moved down the anterior portion of the magnum, the activity started to decrease gradually were the first concentric coats and layers of albumin are added. In the posterior portion of the magnum, 2.5 hours postovulation, when almost all the layers of albumin are deposited, there was a decline in the activity of lysozyme which continued until the ovum has entered the shell gland were the activity has reached its minimum values. For the egg white, the activity appeared in the sample from the anterior portion of the magnum. It increased sharply when the ovum was found in the posterior portion of the magnum, and then the activity started to decrease as the ovum entered the magnum isthmus junction, and no change in the activity was detected in the egg white from the isthmus. Throughout the presence of the ovum in the shell gland, the activity of lysozyme decreased with time.

3.1.3SDS-PAGE

Using SDS- PAGE, a lysozyme band (14.4 kDa) was not detectable in all the follicular stages of chicken oogenesis (results not shown). It should be noticed however that lysozyme activity was detected in those stages of follicular development in the very sensitive lysozyme activity assays. This suggests the presence of lysozyme in those stages but in a very low concentration. A very faint lysozyme band was detected in all the yolk fractions collected from the different segments of the oviduct. This could be due to low lysozyme concentrations in these fractions and the low sensitivity of the stain used. For the egg white a band having a molecular weight of 14.4 kDa started to appear in the fractions which were collected from the anterior portion of the magnum (Fig. 3).

The band remained present in the subsequent fractions collected from other portions of the oviduct. This band comigrated with the standard chicken egg white lysozyme; it was deduced that it is a c-type lysozyme.

3.1.4 Non-denaturing gel electrophoresis

Egg yolk and egg white were assayed for lysozyme activity during different stages of oogenesis by the gel overlay method at pH 4.3. All yolk samples obtained from the different ovarian follicles including the small white prehierarchal follicles (2-5mm in diameter), the small yellow prehieracchal follicles (6-8mm in diameter), and the preovulatory follicles (9-32mm in diameter) showed clear cut lytic lysozyme activity bands on the overlay activity profiles (Fig.4). The activity bands were highly reduced in the ovarian preovulatory follicles (24.8 to 32mm in diameter) (Fig. 4). This could be due to great yolk deposition in these stages of ovarian follicle development. All of the bands in these stages did not correspond to protein bands in the leftover native gel (results not shown). This suggests the presence of lysozyme in the earliest stages of chicken oogenesis but at very low concentrations.

The egg yolk samples obtained from the different segments of the oviduct showed a clear lytic lysozyme activity band (Fig. 5). This indicates the presence of lysozyme in the mature yolk throughout its transport in the chicken oviduct. All of these bands corresponded to very faint protein bands in the leftover protein gel suggesting an increase in lysozyme concentration compared to very low concentrations of lysozyme in the earliest follicular stages of oogenesis. Lysozyme activity bands also appeared in the egg white samples collected from the different sequential segments of the chicken oviduct (Fig.5).

4. Discussion

4.1. The activity levels of lysozyme in different stages of the follicular hierarchy of chicken oogenesis :

The activity of lysozyme was determined for egg yolk fractions collected from the various ovarian follicles during sequential stages of the follicular hierarchy by the lysoplate assay, turbidimetric lytic assay and non-denaturing gel electrophoresis using *Micrococcus Leteus* as a substrate.

Using the lysoplate assay, the activity of lysozyme was detected in all the yolk fractions obtained from chicken ovarian follicles regardless of their diameter as indicated by the different lysis zones. These follicular stages included the small white prehierarchal follicles (2-5.9mm in diameter), small yellow prehierarchal follicles (6-8.9mm in diameter) and the preovulatory follicles (9-32mm in diameter). Based on the diameter of the lysis zones, yolk fractions obtained from these ovarian follicles exhibited a low lysozyme activity compared to the activity observed for standard chicken lysozyme (1.0mg/ml) used in the assay, reflecting a very low concentration of lysozyme in these samples.

In order to further assess the levels of lysozyme activity in these follicular stages, the turbidimetric lytic assay and overlay assay on non-denaturing gels were applied.

Based on the results of turbidimetric assay, these stages of development did not show any levels of activity. On the other hand, the same stages showed very clear activity bands in the overlay assay. The appearance of activity by this method and the absence of activity by the turbidimetric assay are due to the sensitivity of the overlay assay to very low concentrations of lysozyme. No corresponding lysozyme bands were detected in SDS PAGE. This can be interpreted by the very low concentration of the enzyme in these fractions and the very low sensitivity of the stain used in these gels (results not shown).

