UNIVERSITI TEKNOLOGI MARA

PURIFICATION OF RECOMBINANT PHOSPHOLIPASE A_2 ENZYME IN *ESCHERICHIA COLI*

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ABSTRACT

Phospholipase A_2 is a lipolytic enzyme that specifically hydrolyze sn-2 fatty acyl ester bond of phospholipids to yield free fatty acids and lysophospholipids. It was widely use in the several industry including pharmaceutical, food and biotechnology. Therefore, this study was done to purify the phospholipas A_2 enzyme by using hybrid protocol. This method consist of two combination method which is denaturing and native protocol and has ability to purified and retained biological activity of the desired protein. There were two clones involved in the study which is pBADTOPO pla_2 clone 5 and pBADTOPO pla_2 clone 8. They were purified at the 37 °C. Result indicated, only small amount of bioactive protein were recovered. Poor recovery of bioactive protein from inclusion bodies may result from the loss of secondary structure during solubilization procedure and interaction among the denatured protein molecules during refolding.

CHAPTER 1

INTRODUCTION

1.1 Introduction

More than the past two decades, numerous PLA_2 have been recognized and characterized (Dennis, 1994; Six & Dennis, 2000; Laye & Gill, 2003). Phospholipase A_2 is a lipolytic enzyme that specifically hydrolyze sn-2 fatty acyl ester bond of phospholipids to yield free fatty acids and lysophospholipids (Dennis, 1994). The hydrolytic reaction promoted by PLA, has importance effect in biotechnology fields because the lysophospholipid derivative products have strong bioemulsifying properties. In addition, phospholipids are amphiphilic molecules and it can exist in many different aggregated forms in water depending on the polar head group and hydrophobic chain length. (Lichtenberg *et al.*, 1983; Reynolds *et al.*, 1991).

The use of lysophospholipids as food additives is well known (Van Nieuwenhuyzen, 1976), such as in preventing the contamination of foods with microbial spore (Maeda & Murata, 1995) and these compounds are used in the pharmaceutical and biomedical industry (Mukhejee, 1990). The family of mammalian PLA_2 enzymes includes cytosolic, secretory, and calcium-independent forms. The PLA_2s enzyme from porcine pancrease (Puijk *et al.*, 1977; Seilhamer *et al.*, 1986) is the most commonly used in the industry because enzymes isolated from other microorganisms shown to have less activity than