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Partial Purification of Total Body Lipase from *Gryllus campestris* L. (Orthoptera: Gryllidae)

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Abstract: A Total Body Lipase (TBL) (E. C. 3.1.1.3) was partially purified from abdomen homogenate of *Gryllus campestris* L. (Orthoptera: Gryllidae) by a simple and rapid method. The purification process consisted of three steps: preparation of the total body homogenate, ammonium sulphate precipitation and Sephadex G-100 gel filtration column chromatography. Through the purification procedure, enzyme was purified with a yield of 21.84 % and a purification coefficient of 0.81 fold, having the specific activity of 1.03±0.03 enzyme units (EU / mg protein).

The molecular weight of *Gryllus campestris* body lipase was estimated to be about 76.5 kDa by using Sephadex G-100 gel filtration chromatography. Gel filtration was found to be a useful technique in determining molecular weight of insect TBL.

Key words: *Gryllus campestris*, Total Body Lipase, Purification.

Gryllus campestris L. (Orthoptera: Gryllidae)' den Toplam Vücut Lipazının Kısmi Saflaştırılması

Özet: Basit ve hızlı bir metot yoluyla *Gryllus campestris* L. (Orthoptera: Gryllidae)'nin abdomen homojenatından Toplam Vücut Lipazı (TBL) kısmi olarak saflaştırıldı. Saflaştırma süreci üç adımdan oluştu: toplam vücut homojenatının hazırlanması, amonyum sülfat çöktürmesi ve Sephadex G-100 jel filtrasyon kolon kromatografisi. Saflaştırma prosedürü boyunca, enzim 21.84 %'lik bir verim ve 1.03±0.03 enzim ünitelik (EU / mg protein) spesifik aktiviteye sahip olan 0.81 katlık bir saflaştırma katsayısı ile saflaştırıldı.

Sephadex G-100 jel filtrasyon kromatografisi kullanılarak, *Gryllus campestris* vücut lipazı moleküler ağırlığının yaklaşık 76.5 kDa olduğu tahmin edildi. Jel filtrasyonunun böcek TBL' sinin moleküler ağırlığını belirlemede kullanışlı bir teknik olduğu bulundu.

Anahtar Sözcükler: *Gryllus campestris*, Toplam Vücut Lipazı, Saflaştırma.

Introduction

Lipases (triacylglycerol acylhydrolase; EC 3.1.1.3), which catalyses the hydrolysis of fatty acid ester bonds, are widely distributed among animals, plants and microorganisms [1]. It is indicated that lipases can also hydrolyze a variety of esters in organic solvent systems and thus they can be widely used in many industrial areas, e.g., dairy industry, food, detergent and biofuel industry [2, 3]. The most characteristic property of lipases is that they act with a substrate at the interface between the aqueous and the lipid phase [4].

To date, many research groups have been carried out the isolation and purification of lipases from various sources, mainly microorganisms, fish, fungi, milk, including plants [5, 6, 7, 8, 9, 10]. Recently lipid mobilization and transport in insects is under investigation, especially lipases and lipophorin, which is a reusable lipoprotein particle in insect systems because of their roles in energy production and transport of lipids at flying activity [11, 12, 13]. Since the majority of stored lipids in insects are found in the fat body, which combines some properties and functions of vertebrate liver and adipose tissue it has been used to purify lipases [14, 15]. Although stored lipids in vertebrate adipose tissue are released as free fatty acids, in insects most fatty acids are released as *sn-1,2*-diacylglycerols [16] and mobilization of lipid reserves from insect fat body is under the control of adipokinetic hormone [11].

For these purposes, the two best characterized insects are the locust, *Locusta migratoria* and the tobacco hornworm, *Manduca sexta* due to their large size and ample

hemolymph amounts. However, lipid biochemistry studies in insects was time-consuming and moved on very slowly due to incredible diversity of insects [17] and changes in the lipid composition and lipophorin present in hemolymph during metamorphosis from larva to pupa [18].

