*Int. J. Indust. Entomol.* Vol. 16, No. 2, 2008, pp. 57~59

**International Journal of *Industrial Entomology***

# Pancreatic lipase Inhibitory Compound from *Apis mellifera* venome

**Jun-Ran Kim[1] and Shin-Duk Kim***

*Department of Biological Engineering, Seokyeong University, Seoul 136-704, Republic of Korea*
*[1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea*

*(Received 13 April 2008; Accepted 2 May 2008)*

**While searching for pancreatic lipase inhibitors, the active compound was found in a methanol extract of *Apis mellifera* venome. The active compound was isolated by Diaion HP-20 column chromatography, thin layer chromatography and HPLC. The active compound is stable to the extreme pH and heat. There is no loss of activity both in acidic and alkaline solution in the pH range of 2 to 11 by heating for 15 minutes at 90°C. The rf value of the compound was 0.51 at TLC with butanol : methanol : water (4 : 1 : 2) solvent system. The molecular weight of the compound was determined to be 293 by EI-MS.**

**Key words:** Pancreatic lipase inhibitory activity, *Apis mellifera*, Venome

## Introduction

Pancreatic lipase is a key enzyme for dietary triacylglycerol breakdown that leads to the absorption, hydrolyzing triacylglycerols to 2-monoacylglycerol and fatty acids (Embleton and Pouton, 1997). Pancreatic lipase, one of the exocrine enzymes of pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long-chain fatty acids. Short-chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles (Carey *et. al.*, 1983). The micelles are absorbed into the mucosal cells of the intestine, and the fatty acids and monoglycerides are resynthesized into triglycerides. Dietary triglyceride is usually stored in the adipose tissue. The excessive intake of dietary triacylglycerol is relevant to the development of hypertriglyceridemia, hypercholesteromia and obesity, leading to a variety of serious diseases. Therefore, much effort has been directed to develop the pharmacological agents that reduce the absorption of dietary triacylglycerol, thereby reducing the probability of the formation of atherosclerotic plaque (Ballinger and Peikin, 2002). The inhibitors of the pancreatic lipase exhibit a high promise as therapeutic agents to prevent obesity by preventing the digestion and absorption of dietary triacylglycerol (Shi and Burn, 2004).

In the course of screening for the pancreatic lipase inhibitors from the oriental medicines, the active compounds were found from the venom of *Apis mellifera*. The present paper deals with the isolation and the partial characterization of the active compounds.

*To whom the correspondence addressed
Department of Biological Engineering, Seokyeong University, 16-1 Jungneung-Dong, Sungbuk-Ku, Seoul 136-704, Republic of Korea. Tel: +82-2-940-7171; Fax: +82-2-919-0345; E-mail: sdkim@skuniv.ac.kr

## Materials and Methods

### Materials

*Bombycis corpus*, *Reticulitermes Speratus*, and *Buthus martensi Karsh* were purchased from Kyung Dong Market (Seoul, Korea). The venom of *Apis mellifera* was obtained from Korea Research Institute of Science and Biotechnology (Daejeon, Korea).

Lipase substrate emulsion, pancreatic lipase and spray reagents were purchased from Sigma-Aldrich. All other chemicals and reagents were of analytical grade and available commercially.

### Instrumentation

A High performance liquid chromatograph (HPLC) (Hewlett-Packard, Santa Clara, CA) was used for purification of lipase inhibitor compound. The UV-Visible absorption spectrum was measured with a Shimazu model 210. EI-MS spectra were obtained on a JEOL JMS AX505 WA spectrometer.

**Pancreatic lipase enzyme assay**

The dried *Bombycis corpus*, *Reticulitermes Speratus*, and *Buthus martensi Karsh* were extracted with 100% methanol and the methanol extracts were filtered through the Whatman No. 2 filter paper. The venom of *Apis mellifera* was extracted with 50% methanol. The extracts were concentrated to dryness by rotary evaporation at 40°C and dissolved in a small volume of methanol.

An inhibitory effect on pancreatic lipase activity was determined by titrimetric method. The reaction mixtures containing 0.2M Tris buffer solution (pH 7.7), 1 ml of lipase substrate, 1ml of sample solution, and the 3 units of lipase were incubated at 37°C for 30 min. Following incubation, 3 ml of 0.9%(w/v) thymolphthalein indicator in 95% ethanol was added and the fatty acid produced was titrated with 50 mM NaOH to a light blue color.

