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THE EFFECTS OF TEMPERATURE ON THE EXPRESSION OF RECOMBINANT PHOSPHOLIPASE A_2 IN *Escherichia coli*

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THE EFFECTS OF TEMPERATURE ON THE EXPRESSION OF RECOMBINANT PHOSPHOLIPASE A_2 IN *Escherichia coli*

The aim of the research is to study the effects of temperature on the expression of recombinant *Pla_2* in *E. coli*. There were two clones of *E. coli* used in this study. Both clones were incubated at 27 °C and 37 °C respectively. The method of this study was started with 100 µL overnight cultured was transferred into LB broth. After two hours, the cultures were supplemented with 0.2 % arabinose to induce the expression of the PLA_2 and incubated at two different temperatures. Samples (PLA_2 protein) that have been collected were analysed through SDS-PAGE, Bradford protein quantitation and $sPLA_2$ protein activity. Prior to performing analysis study, the protein must be purified from the host. Purification was done using Maxwell 16 polyhistidine protein purification kit. The finding shows that clone pBAD Thio-TOPO *Pla_2* (-TAG 5) and pBAD Thio-TOPO *Pla_2* (-TAG 8) produced PLA_2 protein. Result showed that 37 °C incubation temperature produced higher protein amount when compared to 27 °C incubation temperature. It can be concluded that temperature was found to give effect towards the amount of protein being expressed.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Phospholipase A_2 (PLA_2) is a family of enzyme that specifically hydrolyze the sn-2 ester bond of phospholipids to release unsaturated fatty acids (Deenen & Haas 1963). This reaction is of particular importance if the esterified fatty acid is arachidonic acid (AA) (n6, 20:4), which is converted by downstream metabolic enzymes to various bioactive lipophilic compounds called eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs). The other reaction products, lysophospholipids such as lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC), are also biologically active by themselves and are the precursors of other potent bioactive mediators (Ramirez & Jain 1991).

This enzyme has been classified as $cPLA_2$ and $sPLA_2$ due to it's widely distribution in nature. Intracellular PLA_2s are often membrane associated and are involved in phospholipid metabolism and signal transduction. Extracellular PLA_2s are abundant in mammalian pancreatic juices and in the venom of snakes and insects which display diverse roles, including blood platelet aggregation, anticoagulant and hypotensive effects (Mukherjee *et al.*, 1994).