



**Purification and Characterization of an Intracellular  
Lipase from the Greater Wax Moth, *Galleria mellonella*  
(Lepidoptera: Pyralidae)**

**A Thesis**

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**By**

**Rahma Raafat Zaky Mahdy**

**(B.Sc.)**

**2012**

**Demonstrator in Entomology Department, Faculty of Science,  
Ain Shams University**

**Supervisors**

**Prof. Dr. Emad M. S. Barakat**

**Professor of Insect Physiology, Entomology Department,  
Faculty of Science, Ain Shams University**

**Dr. Marah M. Abd El-Bar**

**Associate Professor of Entomology, Entomology  
Department, Faculty of Science, Ain Shams University**

**Dr. Shaimaa Ahmed Ahmed Mo'men**

**Lecturer in Entomology, Entomology Department, Faculty  
of Science, Ain Shams University**

**(2018)**

## **Biography**

**Name:** Rahma Raafat Zaky Mahdy

**Date and place of birth:** 15/12/1991, Cairo, Egypt

**Degree awarded:** B. Sc. (Entomology)

**Department:** Entomology

**Faculty:** Science

**University:** Ain Shams University

**Date of graduation:** June, 2012

**Date of Appointment:** February, 2013

**Present Occupation:** Demonstrator, Entomology Department,  
Faculty of Science, Ain Shams University

**Date of Registration:** July, 2015

## THESIS EXAMINATION COMMITTEE

| NAME  | TITLE | SIGNATURE |
|-------|-------|-----------|
| ..... | ..... | .....     |
| ..... | ..... | .....     |
| ..... | ..... | .....     |
| ..... | ..... | .....     |

## SUPERVISORS

**Prof. Dr. Emad Mahmoud Said Barakat**

Professor of Insect Physiology, Faculty of Science  
Ain Shams University

**Dr. Marah M. Abd El-Bar**

Associate Professor, Faculty of Science  
Ain Shams University

**Dr. Shimaa A. Mo' men**

Lecturer in Entomology, Faculty of Science  
Ain Shams University

## **Approval sheet**

### **M.Sc. thesis**

**Name:** Rahma Raafat Zaky Mahdy

**Title:** Purification and Characterization of an Intracellular Lipase from the Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae)

### **Supervisions committee:**

**Prof. Dr. Emad M. S. Barakat**

Professor of Insect Physiology, Entomology Department,  
Faculty of Science, Ain Shams University

**Dr. Marah M. Abd El-Bar**

Associate Professor of Entomology, Entomology Department,  
Faculty of Science, Ain Shams University

**Dr. Shaimaa Ahmed Ahmed Mo'men**

Lecturer in Entomology, Entomology Department, Faculty of  
Science, Ain Shams University

### **Examination committee:**

**Prof. Dr. Ahmed Salem Mohammad Ahmed Elebiary**

Emeritus Professor of Entomology, Faculty of Science, Helwan  
University

**Prof. Dr. El-Sayed H. Shaurub**

Professor of Entomology, Faculty of Science, Cairo University

**Prof. Dr. Emad M. S. Barakat**

Professor of Insect Physiology, Entomology Department,  
Faculty of Science, Ain Shams University

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## ABSTRACT

The present study has been conducted to purify intracellular fat body lipase and midgut lipase, for the first time, from last larval instar of *Galleria mellonella*. Purification methods by combination of ammonium sulfate precipitation and gel filtration using Sephadex G-100, yielded a 98.9 and 49.15- fold purity and recovery of 50.81% and 18.3% for fat body lipase and midgut lipase, respectively. SDS-PAGE and zymogram revealed that the molecular weight of midgut lipase was 104.23 kDa, while fat body lipase showed two monomers with molecular weights of 178.8 and 62.6 kDa. Furthermore biochemical characterization of, fat body lipase and midgut lipase, was carried out through testing their activities against several factors such as; different temperatures, pHs, metal ions and inhibitors ending by determination of their kinetic parameters with the use of *p*-Nitrophenyl butyrate (PNPB) as a substrate. The highest activities of enzyme were determined at the temperature ranges of 35-37°C and 37-40°C and pH ranges of 7-9 and 7–10 for midgut and fat body lipase, respectively. The partially purified enzymes showed significant stimulation by  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$  and  $\text{Na}^{+}$  metal ions indicating that both enzymes are metalloproteinases. Also, lipase activity was strongly inhibited by some inhibitors; phenylmethylsulfonyl fluoride(PMSF), ethylene-diaminetetracetic acid (EDTA) and ethylene glycoltetraacetic acid (EGTA) providing an evidence of

presence of serine residue and activation of enzymes by metal ions. Kinetic parameters were 301.95mM  $K_m$  and 0.361Umg<sup>-1</sup>  $V_{max}$  for fat body lipase and 381.46 mM  $K_m$  and 0.7893Umg<sup>-1</sup>  $V_{max}$  for midgut lipase.

