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ISOLATION AND CHARACTERIZATION OF DUCK EGG WHITE OVOMUCIN

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ABSTRACT: In the current study, ovomucin was isolated from Egyptian duck and characterized by several methods including SDS-PAGE, Urea-PAGE, and amino acids composition. Furthermore, Antibacterial activity was estimated against some gram positive and gram negative bacteria. Based on SDS-PAGE, the ovomucin consists of 2 subunits β-ovomucin and α-ovomucin. The molecular masses of these subunits are 400 and 210 KDa, respectively. The migration in Urea-PAGE from anode to cathode direction indicated that ovomucin was much faster than their respective duck egg white protein referring to bigger positive charges and basic amino acids. The content of the hydrophobic amino acids residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) is 35.02% and this represents 35.78% of the total amino acids. The content of the acidic amino acids residues (asp + glu) is 22.5% and the case lower than that of the basic amino acids (arg + lys + his; 27.99%). The minimum inhibitory concentration (MIC) of ovomucin from duck egg white was 50 µg/ml against *Bacillus subtilis* and 100 µg/ml against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. This study elucidated that the ovomucin isolated from duck egg white has potent antibacterial activity against selected pathogenic bacteria.

Key words: Duck egg white, ovomucin, antibacterial activity, amino acids composition.

INTRODUCTION

Egg albumen is vastly used in food industry due to its several functional properties such as solubility, foaming, and emulsifying properties **(Wang and Wang, 2009; Mleko *et al*., 2010; Shan *et al.*, 2012)**. The main component in egg white is ovomucin which making up 2-4% (*W/W*) from egg white **(Omana and Wu, 2009; Omana *et al*., 2011; Wang and Wu, 2012)**. Ovomucin consists of two subunits. The first unit is α- ovomucin (carbohydrate- poor subunit) containing 11–15% (*W/W*) carbohydrate and the second unit is β-ovomucin (carbohydrate-rich subunit) containing 50–57% (*W/W*) carbohydrate **(Offengenden *et al.,* 2011).** Two forms of ovomucin were identified based on the solubility profile: the insoluble shape (23×10^6 Da) and the soluble shape (83×10^5 Da) **(Tominatsu and Donovan, 1972; Hayakawa and Sato, 1976)**. Many publications have been made on the relations between the structural and functional attributes of ovomucin like emulsifying and foaming properties **(Kato *et al.,* 1985; Mine, 1995; Alleoni, 2006; Hammershøj *et al.,* 2008; Omana *et al.,* 2010)**. In addition, ovomucin presented anti-bacterial activity against *Helicobacter pylori* **(Kodama and Kimura, 2001).** Abundant studies have focused on egg white proteins particularly from chicken because chicken eggs constitute one of the main protein sources of our diet **(Miguel *et al.,* 2005)**. In the current study ovomucin was isolated from Egyptian duck and it was characterized by several methods such as SDS-PAGE, Urea-PAGE, and amino acids composition. Furthermore, Antibacterial activity was estimated against some gram positive and gram negative bacteria.

MATERIALS AND METHODS

Materials

Fresh eggs laid within 24 hr., from Egyptian ducks were collected in the morning from Abou-

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Hamad, Sharkia diskict, Egypt and used within the same day for ovomucin preparation.

Methods

Egg White and Ovomucin Isolation

Protein separation was performed according to the protocol recorded by **Omana *et al.* (2011)**, with slight modification. Egg white was minutely separated from yolk and softly homogenized with a magnetic stirrer for 20 min to reduce the viscosity. The white homogenate was lyophilized and preserved at -20°C until used. Ovomucin was isolated from lyophilized egg white according to **Omana and Wu (2009)** as presented in Fig. 1.

SDS-PAGE

SDS–PAGE was subjected on stacking gel (3%) and resolving gel (15%) which were prepared from acrylamide (30%, *W/V*) and N, N-bis methylene acrylamide (0.8%, *W/V*) solution. according to **Laemmli (1970)**. The electrode buffer pH 8.3 consists of 0.192 M glycine, 0.1% SDS and 0.025 M Tris. Ten milligrams of each sample (duck egg white and ovomucin) were added to 1 ml of 0.03 M Tris buffer (pH 8.0) for 20 min with vortexing and the extract was centrifuged for 10 min at 10000 x g. Twenty microliters from the supernatant were mixed with 20 µl of SDS-loading sample buffer, heated at 96°C for 5 min and an aliquot (15 µl) from the final mix was subjected to electrophoresis. Running was performed at 10 mA for stacking gel and 20 mA for resolving gel. Staining was completed with Coomassie Brilliant Blue R-250 dye. The molecular weight of bands was measured using the conforming protein marker (10 -500 KD).