The ovarian follicle of the laying hen is a highly vascularised part of the hen ovary; which serves a nutritive function during the process of oogenesis. Blood vessels enter the follicle through the pedicle or the stalk and extend into all portions if the follicle, except for a narrow band, the stigma, opposite the pedicle. Later, when the most rapid deposition of yolk is taking place, the follicle passes the materials necessary for yolk formation from the blood to the oocyte (Romanoff and Romanoff, 1949). Furthermore the follicle may possibly elaborate some of the materials and nutritive substances across the intercellular bridges to the cytoplasm of the oocyte (Sturkie, 1976). So it can be deduced that lysozyme may probably be one of the intracellular enzymes in the ovarian follicles, and is produced by the granulose cells with the materials needed for yolk formation. It also may pass via blood circulation inside the follicle. Lysozyme distribution in rabbit and human blood among different blood elements and serum has also been studied (Flanagan and Lionetti, 1955). Appreciable values of lysozyme activity were found in both leukocytes, especially the granulocytes, and blood plasma. This indicates that lysozyme is an integral part of the cell and that the liberation of the activity into the plasma is a good index of the white blood cell integrity. In a previous investigation (Margaret *et al*., 1950), lysozyme distribution in chicken blood serum and egg yolk has been studied by a quantitative immunological technique. In this method, rabbit antisera were produced specifically against lysozyme and no lysozyme could be detected in these systems. Moreover, lysozyme presence was detected in chicken blood serum using the turbidimetric assay (Bazlamit, 2009). These inconsistencies could be due to differences in the methods applied; immunological reactions need certain concentrations of the protein to give positive results compared to very specific technique used for lysozyme activity. Our results demonstrate, for the first time, that chicken lysozyme is synthesized in the ovarian follicles during early stages of oogenesis but at considerably lower levels compared to later stages of this process.

4.2. The activity levels of lysozyme in different postovulatory stages of chicken oogenesis:

For the egg white and egg yolk from the different postovulatory stages, the fractions collected from different portions of the chick oviduct were assayed for lysozyme activity by the lysoplate assay, the overlay assay and the turbidemetric assay as described in methods. For the egg yolk, the activity was detected in all the fractions examined. It was higher than the activity observed in the counterpart fractions collected from the ovarian follicles as indicated by the lysis zones. For the egg white high detectable levels of lysozyme were also observed in all fractions examined.

Using the overlay assay on native gels, a definite lysozyme activity band was detected in all egg white and egg yolk fractions examined. To further determine the activity of lysozyme in these fractions, the turbidimetric assay was also performed as described in methods.

For the egg white, the highest level of activity was noticed in the egg white sample collected from the posterior portion of the magnum reaching as high as 1485 units per ml as would be expected, since the magnum of the laying hen is the site were the lysozyme is secreted and concentrated (Gilbert, 1971). The egg white from the magnum isthmus junction and the isthmus showed a lower level of activity (806 units per ml). A notable decrease in the activity was observed in the shell gland were water is added in a considerably rapid rate during the process of plumbing (King and McLelland, 1975). This aids in the dilution of egg white and eventually the decrease in the total number of lysozyme molecules per volume of egg white. From the result obtained, the activity levels in the egg yolk were decreasing as the ovum moved down the oviduct. This gradual decrease in activity can be interpreted as being the result of transfer of some egg white proteins to the egg yolk during the passage of the ovum along the oviduct (Mann *et al*., 2008). On the other hand, lowest levels of the enzyme activity were noticed in the shell gland, this is also a result of yolk dilution and passage of liquid to the yolk during the plumbing process.

Using the SDS-PAGE, a clear band was observed in the egg white fractions collected from the anterior part of the magnum up to the shell gland with very faint bands in the yolk fractions. On the other hand remarkable activity bands for lysozyme were observed in all the yolk fractions using the very sensitive lysoplate and overlay assays. The presence of lysozyme in egg yolk was recently studied by proteomic analysis. This analysis has identified 119 different proteins in the egg yolk. Lysozyme c was one of the major proteins identified (Mann *et al*., 2008).