Key words: *Galleria mellonella*, purification, characterization, digestive lipase, midgut lipase, intracellular lipase and fat body lipase.

# CONTENTS

|                                                                  | Page |
|------------------------------------------------------------------|------|
| <b>I-INTRODUCTION</b>                                            | 1    |
| <b>II-LITERATURE REVIEW</b>                                      | 9    |
| 1- Insect lipids                                                 | 9    |
| 1.1-Fat body and haemolymph lipids                               | 11   |
| 1.1.1- Fat body lipids                                           | 12   |
| 1.1.2- Haemolymph lipids                                         | 15   |
| 1.2- General aspects of insect lipid metabolism                  | 20   |
| 1.3- Release of lipids from fat body                             | 21   |
| 1.4- Fat body-haemolymph interrelationships during lipid release | 23   |
| 2- Hydrolases                                                    | 23   |
| 3- Lipases                                                       | 24   |
| 3.1- Microbial lipases                                           | 25   |
| 3.2- Plant lipase                                                | 26   |
| 3.3- Vertebrate lipase                                           | 28   |
| 3.4- Insect lipases                                              | 29   |
| 4- Detection of lipolytic activity                               | 33   |
| 5- Purification of lipase                                        | 33   |
| 6- Characterization of purified lipase                           | 36   |
| 6.1- Molecular weight                                            | 36   |
| 6.2- Effect of pH and temperature                                | 37   |
| 6.3- Effect of metal ions and other compounds                    | 40   |
| 6.4- Enzyme kinetics parameters ( $K_m$ and $V_{max}$ )          | 43   |
| 7- Biotechnological applications of lipases                      | 43   |
| 7.1- Food industry                                               | 45   |
| 7.2- Organic chemistry                                           | 45   |
| 7.3- Chiral resolution                                           | 46   |
| 7.4- Lipase as biosensor                                         | 47   |
| 7.5- Lipases in bioremediation                                   | 47   |
| 7.6- Detergency and cleaning                                     | 48   |
| 7.7- Paper industry                                              | 48   |
| 7.8- Diagnostic tool                                             | 48   |
| 7.9- Medical applications                                        | 49   |
| 7.10- Other applications                                         | 49   |
| <b>III- MATERIALS AND METHODS</b>                                | 50   |
| 1- Rearing and maintenance of experimental insect                | 50   |

|                                                                                                             |    |
|-------------------------------------------------------------------------------------------------------------|----|
| 2- Processing of larval tissue homogenates                                                                  | 52 |
| 2.1- Larval tissue collection                                                                               | 52 |
| 2.1.1- Midgut collection                                                                                    | 52 |
| 2.1.2- Fat body collection                                                                                  | 52 |
| 2.2- Preparation of larval tissue homogenates                                                               | 53 |
| 3- Determination of protein concentration                                                                   | 53 |
| 3.1- Preparation of the protein reagent                                                                     | 54 |
| 3.2- Preparation of standard protein solution                                                               | 54 |
| 3.3- Construction of standard calibration curve                                                             | 54 |
| 3.4- Estimation of the protein content in unknown sample                                                    | 55 |
| 3.5- Calculation                                                                                            | 56 |
| 4- Determination of lipase activity                                                                         | 56 |
| 5- Purification of fat body and midgut lipase                                                               | 57 |
| 5.1- Ammonium sulphate $[(\text{NH}_4)_2\text{SO}_4]$ precipitation                                         | 58 |
| 5.2- Sephadex G-100 gel filtration chromatography                                                           | 61 |
| 6- Determination of molecular weight and purity of the purified lipase (electrophoretic analysis)           | 62 |
| 6.1- Preparation of electrophoretic solutions                                                               | 62 |
| 6.2- Preparation and casting of the gel                                                                     | 63 |
| 6.3- Sample preparation                                                                                     | 64 |
| 6.4- Electrophoresis                                                                                        | 65 |
| 6.5- Staining                                                                                               | 65 |
| 6.6- Scanning and analysis of the bands                                                                     | 65 |
| 7- Zymogram analysis                                                                                        | 66 |
| 8- Determination of biochemical characteristics of the purified lipases                                     | 66 |
| 8.1- Effect of pH on lipase activity                                                                        | 66 |
| 8.2- Effect of temperature on lipase activity                                                               | 67 |
| 8.3- Effect of mono- and di-valent cations on lipase activity                                               | 67 |
| 8.4- Effect of specific inhibitors on lipase activity                                                       | 68 |
| 8.5- Kinetic parameters measurements                                                                        | 68 |
| 9- Statistical analysis                                                                                     | 69 |
| <b>IV- EXPERIMENTAL RESULTS</b>                                                                             | 70 |
| 1- Rearing and maintenance of <i>G. mellonella</i>                                                          | 70 |
| 2- Protein content and lipase activity in larval midgut and fat body of <i>G. mellonella</i>                | 73 |
| 3- Partial purification of intracellular fat body lipase and midgut lipase from <i>G. mellonella</i> larvae | 74 |
| 4- Determination of molecular weight and purity of the purified lipases from <i>G. mellonella</i> larvae    | 81 |
| 5- Biochemical characterization of purified intracellular fat body lipase                                   | 95 |