**Isolation of the active compounds from honeybee venom**

The methanol extract of the *Apis mellifera* venom was subjected to Diaion HP-20 column chromatography and eluted stepwise with 30% methanol, 50% acetone, 70% acetone, and 70% acetone+0.1N $NH_4OH$ solution. The active fraction (70% acetone+0.1N $NH_4OH$ eluant) was pooled and concentrated *in vacuo*. Then, the residues were subjected to preparative TLC (Merck, Kieselgel 60 $F_{254}$ ) developed with butanol-methanol-water (4:1:2). The active band (rf=0.74) was scraped from the plate and extracted with methanol from the silica gel adsorbent. Final purification was achieved by semi-preparative HPLC on $C_{18}$ reverse phase column (Waters, uBondapak, 7.8×300 mm)eluting with 15% methanol isocratic solvent system. The effluents were monitored by absorbance at 206 nm.

**Table 1.** Pancreatic lipase inhibitory activities of *Apis mellifera* venom, *bombyx batrytictus*, and *Reticulitermes speratus*

| | Inhibition of pancreatic lipase (%)[a] |
|---|---|
| Venome of *Apis mellifera* | 10 |
| Bombycis corpus | - |
| Reticulitermes Speratu | - |
| Burthus martensi Karsch | - |

[a]The pancreatic lipase activity was measured by the quantity of alkali required to reach the thymolphthalein endpoint. The inhibitory activity (%) was calculated by the following formula: (1-titrant volume of treatment/ titrant volume of the control)×100.

*Apis mellifera* venom
- 50% MeOH extraction
- Concentrated *in vacuo* to dryness

Residues

Diaion HP-20 column chromatography
- washed with 50% acetone solution
- eluted with 70% acetone + 0.1 N $NH_4OH$ solution

Active fractions
- Pooled and concentrated *in vacuo*
- TLC(butanol:methanol:water=4:1:2)
- HPLC $C_{18}$ reverse phase
- methanol:$H_2O$ (15:85) isocratic system
- UV detection at 206nm

The active compound

**Fig. 1.** Isolation procedure of the pancreatic lipase inhibitory compound from the *Apis mellifera* venom.

## Results and Discussion

**Pancreatic lipase inhibitory activity**

As part of our screening process for the pancreatic lipase inhibitory compounds from oriental medicinal insects, we tested the extracts of the whole bodies of *Bombycis corpus*, *Reticulitermes Speratus*, *Buthus martensi Karsh*, and venome of *Apis mellifera*. Among them, only the 50% methanol extracts of venom of *Apis mellifera* exhibited the activity (Table 1). The inhibitory activity was determined by titrimetric method. The quantity of fatty acids released was measured by titration with 0.05M NaOH solution. The quantity of alkali required to reach the thymolphthalein endpoint is proportional to lipase activity.

**Table 2.** The physico-chemical properties of the pancreatic lipase inhibitory compound from *Apis mellifera* venom

| | |
|---|---|
| U.V in MeOH | End absorption |
| Rf value | 0.51 silica gel TLC<br>butanol:methamol:water (4:1:2) |
| Color reaction | positive : Dragendorff reagent<br>anisaldehyde<br>ninhydrin<br>dimethylaminobenzaldehyde |
| | negative : orcinol ferric chloride<br>aniline-diphenylamine<br>Ehrlich reagent<br>antimony chloride<br>bromcresol green<br>rhodamine B |