Table 1. Lysozyme activity during different postovulatory (oviductal) stages of chicken oogenesis by the turbidimetric lytic assay

Site of sample collection from the oviduct	Lysozyme activity (units/ml)		Lysozyme activity/fraction		Total lysozyme activity/egg
	Egg white (units/ml)	Egg yolk (units/ml)	Egg white	Egg yolk	
Infundibulum	ND*	105	ND*	1575	1575
Anterior portion of the magnum	696	70	1392	1085	2477
Posterior portion of the magnum	1485	60	21622	887	22509
Magnum-Isthmus junction	806	36	12902	582	13485
Isthmus	806	26	16934	441	17375
Shell gland(1)**	672	17	18816	336	19152
Shell gland(2)***	546	17	19110	336	19446

* Not determined; because the amount of egg white collected was too small to use in the biochemical assays.

** Egg white and egg yolk fractions collected from the shell gland 10 hours post-ovulation.

***Egg white and egg yolk fractions collected from the shell gland 18 hours post-ovulation.

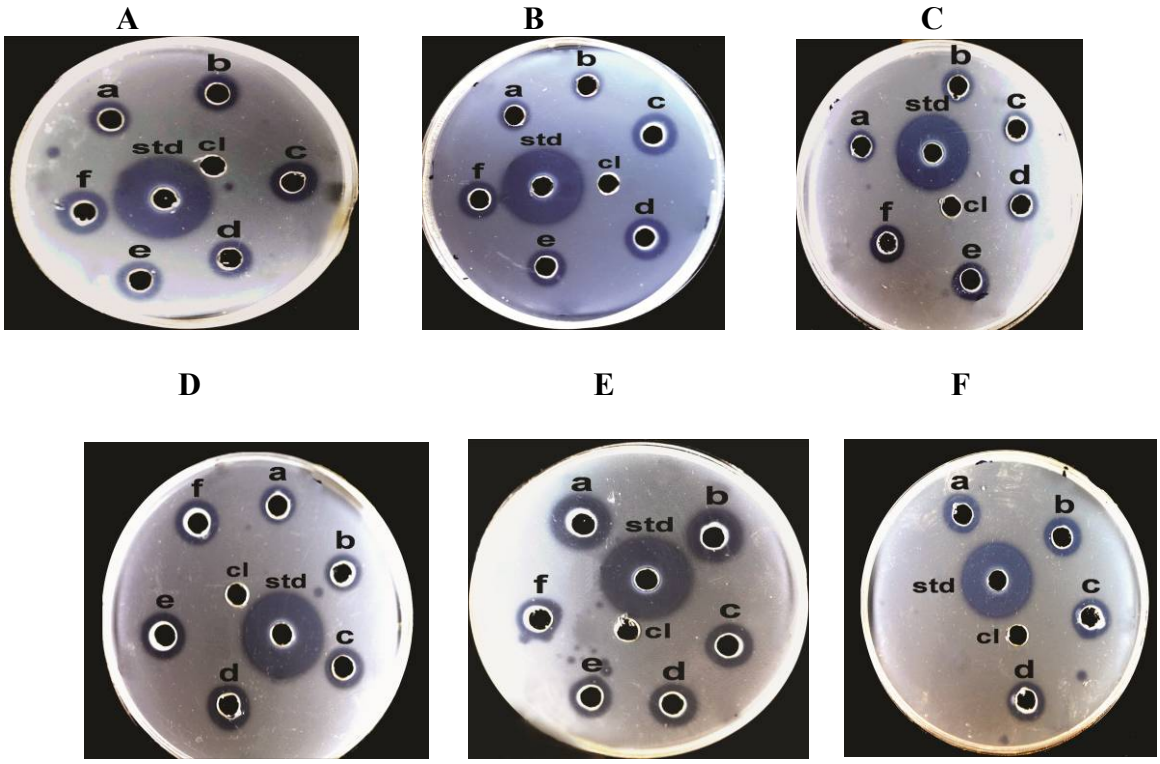

Figure 1. Lysoplate results of lysozyme activity in yolk samples during successive preovulatory (follicular) stages of Oogenesis

Lysoplate assay was done as described in methods. 17 follicles were assayed in duplicates ranging in size from 2-32mm in diameter as shown in A to F successively. a and b, c and d, e and f are duplicates in each case. Std is a positive control and cl is a negative control.