Although there are many studies on animal, microorganism and plant lipases in the literature, there is information paucity on insect lipases and studies conducted on insect lipases have been only focused on fat body [14, 19]. It has been reported that mid gut cells in insects produce lipases that hydrolyze dietary triacylglycerols [15]. At the onset of this study, no reports existed on *Gryllus campestris* lipase and the main objective of this study was to investigate the existence of a total body lipase as a first and preliminary study on *Gryllus campestris.*

Material and Methods

Insect material

Wild-type *G. campestris* L were collected from Cumhuriyet University campus between May and June in 2001. Female and male insects reared on a mixture of wheat scab and lettuce based food in glass insectariums (15x 15 x 30 cm). Adult and larval individuals of the insect were maintained at 30°C and 45-50% relative humidity, with a 14:10 light and dark photo period, respectively. From the stock culture, only female insects (n=20) at ninth nymph stage of the growth were used to determine enzymatic activity.

Preparation of homogenate and centrifuging process

Only abdomen parts of *G. campestris* were pounded in a ceramic plate and were suspended in 50 mM Tris-HCl (pH=7.40) buffer containing 1mM Na_4EDTA and 0.25 mM -D Mannit. Later, the pounded tissue was homogenized with Electromag M II type homogenizer at 1500 rev/min., with a few strong strokes. With this process, 22 ml of homogenate were obtained. The crude extract was centrifuged at 14,800 x *g* for 25 min at 4°C (Beckman J2-21 type cooling centrifuge) and supernatant was removed. 20 ml of supernatant obtained was centrifuged at 21,000 x *g* for 45 min at 4°C and this supernatant was used as a source of enzymes. After each centrifugation step, floating cake was filtered and removed on glass wool.

Ammonium Sulfate [$(NH_4)_2SO_4$] precipitation

All the steps were carried out at 4°C. The supernatant was subjected to ammonium sulfate precipitation. For this purpose, solid ammonium sulfate was slowly added to supernatant with a constant stirring for 30 min, to a first concentration step of 10% (w/v). Suspension was centrifuged at 14,800 x *g* and supernatant obtained was indicated as S III and precipitate P III. In the final step, solid ammonium sulfate necessary to make from concentration of 10% (w/v) to a final concentration of 65% (w/v) was slowly added to S III in similar way and centrifuged at 21,000 x *g* for 20 min. Supernatant and precipitant was recorded as S IV and P IV, respectively. Precipitate III and IV were suspended in Tris-HCl buffer used in homogenization process. In all precipitation steps, the enzyme activity was determined both in supernatant and precipitate. Important amounts of the enzyme were observed to precipitate at 65% precipitation.

Sephadex G-100 gel filtration chromatography and molecular weight estimation

Dried Sephadex G-100 was used to purify insect total body lipase. The dried gel in sufficient amount was incubated in distilled water for 5 h at 90°C. After cooling and removal of air in the gel; it was loaded onto the column (12 x 2 cm). Then the column was equilibrated with 50 mM Tris-HCl + 50 mM Ammonium sulfate (pH=7.80) buffer. The void volume in the gel was observed with Blue Dextrane. Myoglobin 17,8 kDa and glucose-6-phosphate 104 kDa were used as standards for determining of molecular weight of insect TBL. After dilution of the sample (Precipitant IV) to 20 ml of final volume, it was applied to the column at a flow rate of 0.3 ml/min. Each of the standards and sample was collected as elution of 1.5 ml. K average values (K_{av}), elution volumes of standards and insect TBL is summarized in Table 1. Absorbance values of the elution tubes were determined at 280 nm.

Activity determination

Enzymatic activity was determined spectrophotometrically with a Cecil 5000 series spectrophotometer, at 28ºC, according to the method described by Bülow and Mosbach [20] with some modifications. The assay system consisted of 50 mM Tris-HCl containing 4% ethanol and 1 % asetonitrile, 27 mM paranitrophenyl butyrate (pNPB)

and enzyme solution. Blank tube was only devoid of the enzyme solution. One enzyme unit is defined as the amount of enzyme necessary to liberate 1 µmol of paranitrophenyl from pNPB per minute at 28ºC and 405 nm under assay conditions.

Protein determination

The protein concentration was determined by the method Bradford [21] at 595 nm and bovine serum albumin (BSA) was used as a standard.