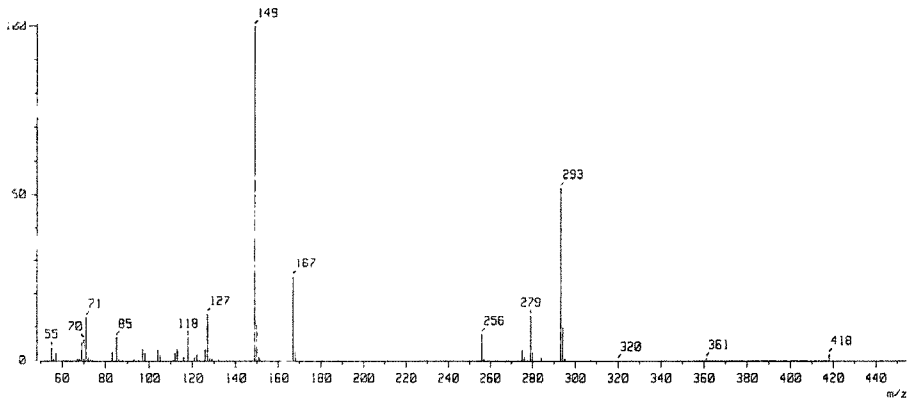


**Fig. 2.** EI-MS spectrum of the pancreatic lipase inhibitory compound from the *Apis mellifera* venom.

**Isolation and purification of the active compound**

The active compound was isolated from methanol extract of *Apis mellifera* venom by the activity-based fractionation using various chromatographic methods. The isolation procedure was outlined in Fig. 1. The purified active compound migrated on silica gel plate as a single spot in several solvent systems (*vide infra*) and eluted from a reverse phase $C_{18}$ column as a single peak at 3.2 min with 15% methanol in water.

**The physico-chemical properties of the active compound**

The physico-chemical properties of the active compound were summarized in Table 2. The active compound was stable in neutral, acidic or alkaline solutions, giving no loss of activity in the pH range of 2 to 11 by heating for 15 minutes at 90°C. In TLC on silica gel 60 F254 with butanol-methanol-water (4 : 1 : 2) as the solvent, the Rf value of the active compound was 0.51. Spraying the plates with anisaldehyde, dimethyl-aminobenzaldehyde, dragendorff's reagent and ninhydrin reagent produced the colored spots, indicating that the active compound might be the amino group containg indole. The negative color obtained from the reactions with aniline-diphenylamine, antimony trichloride, orcinol-ferric chloride, rhodamine B and bromcresol green. The UV spectrum of the compound in methanol exhibited end absorption. The molecular weight of the compound was estimated as 293 by EI-MS (Fig. 2).

Obesity is becoming one of the greatest threats to health, but only a few medications are currently on the market. Obesity is basically regarded as a disorder of energy homeostasis. One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption to reduce energy intake. Since excessive consumption of dietary lipids plays a major role in the development of obesity or hyperlipidemia, leading to atherosclerosis, hypertension and diabetes, the inhibition of the pancreatic lipase could be a good approach for the development of antiobesity agents. The potentials of natural products for the treatment of obesity are tremendous (Bhutani *et al.*, 2007) and especially traditional oriental medicinal plants and insects are excellent sources for the development of safe and effective antiobesity drugs. As part of the continuing search for biologically active antiobesity agents, various medicinal insects have been screened and *Apis mellifera* venom was found to inhibit pancreatic lipase. The *Apis mellifera* venom has been known to contain several biological activities (Castro *et al.*, 1994). The two major components of honeybee venom are mellitin and phospholipase A2. Other components include histamine, oligopeptides, saccharides, neurotoxic apamine, mast cell degranulating protein, hyaluronidase and various catecholamines. However, this is the first report to find pancreatic lipase inhibitory compound from honeybee venom. The structure elucidation of the active compound is under progress.

## References

Ballinger, A. and S. R. Peikin (2002) Orlistat; its current status as an antiobesity drug. *Eur. J. Pharmacol.* **440**, 109-117.

Bhutani, K.K (2007) Potential antiobesity and lipid lowering natural products. *Nat. Product Commun.* **2**, 331-348.

Carey, M. C. and D. M. Small (1983). Lipid digestion and absorption. *Annu. Rev. Physiol.* **45**, 651-677.

Castro, F., M. S. Palma, M. R. Brochetto-Braga, O. Malaspina, J. Lazaretti, M. Baldo, M. Antila, W. Cossermelli (1994). Biochemical propertiesand study of antigenic cross-reactivity between Africanized honey bee and wasp venom. *J. Investig. Allergol. Clin. Immunol.* **4**, 37-41

Embleton, J. K. and C. W. Pouton (1997) Structure and function of gastrointestinal lipases. *Advanced Drug Delivery reviews* **25**, 15-32.

Shi, Y. and P. Burn (2004) Lipid metabolic enzymes: Emerging drug targets for the treatment of obesity. *Nat. Rev. Drug Discov.* **3**, 695-710.