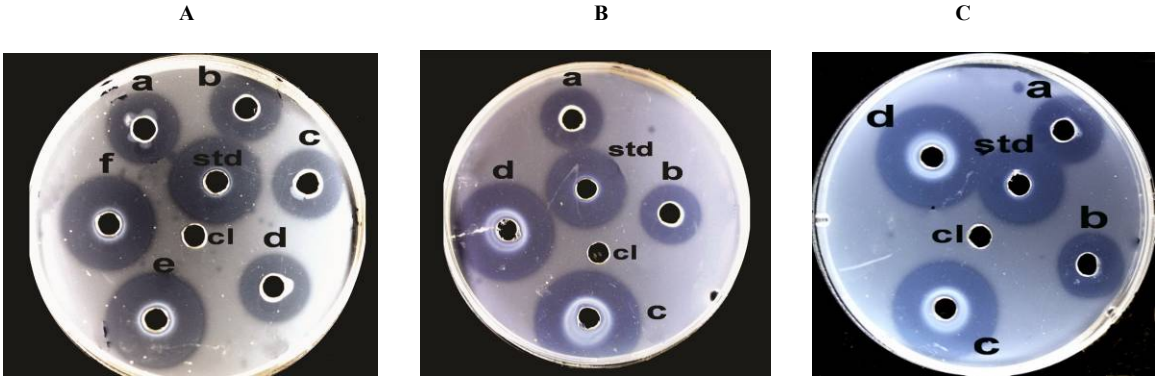

Figure 2. Lysoplate results of lysozyme activity during successive postovulatory (oviductal) stages of oogenesis

The results include both egg white and egg yolk. Each plate included a positive (std) and a negative (cl) control. The stages are:

A: a and b contained egg yolk in the ova from the posterior region of the infundibulum; c and d contained egg yolk in the ova from the anterior part of the magnum; e and f contained the egg white in the ova from the anterior part of the magnum.

B: a and b contained the egg yolk in the ova from the posterior part of the magnum; c and d contained the egg white in the ova from the posterior part of the magnum.

C: a and b contained the egg yolk in the ova from the magnum-isthmus junction; c and d contained the egg white in the ova from the magnum-isthmus junction.

***Figure 2 continues next page…….**

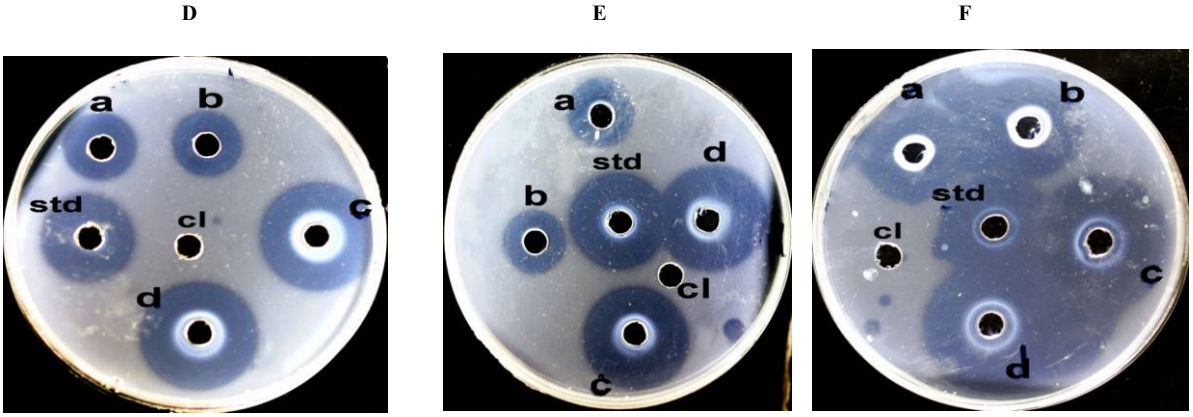

Figure 2. Lysoplate results of lysozyme activity during successive postovulatory (oviductal) stages of oogenesis

The results include both egg white and egg yolk. Each plate included a positive (std) and a negative (cl) control. The stages are:

D: a and b contained the egg yolk in the ova from the isthmus; c and d contained the egg white in the ova from the isthmus.

E: a and b contained the egg yolk in the ova from the shell gland 10 hrs post ovulation; c and d contained the egg white in the ova from the shell gland 10 hrs post ovulation.