Results & Discussion

In this study, purification of total body lipase (TBL) from abdomen parts of *G. campestris* was purposed.

Purification scheme of TBL from *G. campestris* has been outlined in Table 1. In initial step, S II phase, enzyme had specific activity of 1.27±0.22 EU/mg protein. For further purification of the enzyme, 10 and 60 % ammonium sulphate precipitation procedures were conducted on S II phase, respectively. In the first stage, with the addition of 10% ammonium sulphate, total enzyme activity in upper phase III (S III) was found to be 15.85±1.81 EU while specific activity was found 8.96±1.02 EU/mg protein. It was found that pellet III (P III) had 8.88±1.83 EU of total activity and 11.68±2.41 EU/mg protein of specific activity. Upper phase IV (S IV) obtained from S III phase by adding necessary solid ammonium sulphate was found to be 3.70±0.48 EU of total activity with a yield of 19.51%. It was found that the majority of the lipase activity was located pellet IV phase (P IV), which have a total activity of 13.71±0.84 EU with a yield of 72.31% and P IV phase was applied onto Sephadex G-100 gel filtration column. All data found in our study reveals that *G. campestris* lipase is located in cytosolic part of the cell and this data harmonizes with other studies. Previous studies carried out on lipase purification including fish, plants and micro organisms confirmed that lipases found in cytosolic fraction of the cell [8, 10, 22]. A comprehensive study conducted on fat body lipase of *M. sexta* was also revealed that the enzyme was cytosolic [14].

Sephadex G-100 column chromatography elution profile is shown in Figure 1. This technique was used in determining the molecular weight of the insect TBL as described in material method section. Data used in determining molecular weight of

TBL and protein standards whose molecular weights are known and their K average values (K_{av}) can be seen in Table 2. Using this table, relationship between molecular weights and K_{av} values of protein standards and insect TBL were summarized in Figure 2.

In the result of elution patterns on sephadex G-100 column chromatography, *G. campestris* TBL activity was found to be between elution tubes 13 to 15. With Sephadex column chromatography in the final step, enzyme was purified with a yield of 21.84 % and a purification coefficient of 0.81 fold, having the specific activity of 1.03±0.03 enzyme units (EU / mg protein) and the molecular weight of body lipase was estimated to be about 76.5 kDa. It has been found that the major triglyceride lipase of fat body of *M. sexta* is a cytosolic enzyme with a molecular mass of 76 kDa and this enzyme is phosphorylated by cAMP dependent protein kinase A. [14, 19]. The triacylglycerol-hydrolyzing capacity in several tissues of the American cockroach, *Periplaneta americana* L. was investigated for midgut, fat body, thoracic musculature and haemolymph. The highest lipase activity was found to be in midgut homogenates between the tissues investigated [23].

Insect lipase purification studies are a newly field of scientific research and any study could not provide sufficient data by using only one chromatographic purification step. Further studies can reveal the properties and biochemical characteristics of the enzyme and characteristic features of insect lipases such as optimal pH, temperature endurance are not well known yet. From our study and other studies, it appears that combined chromatographic techniques must be used to obtain highly purified insect lipase.

Table 1: Purification scheme of total body lipase from *Gryllus campestris.*

Fractions	**Total Activity (µ mol)** Mean* ±SE	**Total Protein (mg)** Mean* ±SE	**Specific Activity (µmol/mg. protein)** Mean* ±SE	**Yield (%)**	**Purification (fold)**
21000xg supernatant	18.96±3.24	14.97±0.72	1.27±0.22	100.00	1.00
14800xg %10 A. S.** supernatant III	15.85±1.81	1.77±0.21	8.96±1.02	83.60	7.06
14800xg % 10 A. S. precipitant III	8.88±1.83	0.76±0.06	11.68±2.41	46.84	9.20
21000xg % 65 A. S. supernatant IV	3.70±0.48	0.63±0.30	5.88±0.75	19.51	4.63
21000xg %65 A. S. precipitant IV	13.71±0.84	11.72±2.03	1.17±0.07	72.31	0.92
Sephadex G-100 elution tubes of 13 to 15	4.14±0.12	4.03±0.09	1.03±0.03	21.84	0.81

*: Each value represents the mean of two experiments. **: Ammonium Sulphate. SE: Standard Error.