|                                                                                                            |     |
|------------------------------------------------------------------------------------------------------------|-----|
| and midgut lipase                                                                                          |     |
| 5.1- The effect of pH on lipase activity                                                                   | 95  |
| 5.2- The effect of temperature on lipase activity                                                          | 98  |
| 5.3- Effect of mono- and di-valent cations on lipase activity                                              | 101 |
| 5.4- Effect of specific inhibitors on lipase activity                                                      | 108 |
| 5.5- Kinetic parameters of purified lipase from larval fat body and midgut tissues of <i>G. mellonella</i> | 115 |
| <b>V- DISCUSSION AND CONCLUSION</b>                                                                        | 117 |
| 1- Rearing and maintenance of <i>G. mellonella</i> larvae                                                  | 117 |
| 2- Protein content and lipase activity in larval midgut and fat body of <i>G. mellonella</i>               | 119 |
| 3- Purification of intracellular lipase and midgut lipase from <i>G. mellonella</i> larvae                 | 122 |
| 4- Determination of molecular weight and purity of the purified lipases from <i>G. mellonella</i> larvae   | 124 |
| 5- Effect of pH on lipase activity                                                                         | 125 |
| 6- Effect of temperature on lipase activity                                                                | 126 |
| 7- Effect of mono- and di-valent cations on lipase activity                                                | 128 |
| 8- Effect of specific inhibitors on lipase activity                                                        | 129 |
| 9- Determination of kinetic parameters of digestive and intracellular lipase                               | 131 |
| <b>Conclusion and perspectives</b>                                                                         | 133 |
| <b>VI- SUMMARY</b>                                                                                         | 135 |
| <b>VII- REFERENCES</b>                                                                                     | 140 |
| <b>Arabic Summary</b>                                                                                      |     |

## ABBREVIATIONS

|                             |                                                                        |
|-----------------------------|------------------------------------------------------------------------|
| <b>AgNO<sub>3</sub></b>     | : Silver nitrate                                                       |
| <b>AKH</b>                  | : Adipokinetic hormone                                                 |
| <b>BSA</b>                  | : Bovine serum albumin                                                 |
| <b>CaCl<sub>2</sub></b>     | : Calcium chloride                                                     |
| <b>cAMP</b>                 | : Cyclic adenosine monophosphate                                       |
| <b>CBB</b>                  | : Coomassie brilliant blue                                             |
| <b>Cm<sup>3</sup></b>       | : Cubic centemeter                                                     |
| <b>Conc.</b>                | : Concentration                                                        |
| <b>DAG</b>                  | : Diacylglycerol                                                       |
| <b>DAGs</b>                 | : Diacylglycerides                                                     |
| <b>EDTA</b>                 | : Ethylenediamine tetraacetic acid                                     |
| <b>EGTA</b>                 | : Ethylene glycol-bis(β-aminoethylether) N,N,N',N'<br>tetraacetic acid |
| <b>FeCl<sub>3</sub></b>     | : Ferric chloride                                                      |
| <b>FeSO<sub>4</sub></b>     | : Ferrous sulfate                                                      |
| <b>FFAs</b>                 | : Free fatty acids                                                     |
| <b>g</b>                    | : Gram                                                                 |
| <b><i>G. mellonella</i></b> | : <i>Galleria mellonella</i>                                           |
| <b>h</b>                    | : Hour                                                                 |
| <b>HDL-C</b>                | : High-density lipoprotein cholesterol                                 |
| <b>KCl</b>                  | : Potassium chloride                                                   |
| <b>K<sub>m</sub></b>        | : Michaelis-Menten constant                                            |
| <b>LIPC</b>                 | : Gene produce hepatic lipase                                          |
| <b>LIPG</b>                 | : Gene produce endothelial lipase                                      |
| <b>LIPL</b>                 | : Gene produce lipoprotein lipase                                      |
| <b>mA</b>                   | : Milliamper                                                           |
| <b>MAG</b>                  | : Monoacylglycerol                                                     |
| <b>MAGs</b>                 | : Monoacylglycerides                                                   |
| <b>μg</b>                   | : Microgram                                                            |
| <b>μl</b>                   | : Microliter                                                           |
| <b>μM</b>                   | : Micromolar                                                           |
| <b>μmol/min/mg</b>          | : Micromole per minute per milligram                                   |
| <b>mM</b>                   | : Millimolar                                                           |
| <b>mm</b>                   | : Millimeter                                                           |
| <b>MUF-butyrate</b>         | : 4-Methylumbelliferyl butyrate                                        |
| <b>mV</b>                   | : Millivolt                                                            |
| <b>NaCl</b>                 | : Sodium chloride                                                      |
| <b>NaOH</b>                 | : Sodium hydroxide                                                     |
| <b>NL</b>                   | : Neutral lipids                                                       |