F: a and b contained the egg yolk in the ova from the shell gland 18 hrs post ovulation; c and d contained the egg white in the ova from the shell gland 18 hrs post ovulation.

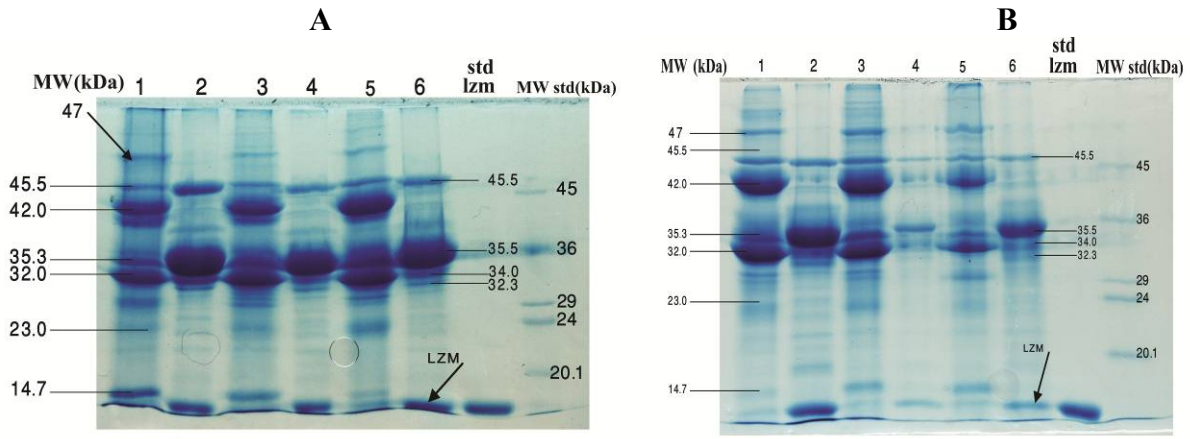

Figure 3. SDS-PAGE results of lysozyme in the egg yolk and egg white of successive postovulatory (oviductal) stages of oogenesis

A: lanes 1 and 2 contained egg yolk and egg white in the ova from the anterior part of the magnum, respectively.Lanes 3 and 4 contained egg yolk and egg white in the ova from the posterior part of the magnum, respectively. Lanes 5 and 6 contained egg yolk and egg white in the ova from the magnum-isthmus junction, respectively.

B: lanes 1 and 2 contained egg yolk and egg white in the ova from isthmus respectively.

Lanes 3 and 4 contained egg yolk and egg white in the ova from the shell gland 10 hrs post ovulation. Lanes 5 and 6 contained egg yolk and egg white in the ova from the shell gland 18 hrs post ovulation. STD is standard chicken lysozyme and MW is molecular weight standard

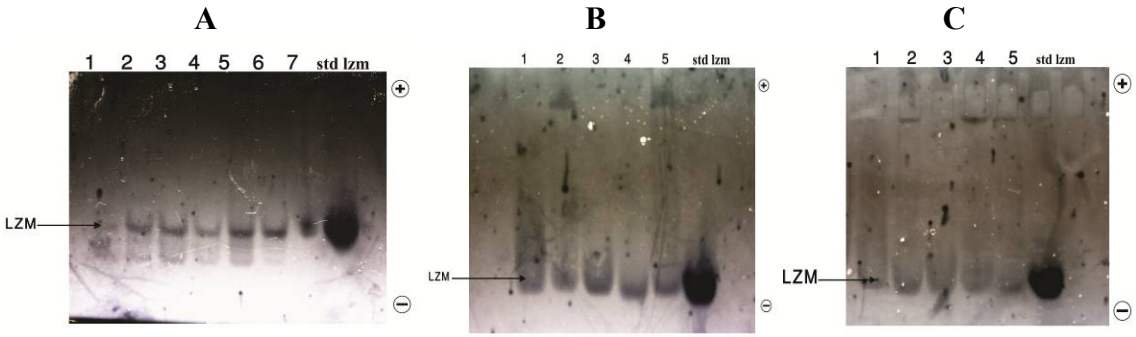

Figure 4. Non-denaturing gel electrophoresis coupled with the overlay lysozyme activity gel for successive preovulatory (follicular) stages of oogenesis. 17 successive follicular stages ranging in size from 2-32 mm in diameter were assayed as shown in A, B and C. A distinct lysozyme activity bands is shown in all stages.

