UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT AND CHARACTERIZATION OF A DIAMINE OXIDASE-BASED HISTAMINE BIOSENSOR

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DEVELOPMENT AND CHARACTERIZATION OF A DIAMINE OXIDASE-BASED HISTAMINE BIOSENSOR

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2006

Dedicated to Papa, Mama…..
Ling, Peng, Boy, Lee, Fun, Yuan and Jackson

Extended to Francis Wong

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPMENT AND CHARACTERIZATION OF A DIAMINE OXIDASE-BASED HISTAMINE BIOSENSOR

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Chairman: Associate Professor Fatimah Abu Bakar, PhD

Institute: Bioscience

Histamine levels have been suggested as a rapid fish spoilage indicator. Therefore, histamine biosensors based on immobilization of diamine oxidase (DAO) in photocurable poly (2-hydroxyethyl methacrylate) (photoHEMA) film was developed. Histamine was oxidized by immobilized DAO at 0.35 volt on the surface of carbon-paste screen-printed electrode (SPE) versus conventional Ag/AgCl reference, and with platinum rod as a counter electrode, which named as macro electrode system histamine biosensor. No leaching of the immobilized DAO was observed during histamine detection using the biosensor. The optimized histamine biosensor displayed a linear response over the range of 0 to 60 ppm histamine with correlation coefficient (R^2) equals to 0.9946 (RSD < 11.19%). The sensitivity obtained was 5.56 nA ppm^{-1} and the limit of detection was 0.65 ppm of histamine with the response time of 50 seconds. The histamine biosensor exhibited repeatability and reproducibility characteristic with RSD values equals to 14.06 and 7.80% (n = 10) respectively. The histamine biosensor was applied to determine histamine in tiger prawns *(Penaeus*

monodon) and the results were agreeable with a conventional high performance liquid chromatography (HPLC) method, where a correlation of R^2 = 0.9612 (Y = 0.9614 x + 5.5813) was obtained. The developed histamine biosensor showed recovery of added histamine in the range of 93.11 to 100.58%.

Home made miniaturized histamine biosensor (30 mm x 10 mm) was then developed by screen-printed of carbon as working and counter electrodes together with Ag/AgCl reference electrode on the polyester substrate {named SPE (i)}. Miniaturized histamine biosensor with SPE (i) operated at 0.25 volt exhibited a linear range from 0 to 100 ppm of histamine with R^2 = 0.9577 (RSD < 9%) and sensitivity of 0.03 $nAppm^{-1}$ with the limit of detection of 2.46 ppm of histamine. The miniaturized histamine biosensor with a SPE (ii) was operated at 0.35 volt and it showed a linear range from 0 to 50 ppm histamine with R^2 of 0.9766 (RSD < 16%) and sensitivity of 0.40 $nAppm^{-1}$. The limit of detection of histamine was 4.64 ppm. The miniaturized SPE (i) was then modified with $K_3Fe(CN)_6$ and operated at 0.35 volt. This biosensor could detect the histamine in the linear range of 0 to 80 ppm with the R^2 = 0.9931 and sensitivity 5.31 $nAppm^{-1}$. The limit of detection for this modified histamine biosensor was 2.11 ppm histamine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBINAAN DAN PENCIRIAN HISTAMIN BIOSENSOR YANG BERDASARKAN DIAMINE OXIDASE

Oleh

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November 2006

Pengerusi: **Profesor Madya Fatimah Abu Bakar, PhD**

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Tahap histamin telah dicadangkan sebagai penunjuk ikan rosak secara cepat. Oleh itu, penderiabio histamin telah dibina berpandukan kepada pemegunan enzim diamine oxidase (DAO) dalam poli (2-hidroksietil metaakrilat) (fotoHEMA) boleh terawat-foto telah dibangunkan. Histamin telah dioksidakan oleh DAO yang terpegun pada 0.35 volt atas permukaan elektrod skrin bercetak pes-karbon melawan dengan rujukan Ag/AgCl konvensional, dan rod platinum sebagai elektrod lawan. Tiada larutresap semasa pengesanan histamin menggunakan penderiabio diperhatikan bagi DAO terpegun ini. Penderiabio yang dalam keadaan optimum telah menunjukkan julat respon linear dari 0 hingga 60 ppm histamin dengan koeffisien korelasi (R^2) 0.9946 (RSD < 11.19%). Sensitivity penderiabio ini adalah 5.56 $nAppm^{-1}$ dan had pengesanan 0.65 ppm dengan masa tindak balas selama 50 saat. Penderiabio ini telah menunjukkan sifat kebolehulangan dan kebolehasilan dengan RSD 14.06 dan 7.80% (n = 10) masing-masing. Penderiabio histamin ini telah digunakan untuk mengesan

