UNIVERSITI TEKNOLOGI MARA

CLONING AND EXPRESSION OF HETEROLOGOUS HUMAN CYTOCHROME P450 3A4-NADPH P450 REDUCTASE IN *ESCHERICHIA COLI*

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

Faculty of Pharmacy

JANUARY 2010

ABSTRACT

Cytochrome P450 3A4 (CYP3A4) is one of the Cytochrome P450 monooxygenases, dominant in drug metabolism in human liver by virtue of its high levels of expression in liver and other tissues. It represents about 30% of total CYP450. The aim of this study is to develop a heterologous *in vitro* drug metabolizing enzyme system for human CYP3A4 with NADPH-P450 reductase using *Escherichia coli* (*E. coli*). The advantages of this bacteria system include low maintenance cost, ease of use and the high yield of enzymes. The gene encoding *CYP3A4* was identified from database and specific primers were designed. The cDNA was then amplified using polymerase chain reaction and the human liver cDNA was the initial template. The recombinant 17αCYP3A4 with the NADPH-P450 reductase expression plasmid was constructed for cloning into *E. coli*. The two plasmids were co-expressed and harvested. The identification and confirmation of the protein was done by SDS-PAGE, Western-Blot and spectral analysis; while the functionality of the enzyme was performed using Vivid® P450 kit. The *CYP3A4* gene sequence and its construct in the expression plasmid were confirmed with direct sequencing. The optimal condition for large scale expression of CYP3A4 protein was 30°C for 48 hours. The pro tein expressed was successfully characterized. The SDS-PAGE and Western-blot confirmed the bands at 57 kDa for CYP3A4 and 80 kDa for NADPH-P450 reductase. The spectral determination of CYP3A4 showed a absorbance peak at the UV wavelength of 450 nm. The yield of this recombinant protein was 71.4286 nmol/ml protein; and the activity was 9442.89 nmol/min/ml protein. The Michaelis-Menten parameters determined from the protein were 35.2 μM for the K_m and 1366.3 RFU/min/pmol CYP3A4 for the V_{max}. An in vitro heterologous system containing co-expression of CYP3A4 with NADPH-P450 reductase in *E. coli* was successfully developed and characterized. The system would be of enormous benefit for elucidation of metabolic pathways for new chemical entities as well as in the study of drug-drug and drug-herb interactions.

Keywords : CYP3A4, NADPH-P450 reductase, heterologous, *Escherichia coli*, construction, SDS-PAGE, Western-blot, spectral determination

ACKNOWLEDGEMENT

First and foremost, all praise and thanksgivings to Allah the Almighty, for gracing me with strength to complete my thesis. Alhamdulillah. My utmost gratitude and appreciation goes to my supervisor Associate Professor Dr. Teh Lay Kek for her encouragement, help, support and personal guidance throughout the period of this research. Her wide knowledge has been of great value for me.

I wish to express my warm and sincere thanks to Professor Dr. Mohd Zaki bin Salleh and Dr. Rosmadi Yusoff for their advices, ideas, help and guidance in molecular field especially for the cloning and expression of protein.

I am deeply grateful to Mr. Lee Wee Leng, for his guidance, ideas, advices and help throughout my research. My sincere thanks are due to Mrs. Riza Afzan Asri for her help and sharing her skills and knowledge with me in the laboratory.

I am grateful to all my labmates, Fazleen, Izuddin, Wan Rosalina, Hazwanie, Izwani, Sharina, Suzana, Kamila, Imma and also to Puan Siti and Puan Junaidah Amir for their assistance and prays for my success. Also my gratitude goes to Hamidah, Zaihana, Suhaiza and Syazwani for their time, support and encouragement.

I owe my loving thanks to my parents, Hj Abdul Malik and for their prayers, patient and support throughout my study. I also would like to express my sincere appreciation to my husband, Mohd Qusyairi Razali for his time, support, prayers and help. Last but not least, my warmest thank goes to my siblings for their loving support. Without their encouragement it would have been impossible for me to complete this research.

Finally, my thank goes to all who have involved in my study. Also, the financial support from MOSTI is gratefully acknowledged.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cytochrome P450 (CYP450) is one of the Phase I drug-metabolizing enzymes found mainly in the liver (Coleman, 2005). It is an integral membrane-bound heme-containing enzyme. CYP450 comprises a superfamily of homeoproteins which function as the terminal oxidase of the mixed function oxidase system. This superfamily is divided into families and subfamilies according to their amino acid sequences (Nelson *et. al.*, 1996). As up to date, eighteen subfamilies of CYPs contribute substantially to the metabolism of drug and non-drug xenobiotics (Miners *et. al.*, 1998).

The expressions of the CYP enzymes vary between individuals due to interaction of the environment with genetic. The variation of the expression and functionality of the enzymes are often complicated by diseases (Miners *et. al.*, 1998). These factors produce inter-individual variation in the rate and metabolic pathways of xenobiotics. Xenobiotics are chemical compounds that do not belong to the normal composition of the human body. These compounds enter the body via diet, air and medication; elimination of xenobiotics from the body occurs after biotransformation (Coleman, 2005). Xenobiotic metabolizing CYP isoforms typically exhibit characteristic, but occasionally overlapping patterns of substrate specificities and inhibitor profiles (Miners *et. al.*, 1998).