Table 2: The relationship between molecular weight, K average (K_{av}) values and elution volumes of protein standards and insect total body lipase according to Sephadex G-100 gel filtration technique.

Proteins	**Molecular Weight (Dalton)**	**K_{av}**	**Elution Volume (ml)**
Myoglobin	17.800	1.22	15.60
Glucose-6-Phosphate	104.000	1.12	30.00
İnsect Total Body Lipase	76.500	1.15	25.50

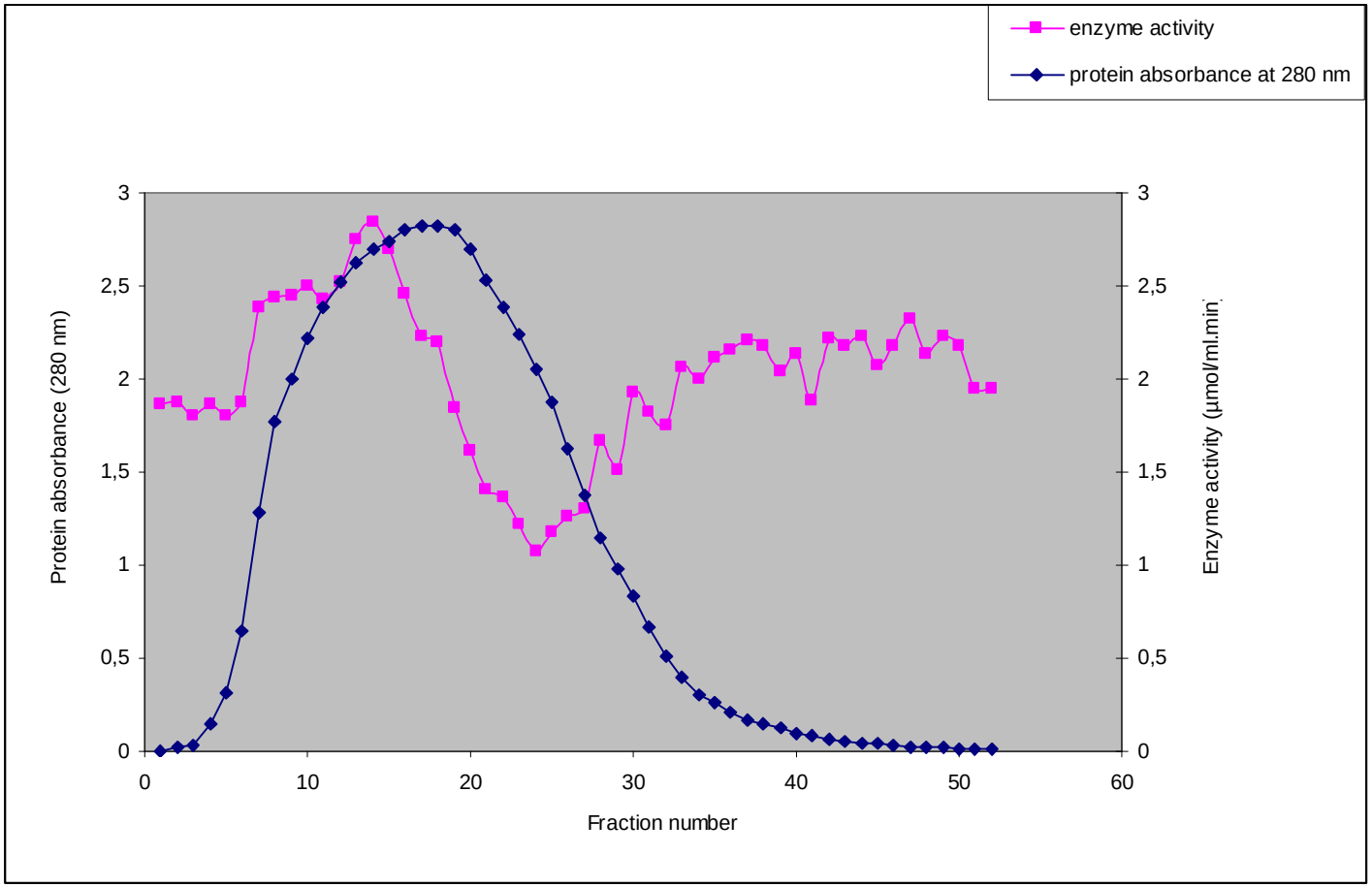

Figure 1: Sephadex G-100 column gel filtration chromatography elution profile
*: Each value represents the mean of two experiments