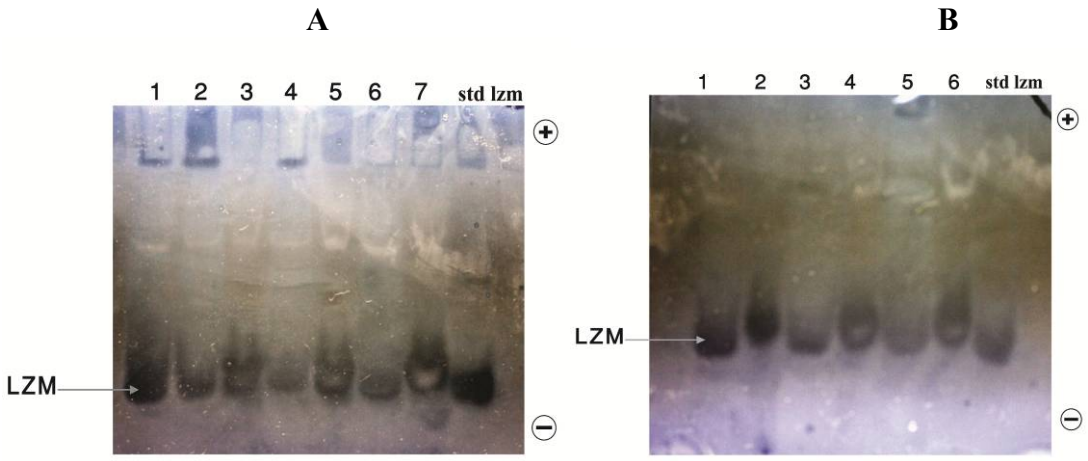

Figure 5. Non-denaturing gel electrophoresis coupled with the overlay lysozyme activity gel for the successive postovulatory (oviductal) stages of oogenesis ranging from posterior part of the infundibulum to the shell gland. Even numbers represent the egg yolk extract and odd numbers represent the egg white extract.

A: lane 1 contained egg yolk from posterior region of the infundibulum. Anterior portion of the magnum: Lane 2 egg yolk and lane 3 egg white. Posterior portion of the magnum: lane 4 egg yolk and lane 5 egg white, magnum-isthmus junction: lane 6 egg yolk and lane 7 egg white.

B: isthmus: lane 1 egg yolk and lane 2 egg white, shell gland 10 hrs post ovulation: lane 3 egg yolk and lane 4 egg white, shell gland 18 hours post ovulation: lane 5 egg yolk, lane 6 egg white.

A distinct lysozyme activity band is seen in all stages.

Acknowledgements

This study was supported by a grant from the Deanship for Scientific Research, University of Jordan. Amman, Jordan.

References

Bazlamit Z. 2009. In: The distribution of lyszyme in certain fluids and tissues of the chick and goose with special reference to polyymorphism.M.Sc. Thesis, University of Jordan, Jordan.

Canfield R E, Collins J C and Sobel J H. 1974. Human leukemia lysozyme. In: Osserman E F, Canfield, R E and Beychok S. (Eds), **Lysozyme**. Academic Press, New York.

Dobson D E, Prager EM and Wilson A C. 1984. Stomach lysozyme of ruminants: Distribution and catalytic properties. *J. Biol. Chem*., **259**: 11607-11616.

Eshbailat S, Haj Hand Ibrahimi I. 2004. Turnover of lysozyme during the development of chicken embryo. *Dirasat, medical and biological sciences*, **31(2):** 103-115.

Flanagan P and Lionetti F. 1955. Lysozyme distribution in blood. *Blood*, **10**: 497-501.

Gilbert A B. 1971. The ovary. In: Bell, D.J. and Freeman, B.M. (Eds), **Physiology and Biochemistry of the Domestic fowl**. Academic press, New York. Pp. 1163-1208.

Guilmineau F, Krause I and Kulndozik U. 2005. Efficient analysis of egg yolk proteins and their thermal sensitivity using sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing and nonreducing conditions. *JAgric Food Chem.*, **53**: 9329−9336.

Harrison JF, Parker RQ and Desilva K L. 1973. Lysozymuria and acute disorders of renal function. *J Clin Pathol*, **26**: 279-284.