|                                       |                                                               |
|---------------------------------------|---------------------------------------------------------------|
| <b>nm</b>                             | : Nano meter                                                  |
| <b>nmol</b>                           | : Nanomole                                                    |
| <b>Pb(NO<sub>3</sub>)<sub>2</sub></b> | : Lead dinitrate                                              |
| <b>PL</b>                             | : phospholipid                                                |
| <b>PMSF</b>                           | : Phenylmethylsulfony fluoride                                |
| <b>P-NPB</b>                          | : <i>p</i> -Nitrophenyl butyrate                              |
| <b>S II</b>                           | : Supernatant II                                              |
| <b>S III</b>                          | : Supernatant III                                             |
| <b>SDS</b>                            | : Sodium dodecyl sulphate                                     |
| <b>SDS-PAGE</b>                       | : Sodium dodecyle sulphate polyacrylamide gel electrophoresis |
| <b>TAG</b>                            | : Triacylglycerol                                             |
| <b>TAGs</b>                           | : Triacylglycerides                                           |
| <b>TEMED</b>                          | : N, N, N', N'-Tetramethylethylenediamine                     |
| <b>Ul</b>                             | : Unitliter                                                   |
| <b>Unit/min/ml</b>                    | : Unit per minute per milliliter                              |
| <b>UV</b>                             | : Ultra violet                                                |
| <b>VLDL</b>                           | : Very low density lipoproteins                               |
| <b>Vmax</b>                           | : Maximum velocity                                            |

# LIST OF TABLES

| Table no. | Table Title                                                                                                                                                 | Page |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1         | Protein content (mg/ml) and lipase activity (u) in fat body and midgut tissues of <i>G. mellonella</i> larvae.                                              | 73   |
| 2         | Purification process of fat body lipase from <i>G. mellonella</i> larvae.                                                                                   | 76   |
| 3         | Purification process of midgut lipase from <i>G. mellonella</i> larvae.                                                                                     | 77   |
| 4         | Comparative analysis of molecular weight (MW) and relative concentration (band %) of SDS-PAGE of purified fat body lipase from <i>G. mellonella</i> larvae. | 84   |
| 5         | Comparative analysis of molecular weight (MW) and relative concentration (band %) of zymogram of purified fat body lipase from <i>G. mellonella</i> larvae. | 85   |
| 6         | Comparative analysis of molecular weight (MW) and relative concentration (band %) of SDS-PAGE of purified midgut lipase from <i>G. mellonella</i> larvae.   | 90   |
| 7         | Comparative analysis of molecular weight (MW) and relative concentration (band %) of zymogram of purified midgut lipase from <i>G. mellonella</i> larvae.   | 92   |
| 8         | Effect of pH on activity of purified fat body lipase from larvae of <i>G. mellonella</i> ..                                                                 | 96   |
| 9         | Effect of pH on activity of purified midgut lipase from larvae of <i>G. mellonella</i> .                                                                    | 96   |
| 10        | Effect of temperature on activity of purified fat body lipase from larvae of <i>G. mellonella</i> .                                                         | 99   |
| 11        | Effect of temperature on activity of purified                                                                                                               | 99   |

|           |                                                                                                                                              |     |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------|-----|
|           | midgut lipase from larvae of <i>G. mellonella</i> .                                                                                          |     |
| <b>12</b> | Effect of selected di- and mono-valent cations on specific activity of lipase purified from larval fat body tissue of <i>G. mellonella</i> . | 102 |
| <b>13</b> | Effect of selected di- and mono-valent cations on specific activity of lipase purified from larval midgut tissue of <i>G. mellonella</i> .   | 103 |
| <b>14</b> | Effect of some inhibitors on specific activity of lipase purified from larval fat body tissue of <i>G. mellonella</i> .                      | 109 |
| <b>15</b> | Effect of some inhibitors on specific activity of lipase purified from larval midgut tissue of <i>G. mellonella</i> .                        | 110 |
| <b>16</b> | Kinetic parameters of purified lipase from larval fat body and midgut tissues of <i>G. mellonella</i> .                                      | 115 |