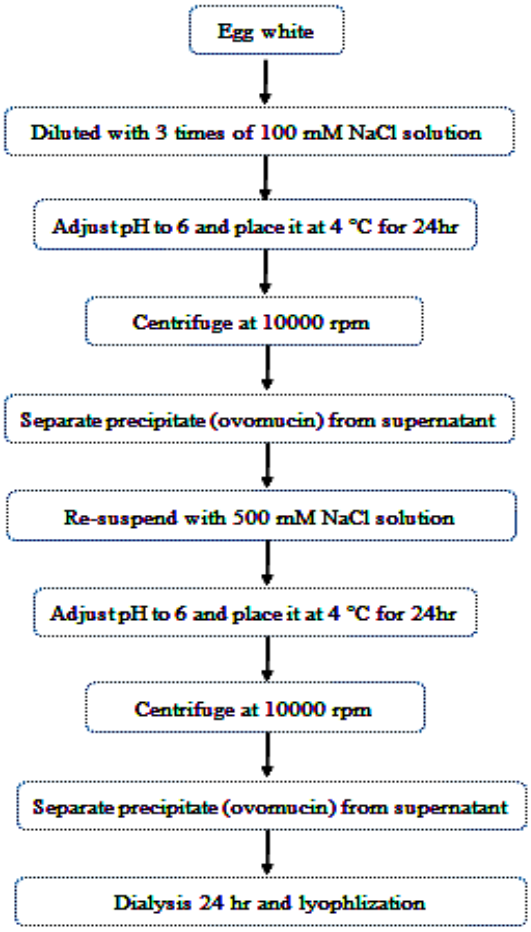

Fig. 1. Diagram for ovomucin isolation

Urea-PAGE

Lyophilized duck egg white and ovomucin (20 mg/ml) were mixed with buffer pH 6.8 containing 0.25 M Tris-base, 50% glycerol and bromophenol blue traces. Samples were centrifuged at 10000 xg for 10 min at 4˚C. Supernatants were subjected to Urea-PAGE (10 µl of protein/lane) in 3% and 12% stacking and running gels, respectively, according to **Evans and Williams (1980)**.

Amino Acids Analysis

The composition of amino acids for ovomucin isolated from duck egg white was evaluated according to **Simpson *et al.* (1976)** by amino acid analyzer instrument model "Eppendorf LC3000" using the following procedure: 0.2 g from ovomucin isolated from duck egg white received in 10 ml HCl (6N) in a sealing tube, and then put in oven at 110°C for 24 hr. Hydrolysates were transported quantitatively into a porcelain dish and HCl was evaporated to dryness at 50-60 °C on a water bath. To remove the excess of hydrochloric acid, 5 ml distilled water was added to the hydrolysates and evaporated to dryness. Finally, the remain was dissolved in 10 ml distilled water and filtrate through filter membrane (0.45 µm). The filtrate was lyophilized with a lyophilizer (Heto power Dry ll 3000 freeze Dryer thermo Electron corporation, Czech Republic), then 10 ml of distilled water were added and the samples lyophilized a second time. One ml of sodium citrate buffer 0.2 N at pH 2.2 was added and the samples stocked frozen in a closed vial until amino acids separation by amino acid analyzer (Column: hydrolysate column Eppendorf LC 3000 (250 × 4.6) the conditions are as follows: temperature is 47 °C; Sample: 30 µl; Buffer system: buffer A: Sodium acetate pH 3.3, buffer B Sodium acetate pH 3.6, buffer C: Sodium acetate pH 4.3 and buffer D: Sodium acetate pH11.0; Flow rate: 0.3 ml/min.). Ninhydrin is utilized for the revelation of amino acids at 440 nm for proline and 570 nm for the other amino acids out of an oxidative decarboxylation reaction. The peak zone and percentage of each amino acid were calculated by computer software AXXIOM CHROMATOGRAPHY- 727.

Antibacterial Activity Estimation

Microorganisms

Two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were kindly gained from the Laboratory of Microbiology, Department of Microbiology, Faculty of Science, Zagazig University, Egypt.

