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Possible Roles of Phospholipase A_2 in the Biological Activities of *Acanthamoeba castellanii* (T4 Genotype)

Parisa Nakhostin Mortazavi[a], Ehud Keisary[a], Lip Nam Loh[a], Suk-Yul Jung[a], and Naveed Ahmed Khan[b,1]

[a]School of Biological and Chemical Sciences, Birkbeck, University of London, Malet Street, Bloomsbury, London, WC1E 7HX, England, UK
[b]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, LE12 5RD, England, UK

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Using phospholipases A_2-specific spectrophotometric assays, it was shown that *A. castellanii* lysates and their conditioned medium exhibit phospholipase activities. The extracellular levels of PLA_2 detected were significantly reduced compared with the cell-associated enzyme ($P<0.05$). Sphinganine, a PLA_2 inhibitor showed robust amoebistatic properties but had no effect on the viability of *A. castellanii*. The potency of sphinganine was demonstrated effectively towards purified PLA_2 derived from porcine pancreas. Using sphinganine, it was observed that PLA_2 is involved in neither binding nor cytotoxicity of the human brain microvascular endothelial cells due to *A. castellanii*. Unlike as was the case for *Dictyostelium* amoebae, PLA_2 appeared to be involved in *A. castellanii* phagocytosis of the fluorescently-labelled polystyrene beads. Horseradish peroxidase was used as a tracer molecule to develop assays to study pinocytosis in *A. castellanii*. The findings revealed that sphinganine impedes phagocytosis but augments pinocytosis in *A. castellanii* suggesting distinct nature of processes. A complete understanding of the role of phospholipases in the biology and pathogenesis of *A. castellanii* infections will determine their potential as therapeutic targets.

Key words: *Acanthamoeba castellanii*; pathogenicity; phospholipase A_2; phagocytosis; pinocytosis; sphinganine.

Introduction

Acanthamoeba castellanii is an opportunistic protozoan parasite, which can cause fatal encephalitis and a blinding keratitis (Khan 2006; Marciano-Cabral and Cabral 2003; Visvesvara et al. 2007). However, the underlying mechanisms of the pathogenesis and pathophysiology of amoebic infections remain incompletely understood. Previous studies have shown the presence of phospholipases in *Acanthamoeba* (Cursons et al. 1978; Victoria and Korn 1975a, b).

Phospholipases are a diverse group of enzymes that hydrolyze ester linkage in glycerophospholipids and are involved in the biosynthesis and degradation of membrane lipids. Thus, the activities of phospholipases can result in membrane dysfunction. The five major known phospholipases are A_1, A_2, B, C, D, and each has the ability to cleave a

[1]Corresponding author; fax +44 1159516440
e-mail naveed.khan@nottingham.ac.uk (N.A. Khan).

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specific ester bond in the phospholipid substrate of the target membrane (Fig. 1) (Akiba and Sato 2004; Exton 2002; Ghannoum 2000; Kudo 2004; McDermott et al. 2004).

Among them, PLA_2 are most extensively studied and are shown to play important roles in a number of diverse, important cellular and physiological functions such as phospholipid degradation leading to membrane remodelling and/or perturbation, cellular metabolism, inflammation, lipid-derived generation of second messengers, pathogenesis and cell signal transduction (Dennis 1994; Ghannoum 2000; Istivan and Coloe 2006; Ivanovska 2003; Six and Dennis 2000; Leslie 1997). On the other hand, phospholipases are proven targets in the prevention and treatment of microbial infections. For example, phospholipase C from *Clostridium perfringens* induced protection against *C. perfringens*-mediated gas gangrene, whilst targeting phospholipases using synthetic compounds successfully treated *Candida* infections (Bryant and Stevens 1996; Hanel et al. 1995; Kameyama et al. 1975). This suggests that an understanding of phospholipases in the biology and pathogenesis of *Acanthamoeba* may identify targets for therapeutic interventions. The overall aim of the present study was to determine PLA_2 activities in *A. castellanii* and demonstrate its possible involvement in the cell biology and pathogenesis of the amoeba.