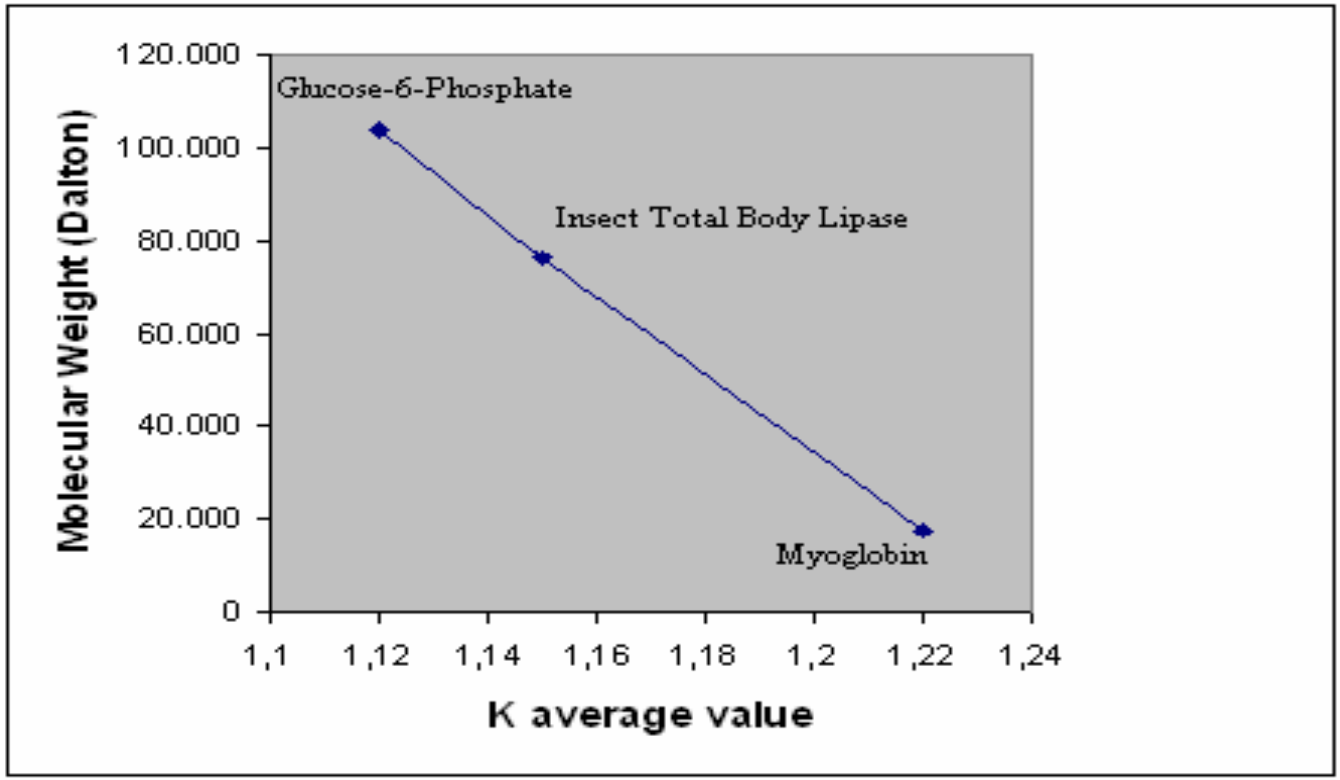

Figure 2: Graphic of K average (K_{av}) values-molecular weights of Myoglobin, Glucose-6-phosphate and purified insect total body lipase.

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