Hayslett J P, Perllie P E and Finch S C. 1968. Urinary muramidase and renal disease: correlation with renal histology and implication for the mechanism of enzymuria. *N Engl J Me*d., **279**: 506-512.

Jollés P and Jollés J. 1984. What's new in lysozyme research? Always a model system, Today as yesterday (Review). *Mol Cell Biochem.*, 63: 165-189.

JollésJ, PragerE M, Alnemri E S, Jollés P, Ibrahimi I M and Wilson A C.1990. Amino acid sequence of stomach and non-stomach lysozyme of ruminants. *J Mol Evol.*, **30**: 370-382.

King A S and McLelland J. 1975. Female Reproductive System. In: **Outline of Avian Anatomy.** The Macmillan Publishing Company Inc., New York, Pp. 65-73.

Lacono V J, Mackay B J, Dirienzo S and Pollock J J. 1980. Selective antibacterial properties of lysozyme for oral microorganisms. *Infection and Immunity*, **29(2):** 523-532.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680-685.

Lee-Huang S, Huang P, Sun Y, Kung H, Blithe D and Chen H. 1999. Lysozoyme and RNases as an anti HIV components in β-core preparations of human chorionic gonadotropin. *PNAS*, **96**: 2678-2681.

Lee-Huang S, Maiorov V, Huang P, Angela N, Lee H, Chang Y, Kallenbach N, Huang P and Chen H. 2005. Structural and functional modeling of human lysozyme reveals a unique nonapeptide H, with anti-HIV activity. *Biochem.*, **44:** 4648-4655.

Mann K, Olsen J V, Maček B, Gnad F and Mann M 2008. Identification of new chicken egg proteins by mass spectrometry-based proteomic analysis. *World's Poultry Science J*, **64**: 209-218.

Margaret E, Marshall E and Deutsch H F. 1950. Distribution of egg white proteins in chicken blood serum and egg yolk. *The J of Biol Chem.*, **24**: 1-9.

Namba Y, Hidaka Y, Taki K and Morimoto T. 1981. Effect of oral administration of lysozyme on digested bacterial cell walls on immunostimulation in guinea pigs. *Infection Immunity*, **31(2):** 580-583.

Osserman E F. 1973. Monocytic and monomyelocytic leukemia with increased serum and urine lysozyme as a late complication in plasma cell myeloma. *British Medical J.*, **8**: 215-222

Pepys M B, Hawkins P N, Booth D R, Vigushin D M, Tennent G A, Soutar A K, Nguyen O, Blake CCF, Terry C J, Zalin A M and Hsuan J J. 1993. Human lysozyme gene mutations cause hereditary systemic amyloidosis. *Nature*, **362:** 553-557.

Prager E M and Jollés P. 1996. Animal lysozyme C and G: An overview. In: Jolles P (Ed), **Lysozyme: Model Enzymes in Biochemistry and Biology**. Birkhauser Venag Baser, Switzerland. Pp. 9-31.

Reisfeld R A, Lewis U J and Williams D E. 1962. Disc electrophoresis of Basic proteins and peptides on polyacrylamide gels. *Nature*, **195**: 281-283.

Romanoff A L and Romanoff A J. 1949. Egg formation. In: **The Avian Egg**. (Edited by John Wiley & Sons, Inc.) New York, Pp. 199-251.

Saleh A and Ibrahimi I. 1995. Electrophoretic polymorphism in rabbit tear lysozyme. *Comp Biochem Physiol.,* **112 B** (1): 21-30.

Salman H M. 2009. In: Protein turnover during chick oogenesis with special reference to lysozyme. M. Sc. Thesis, University of Jordan, Jordan.

Sen D R and Sarin G S. 1982. Immunoassay of tear lysozyme in conjunctivital diseases. *BrJ Ophthalmol.,* **66**: 732-735.

Sturkie P D. 1976. Reproduction in the female and egg formation. In: Sturkie P D. (Ed) **Avian Physiology**. Springer-Verlag, New York Heidelberg Berlin. Pp. 303-326.

Sugahara T, Murakami F, Yamada Y and Sasaki T. 2000. The mode of action of lysozyme as an immunoglobulin production stimulation factor. *Biochimica et Biophysica Acta*, **1475**: 27-34.