Results

A. castellanii Exhibit Cell-associated and Extracellular Phospholipases A_2 Activities

To determine PLA_2 activities in *A. castellanii*, spectrophotometric-based assays were performed. The findings revealed that *A. castellanii* lysates (Fig. 2A and B) as well as their conditioned medium, ACM (Fig. 2C) exhibited PLA_2 activities. As expected, the levels of extracellular PLA_2 activities were lower compared with the cell-associated PLA_2. As expected, maximum PLA_2 activities were observed with increasing numbers of *A. castellanii* (Fig. 2B).

PLA_2 Plays an Important Role in Growth but not Viability of *A. castellanii*

To study the involvement of PLA_2 in growth and viability of *A. castellanii*, a specific inhibitor of the enzyme, sphinganine, was tested. The potency and specificity of sphinganine was observed against purified porcine pancreas PLA_2 (data not shown). When tested against *A. castellanii* lysates and ACM, sphinganine completely abolished PLA_2 activities at concentrations $\geq$ 300 μM (Fig. 3A and B). Interestingly, sphinganine inhibited the growth (P<0.05, using paired T-test, one tail distribution) but not the viability (P>0.05) of *A. castellanii*. Amoebae incubated with $\geq$ 300 μM of sphinganine showed no growth in PYG (Fig. 4A). At 100 μM

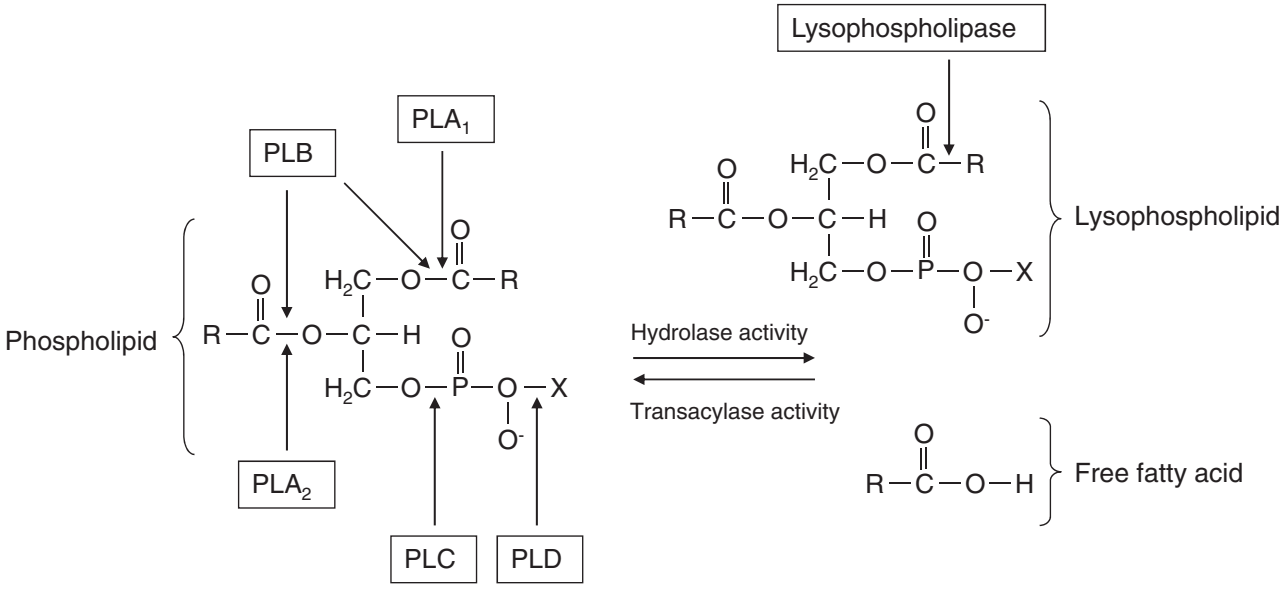

Figure 1. Schematic diagram presenting the activity of various phospholipases and their sites of action on phospholipids. Phospholipase A_1 (PLA_1), Phospholipase A_2 (PLA_2), Phospholipase B (PLB), Phospholipase C (PLC), and Phospholipase D (PLD). Adapted from Ghannoum (2000).