histamin dalam udang harimau (*Penaeus monodon*) dan keputusannya adalah bolehbanding dengan kaedah konvensional kromatografi cecair prestasi tinggi (HPLC), di mana satu korelasi R^2 sama dengan 0.9612 (Y = 0.9614 x + 5.5813) telah diperolehi. Penderiabio histamin yang telah dibina ini telah menunjukkan perolehan-semula dalam julat 93.11 sehingga 100.58% bagi tambahan histamin.

Penderiabio histamin buatan sendiri dengan saiz yang dikecilkan (30 mm x 10 mm) telah dibina dengan karbon bercetak-skrin sebagai elektrod berkerja dan elektrod lawan dan elektrod runjukan Ag/AgCl, di atas substrat poliester {dinamakan SPE (i)}. Penderiabio histamin mini dengan SPE (i) yang berfungsi pada 0.25 volt telah menunjukkan julat respon linear dari 0 sehingga 100 ppm histamin dengan R^2 = 0.9577 (RSD < 9%) dan sensitiviti adalah 0.03 $nAppm^{-1}$ dengan had pengesanan sebanyak 2.46 ppm histamin. Manakala, penderiabio histamin mini dengan SPE (ii) telah beroperasi pada 0.35 volt dan telah menunjukkan julat respon linear 0 sehingga 50 ppm histamin dengan R^2 = 0.9766 (RSD < 16%) dan sensitivitinya adalah 0.40 $nAppm^{-1}$. Had pengesanannya ialah 4.64 ppm histamin. Selepas itu, SPE (i) mini telah diubahsuai dengan $K_3Fe(CN)_6$ dan telah beroperasi pada 0.35 volt. Biosensor ini telah dapat mengesan histamin dalam julat respon linear dari 0 sehingga 80 ppm histamine dengan R^2 = 0.9931 dan sensitivitinya adalah 5.31 $nAppm^{-1}$. Had pengesanan penderiabio histamin ini adalah 2.11 ppm histamin.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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CHING MAI KEOW

Date:

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LIST OF ABBREVIATIONS

1. DAO	Diamine oxidase
2. DMPP	2,2-hydroxyethyl methacrylate
3. polyHEMA	Poly (2-hydroxyethyl methacrylate)
4. photoHEMA	Photo (2-hydroxyethyl methacrylate)
5. UV	Ultra violet
6. HPLC	High Performance Liquid Chromatography
7. CV	Cyclic Voltammatry
8. SPE	Screen-printed electrode
9. AOAC	Associate Official Analytical Chemistry
10. FDA	Food and Drug Administrtation
11. SCE	Saturated calomel electrode
12. RSD	Relative standard deviation
13. BSA	Bovine serum albumin
14. LC	Liquid chromatography
15. PBS	Phosphate buffer saline

LIST OF SYMBOLS

1. mM	Millimolar
2. A	Ampere
3. V	Volt
4. Ox	Oxidation form
5. Red	Reduction form
6. ppm	Part per million
7. ppb	Part per billion
8. µl	Microliter
9. ml	Milliliter
10. g	Gram
11. N	Normality
12. kDa	Kilo Dalton

CHAPTER 1

INTRODUCTION

Histamine is known as a biogenic amine which is low molecular weight and possesses biological activity (Tombelli and Mascini, 1998). This compound was observed to accumulate in fish tissues when bacteria spoilage commenced (Male *et al.,* 1996) without altering the fish normal appearance and odor (Lehane and Olley, 2000). Histamine or 'Scombroid' poisoning is a short-lived and benign food-borne chemical intoxication typically associated with the consumption of food products containing large levels of histamine. Therefore, levels of histamine have been suggested as rapid fish spoilage indicators (Male *et al.,* 1996; Tomballi and Mascini, 1998; Patange, *et al.,* 2005). Histamine poisoning probably occurs frequently in Asia, and was reported in extremely high levels in some salted, and dried fermented products in Asia. Other countries such as Australia, New Zealand, Africa, Canada, Europe, and United State have also reported cases of histamine poisoning. The largest outbreak was recorded in Japan in 1973 (2656 cases) (Lehane and Olley, 2000).

Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal and hematological immunological system and the skin in the course of allergic and other actions such as hypotension, flushing, diarrhea, vomiting and headache (Lehane and Olley, 2000). The symptoms may vary between individuals that exposed to the same dose of histamine in contaminated

fishery products (Bremer, *et al.*, 2003). The earliest record of this disease was in 1828. Since then, the worldwide network for harvesting, processing and distributing fish products has made histamine poisoning as a global problem (Lehana and Olley, 2000). According to Food Safety Information System Malaysia (FOSIM) no guidance of histamine level is quote in Food Regulation 1985 and Food Act 1983. However, International food safety regulation, Food and Drugs Administration (FDA) USA, has quoted 500 ppm as hazardous level of histamine (FDA, 2001). Therefore, it had been considered as an indicator of earlier microbial decomposition. Histamine is generally not uniformly distributed in a decomposed fish (Lehane and Olley, 2000; FDA, 2001). The guidance level of 50 ppm has been set as the chemical index for fish spoilage. If 50 ppm of histamine is found in one section, there is the possibility that other sections may exceed 500 ppm (Lehane and Olley, 2000; FDA, 2001). The fish and fishery products with histamine above that level are prohibited from being sold for human consumption (Gigirey, *et al.*, 1998). Asia Pacific countries such as Australia and New Zealand have quoted 100 ppm of histamine as upper limit in food products (Brinker, *et al.*, 1996). In Canada, the level of histamine in seafood products should not exceed 200 ppm (Ababouch, *et al.*, 2005). Reviews of the oral toxicity to humans suggested that histamine induced poisoning have three stage, slight poisoning at 80- 400 mg/kg (ppm) fish, moderate poisoning at > 400 mg/kg and severe poisoning at > 1000 mg/kg. Based on the analysis of poisoning cases, the following guidance levels have been suggested for histamine content of seafood: (i) < 50 mg/kg (safe for consumption); (ii) 50-200 mg/kg (possibly toxic), (iii) 200-1000 mg/kg

(probably toxic) and (iv) > 1000 mg/kg (toxic and unsafe for human consumption) (Lehane and Olley, 2000).

Several chromatography methods have been proposed for histamine detection (Chemnitius and Bilitewski, 1996; Male *et al.*, 1996; Scott, 1998; Tombelli and Mascini, 1998). However, these methods required complicated and expensive instruments and time consuming. With the current technology, detecting seafood spoilage can be fast, cost effective and highly specific by using amperometric biosensors method (Male *et al.*, 1996; Shin, *et al.*, 1998; Zhang *et al.*, 2002).

Biosensors are devices comprising of an analyte and a selective interface in close proximity or integrated with a transducer. The transducer will converts the biochemical signal into an electronic signal which can be processed as an output (Chaubey and Malthotra, 2002). One of the benefits of biosensors is that they show a very highly selective property. This selectivity is due to the high substrate specificity of the biological material and the interference free indication of the reaction product (Chaubey and Malhotra, 2002). Nowadays, biosensor has moved from the laboratory to field testing and some of the biosensors have been commercialized in US, Europe and Japan. Table 1.1 shows the development of biosensors by different industries (Sharma, *et al.*, 2003).

Table 1.1 Development of biosensors by different industries

Type of biosensor	Manufacturers/ company
Air pollutants of *Candida albicans*	Universal Sensors, USA
Choline biosensor (immobilized on graphite electrode)	Thorn EMI Simtec Ltd., UK
Artificial electron acceptor, Hexacynoferrate (+) II (used with Pt electrode)	Wolverine Medical, USA
Immobilized enzyme membranes with O_2 , NH_4^+ and CO_2 electrode	Universal Sensors, USA
Glucose sensor (Immobilized with O_2 electrode)	Analytical Instruments Co. Japan, Gambro AB, Sweden, Radlkis Electrochemical Instrum., Hungary, Oriental Electric Co. Ltd., Japan
Glucose biosensor (immobilized with H_2O_2 probe)	Fuji Electric Co. Japan, Kyoto Daichi Kagaku, Japan, Omron Toyoba, Japan, Solea-Tacusse., France, Yellow Springs Instruments, Co., USA
Lactate biosensor	YSI Co., USA, Omron Toyoba, Japan
Acetic acid, methanol and ethanol (immobilized with O_2 electrode)	Denki Kagaku Keik Ltd., Japan
Uric acid sensor (immobilized with H_2O_2 electrode)	Fuji Electric Co. Japan
Fish freshness biosensor (immobilized on polarographic electrode)	Pegasus Industrial Specialities Ltd., Canada
BOD biosensors	Nissin Electric Co. Ltd., Japan