UNIVERSITI TEKNOLOGI MARA

CONSTRUCTION OF pBAD/TOPO® Thio Fusion-*pla*$_2$ GENE CASSETTE IN *Escherichia coli*

HANIS HANUM ZULKIFLY

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

Faculty of Pharmacy

November 2006

ACKNOWLEDGEMENTS

I would like to deeply thank the various people who, during the several months in which this endeavor lasted, provided me with useful and helpful assistance. Without their care and consideration, this book would likely not have matured.

Many thanks to my project supervisor, Mrs Norazlina Ahmad, who read my numerous revisions and helped, make some sense of the confusion. Also for her infinite and tireless teachings, her useful comments and suggestions and her willingness to provide constructive criticism that made my project a success.

Grateful acknowledgment is made for the valuable suggestions and help given by Dr. Kalavathy Ramasamy as the coordinator of Research Instrumentation final year project. Also gratitude to Professor Dr Abu Bakar Abdul Majeed, Dean of Faculty of Pharmacy for his help and endless support throughout the completion of my project and not forgetting in providing the approval for this project.

Special thanks to these people that have been most helpful especially to Cik Raja Noorfatihah Raja Lias, Scientific Officer Assistant, Cik Murni Mansor, Post Graduate Student and Nor Aznan Nor Azni, Research Assistant of Life Science Department for their help and teachings in dealing with equipments, important to the project completion.

My gratitude goes to my friends especially Nazhatul Nadiah Md Anuar, Nurhana Ghazali and Hilmi Hassan in providing me favours and endless help in so many ways that I could not have imagined.

And last but not least, my warmest thank you to my family whom endured this long process with me, always offering support and love.

TABLE OF CONTENTS

ABSTRACT

Aim - To construct pBAD/TOPO® Thio Fusion-*pla*$_2$ gene cassette in *Escherichia coli*

Method - The method of this study started of with conformation of the original clone (*pla*$_2$) synthesized by First BASE Laboratories followed by the amplification of the gene (*pla*$_2$) using PCR. Then, the amplified gene was ligated into a vector which is pBAD/TOPO® Thio Fusion. The entire plasmid was then transformed into a bacterial host, *E. coli* strain TOP10. Analysis of positive recombinant was done by restriction digestion, analyzing PCR and DNA sequencing. Prior to performing restriction digestion, PCR and DNA sequencing, the plasmid which carries the gene of interest (GOI) must be extracted from the bacterial host. Extraction was done using commercial DNA extraction kit (Wizard® Plus Minipreps DNA Purification System, Promega).

Conclusion – This study represent the construction of bacterial expression system for heterologous expression of *pla*$_2$ in *E. coli*. It can be concluded that it is a success to construct pBAD/TOPO® Thio Fusion-*pla*$_2$ plasmid in *E. coli*. It has been proven that the nucleotide sequence of *pla2* gene exhibits high homology to the corresponding region of the porcine PLA_2 sequence.

CHAPTER 1

INTRODUCTION

Phospholipase A_2 (PLA_2) belongs to a family of enzymes that catalyze the cleavage of fatty acids from the *sn*-2 position of phospholipids. There are more than 19 different isoforms of PLA_2 in the mammalian system, but recent studies have focused on three major groups, namely, the group IV cytosolic PLA_2, the group II secretory PLA_2 ($sPLA_2$), and the group VI Ca^{2+}-independent PLA_2 (Grace *et al.*, 2004). These PLA_2s are involved in a complex network of signaling pathways that link receptor agonists, oxidative agents, and pro inflammatory cytokines to the release of arachidonic acid (AA) and the synthesis of eicosanoids. PLA_2s acting on membrane phospholipids have been implicated in intracellular membrane trafficking, differentiation, proliferation, and apoptotic processes (Grace *et al.*, 2004).

Secretory PLA_2s constitute a large family of structurally and mechanistically related enzymes with relative molecular masses of 13-16 kDa. They are widespread in various mammalian cells and tissues, as well as in snake, lizard and insect venom, and are divided into several groups and subgroups based on their amino acid sequences, disulfide bonding patterns, tissue distribution, and functional properties. These enzymes perform phospholipid hydrolysis using a His-Asp doublet plus a conserved water molecule as a