Agar well-diffusion assay

Ovomucin isolated from duck egg white was tested for antibacterial activity at different concentrations (0, 25, 50, 100, 150 and 200 µg/ml) against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) by conventional well-diffusion assay **(Nanda and Saravanan, 2009)**. The clear cultures of bacterial strains were sub-cultured on nutrient broth at 37˚C on a rotary shaker at 200 rpm. Every strain was dispersal uniformly onto the single plates using sterile cotton swabs. Wells of 6-mm diameter were made on Müller Hinton Agar (MHA) plates using a gel puncturing tool. Forty µl of each concentration (0, 25, 50, 100, 150 and 200 µg/ml) were carried into each well. After incubation at 37˚C for 24 hr., the diameter of the inhibition zone was recorded by using a ruler.

Minimum inhibitory concentration (MIC)

The antibacterial activity against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) was checked by the conventional well-diffusion assay **(Nanda and Saravanan, 2009)** as described above in section 2.6.2. Minimum inhibitory concentration (MIC) of ovomucin was estimated as recorded earlier **(Abdel-Hamid *et al.*, 2016)**. The lowest concentration of the examined articles that presented visible clear zone on Mueller-Hinton agar plates was regarded as the minimal inhibitory concentration.

RESULTS AND DISCUSSION

SDS-PAGE and Urea-PAGE of Duck Egg White and Ovomucin

SDS-PAGE of duck egg white (lane 1) and ovomucin (lane 2) compared to standard molecular weight (St) are shown in Fig. 2. Duck egg white recorded a broad zone of proportional

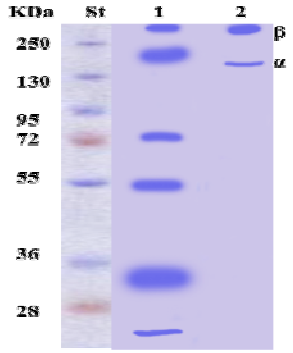

Fig. 2. SDS-PAGE of duck egg white (lane 1) and ovomucin (lane 2) compared to standard molecular weight (St) marker

molecular masses, present in very various concentrations. The major egg white proteins, ovalbumin, ovotransferrin, ovomucoid and lysozyme, constitute, 54, 12, 11 and 3.4% of total egg white proteins, respectively. These results are in agreement with **Mine (1995).** The molecular masses of ovomucin isolated from duck egg white are evaluated from the same gel (Fig. 2). The ovomucin consists of 2 subunits β-ovomucin and α-ovomucin. The molecular masses of these subunits are 400 and 210 KDa, respectively. These results are in agreement with **Robinson and Monsey (1971), Itoh *et al.* (1987), Hiidenhovi *et al.* (1999), Omana *et al.* (2010), Shan *et al.* (2012), Abeyrathne *et al.* (2014), Liu *et al.* (2017)**. The first unit is α-ovomucin (carbohydrate-poor subunit) containing 11–15% (*W/W*) carbohydrate and the second unit is β-ovomucin (carbohydrate-rich subunit) containing 50–57% (*W/W*) carbohydrate **(Offengenden *et al.*, 2011)**.

The migration in Urea-PAGE from anode to cathode direction (Fig. 3) indicated that ovomucin was much faster than their respective duck egg white protein referring to bigger positive charges and basic amino acids. These results are may have the positive impact for the antibacterial activity.

Amino Acids Composition of Duck Ovomucin

The amino acids composition of ovomucin separated from duck egg white are listed in Table 1.

The content of the hydrophobic amino acids residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) is 35.02% and this represents 35.78% of the total amino acids. The content of the acidic amino acid residues (asp + glu) is 22.5% and lower than that of the basic amino acids (arg + lys + his; 27.99%). The content of the essential amino acid residues (Lys, His, Thr, Meth, Ileu, Leu, Tyr and Phe) is 51.17%

Antibacterial Activity of Duck Egg White Ovomucin

The ovomucin from duck egg white was subjected at different concentrations (0, 25, 50, 100, 150 and 200 µg/ml) to Petri dishes containing Müller Hinton Agar infected with two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), After incubation at 37°C for 24 hr., and the obtained inhibition zones diameter (mm) of the resulting are listed in Table 2. The diameter of the inhibition zones has increased with the increment of the extracts concentration.

The minimum inhibitory concentration (MIC) of ovomucin from duck egg white was 50 µg/ml against *Bacillus subtilis* and 100 µg/ml against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Table 3). Ovomucin presented antibacterial activity against *Helicobacter pylori* **Kodama and Kimura (2001).** The antibacterial properties of proteins are dependent on their interaction with the bacterial cell wall and membranes **Hancock (2004)**. Positively charged protein electrostatically binds to lipopolysaccharides on the outer membrane of gram negative bacteria or lipoteichoic acids on the surfaces of gram positive bacteria **Glinel *et al.* (2012)**. Based on the results of Urea-PAGE and amino acids composition reflect the more fundamental nature of the ovomucin and high activity as an antibacterial agent.

