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STUDIES ON THE RECOMBINANT BOVINE LACTOFERRIN AND HUMAN LYSOZYME: STRUCTURE, BIOSYNTHESIS AND ANTIBACTERIAL ACTIVITY IN MILK

BY

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Thesis

Submitted in Partial Fulfilment of the Requirements for the degree of

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in

Agricultural Science

(Dairy Science and Technology)

Dairy Science and Technology Department
Faculty of Agriculture
Cairo University

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Title of Thesis: Studies on the recombinant bovine lactoferrin and human Lyzoyme: Structure, biosynthesis and antibacterial activity in milk

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ABSTRACT

This study was carried out in order to :

- clone the full-length of lactoferrin gene using DNA procedures. The cDNA encoding bovine lactoferrin transferred into epithelial cells to make stable cell lines expressed bovine lactoferrrin.
- study the N-glycosylation, intracellular transport and sorting of recombinant bovine lactoferrin .
- generate the recombinant lactoferricin –B using DNA procedures and pepsin digestion.
- study the biosynthesis, N-glycosylation and sorting of human glycosylated lysozyme mutant.
- study the antimicrobial activity of the recombinant lactoferrin, lactoferricin, human glycosylated lysozyme and lactoferricin with lysozyme and EDTA against the pathogenic and non-pathogenic bacteria

From the obtained results, one can conclude the following :

1-The complete bLFcDNA (2357 bp) was produced using RT-PCR and ligated in the vector and ransformed into *E. coli and* The success in the insertion of Lf-cDNA in the plasmid vector was tested using the restriction enzyme.

2- The bLF cDNA expression vector was transferred into epithelial and the rbLf was detected using SDS-PAGE. The soluble form of lactoferrin in the medium was of higher molecular weight (~80-84 kDa) than protein found in the from lysate (~77-78kDa).

3- The immuno-precipitation of the lactoferrin secrted in the medium was carried out at two p H's ie. PH 7.7 and pH 8. Two bands were observed in SDS-PAGE pattern of lactoferrin precipitated at pH 7.7; Lf-a (~ 80 kDa) and Lf-b (84 kDa) but at pH 8 was one band Lf-b.

4- Lactoferrin was affected more by the endo F hydrolase as a protein band of smaller molecular weight was apparent in the electrophoretic pattern while the lactoferrin was not affect much by by the N-glycosidase H. With the lactoferrin secreted in the medium, molecular weight of the protein was almost unchanged when subjected to endo H hydrolysis, but gave a protein with less molecular weight when treated with N -glycosidase F.

5- Comparing the transport kinetics of lactoferrin, results clearly indicate that the wild type of lactofrrin was processed in the Golgi apparatus after 1-2 hour and the complex glycosylated lactoferrin was apparent in the medium within 2 hours of chasing.

6- The sorting of bovine lactoferrin was carried out by biosynthetic labelling of MDCK cells and the immunoprecipitation in the apical and basolateral medium and cell lysate. Results indicated that the intracellular transport and sorting of bovine lactoferrin (Lf) in epithelial cells was to the apical membrane .

7- The role of N-glycosylation on the sorting of lactoferrin carried out by the culturing of the labeled MDCK cells the presence or absence of glycosidase inhibitors such as tunicmycine, deoxymannjirimycin (dMM), deoxynojirimycin (dNM), Swainsonine and Benzyl.

Ibrahim Abd El Gawad

Name of Candidate : Ahmed Mohamed Abdelsalam Ibrahim **Degree:** Ph.D.

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8- Lfcin cDNA was obtained from in the RT-PCR reaction using Lf cDNA template and the PCR products were electrophoresed on 0.8 % agarose/TBE gels. The Lfcin cDNA was ligated into pcDNA3MAGFP and transformed to E.coli. The results indicated that the ligation has been successful and the pcDNA3MAGFP construct contained the Lfcin insert. Expression of these constructs in mammalian cells have been monitored by the GFP fluorescence, immunoprecipitations with anti-GFP and antipeptides directed against lactoferricin1Also. recombinant of bovine lactoferricin-B was produced from the stable transfected cell lines was used to generation of lactoferricin –B by pepsin digestion.

9- The SDS/PAGE N-glycosylated human lysozyme showed the presence of 3 band corresponding to lysozyme in the medium. These bands had an increasing molecular weight from 18 to 30 kDa .

10-The role of glycosylation on the transport of lysozyme was followed by culturing the cells expressing the glycosylated lysozyme with or without the presence of glycosidase inhibitor Tunicmycine and the obtained data indicated that transport using the immunopricipitation and abelling in the presence or absence of the tunicmycine was similar in lysozyme transported to the apical membrane .

11-The antimicrobial activity of recombinant bovine lactoferrin, and its peptic digest (lactoferricin-B) and recombinant glycosylated human lysozyme rhLz) and mixture of lactoferricin-B and rhlz + 0.25 mM EDTA against *Staphylococcus aureus, Listeria monocytogenious, Escherichia coli, Sallmonella typhirium and Salmonella enteritidis* was carried out using the disk diffusion method.

12-Lactoferrin demonstrated weak inhibitory activity against *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* when used at a concentration of 1mg/ml, and a strong inhibitory activity at a concentration of 2.0 mg/ml. However, recombinant lactoferrin showed inhibitory effect against the two Salmonella strains at these concentrations.

13- The bactericidal activity of recombinant bovine lactoferrin digest (lactoferricin B) against *Staphylococcus aureus, Listeria monocytogenes, Salmonella* and *Escherichia coli* was studied under various conditions. The obtained data showed that lactoferrin digest alone or in combination with lysozyme had little antibacterial action against *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* and no antibacterial action against all bacterial strains grown in fat-free milk medium at the used concentrations (30 µg/ml for lactoferricin and ~0.1 mg/ lysozyme), while this acivity can be easily demonstrated in 1% peptone or other simple growth medium.

14- Human gylcosylated lysozyme mutant demonstrated some bactericidal activity against pathogenic bacteria strains with minimal inhibitory concentrations (MICs) ranging from 0.8 mg/ml for *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* strain to greater than ~ 2,0 mg/ml for *Salmonella* strains.

Ibrahim Abd El Gawad

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Dedication

May I dedicate all work to both my father and mother for their constant assistance for me , as well as my sisters and my brother for their help to me .

My due respect and true dedications of this work should be also given to my wife and my kids.

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