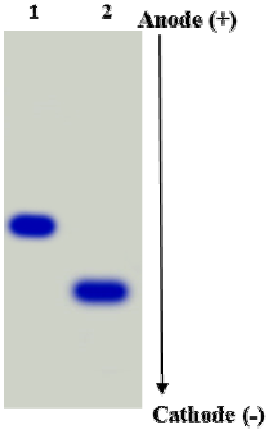

Fig. 3. Urea-PAGE of duck egg white (lane 1) and ovomucin (lane 2)

Table 1. Amino acids composition of duck ovomucin

Amino acid	Concentration (%)
Aspartic	10.00
Threonine*	2.17
Serine	4.87
Glutamic	12.50
Proline	0.11
Glycine	8.12
Alanine	7.87
Valine	3.14
Methionine*	2.65
Isoleucine*	1.78
Leucine*	6.64
Tyrosine*	3.10
Phenylalanine*	7.36
Histidine*	4.12
Lysine*	23.4
Arginine	0.47
Ammonia	1.70

*Essential amino acids

Table 2. The Inhibition zone diameter (mm) induced in two gram positive bacteria and two gram negative bacteria using agar well diffusion assay of ovomucin from duck egg white

Microorganisms	Inhibition zone diameter (mm)					
	0	50	100	200	500	1000
Gram +						
Staph. Aureus	0 ±0	0 ±0	10 ±0	17±0.01	22±0.02	33±0.01
B. subtilis	0 ±0	8 ±0	13 ±0	22±0.02	30±0.1	37±0.01
Gram -						
Pse. aeruginosa	0 ±0	0 ±0	11±0.08	13±0.08	18±0.07	25±0.09
E. coli	0 ±0	0 ±0	9 ±0.04	12±0.06	16±0.05	23±0.08

Table 3. Minimum inhibitory concentration (MIC) of ovomucin from duck egg white against susceptible bacteria[a]

Strains	MIC (µg/ml)
Staph. aureus	100
B. subtilis	50
Pse. aeruginosa	100
E. coli	100

[a] MIC rates are the mean of three freelance experiments; no diversity in MIC values was exposed between experiments.

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عـــــزل وتـوصيـــف الأوفـــوميـــوســـــين مـــن بيـــض البـــــط

هاله حسن عبدالجليل - على عثمان محمد - أحمد محمد أبوعيطه - سيد سليمان السعدنى

قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة الزقازيق – الزقازيق - مصر

فى هذه الدراسة تم فصل الأوفوميوسين من بيض البط البلدى المصرى وعمل توصيف كيميائي له بعدة طرق منها الهجرة فى المجال الكهربى على جيل SDS-PAGE وكذلك الهجرة في المجال الكهربى على جيل Urea-PAGE بالإضافة إلى تقدير محتواها من الأحماض الأمينية، أظهرت نتائج الهجرة في المجال الكهربى باستخدام SDS-PAGE أن الأوفوميوسين يتكون من وحدتين هما ألفا-أوفوميوسين وبيتا-أوفوميوسين تبلغ أوزانهم الجزيئية ٢١٠ و ٤٠٠ كيلودالتون على التوالى، كما أظهرت نتائج الهجرة فى المجال الكهربى باستخدام جيل Urea-PAGE أن الأوفوميوسين يتحرك أسرع من الألبيومين جهة القطب السالب مما يشير الى الطبيعة الموجبة لهذا البروتين، كما تشير نتائج تحليل الأحماض الأمينية أن نسبة الأحماض الأمينية الهيدروفوبية ٣٥,٧٨% من إجمالى الأحماض الأمينية بينما نسبة الأحماض الأمينية الهيدروفيلية (الحامضية والقاعدية) حوالى ٢٢,٥% و ٢٧,٩٩% على التوالى، أظهرت نتائج النشاط المضاد للبكتيريا أن الحد الأدنى من التركيز المثبط لنمو البكتيريا كان ٥٠ ميكروجرام/ملل ضد بكتيريا *Bacillus subtilis* و١٠٠ ميكروجرام/ملل ضد بكتيريا *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* أوضحت هذه الدراسة أن الأوفوميوسين المعزول من بيض البط له نشاط مضاد للبكتيريا التى تمت دراستها.

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