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The Effect of a Muscarinic Antagonist on the Lipase Activity

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Abstract**—**Lipases constitute one of the most important groups of industrial enzymes that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids. Muscarinic antagonist relieves smooth muscle spasm of the gastrointestinal tract and effect on the cardiovascular system. In this research the effect of a muscarinic antagonist on the lipase activity of *Pseudomonas aeruginosa* was studied. Lineweaver–Burk plot showed that the drug inhibited the enzyme by competitive inhibition. The IC50 value (0.16 mM) and Ki (0.03 mM) of the drug revealed the drug bound to enzyme with high affinity. Determination of enzyme activity in various pH and temperature showed that the maximum activity of lipase was at pH 8 and 60°C both in presence and absence of the drug.

Keywords**—**Bacteria, inhibition, kinetics, lipase.

I. Introduction

PSEUDOMONAS aeruginosa is a Gram-negative bacterium which is widely used for bioremediation of organic pollutant in soil and water [1], [2]. *P. aeruginosa* has a large genome, with one of the largest regulatory networks of any bacteria sequenced [3]. It is a useful bacterium for industries, it can aerobically biodegrade some types of toxic materials in soil and water. *Pseudomonas aeruginosa* has also become an important cause of infection, especially in patients with compromised host defense mechanisms. One of the most important reasons that make *Pseudomonas* an emerging opportunistic pathogen in patients is its ability to use various compounds as carbon sources [3]. In this regard, it uses a wide variety of enzymes to degrade simple or complicated carbon sources for earning energy [3].

The major extracellular enzymes generated by *P. aeruginosa* are protease [4], elastase A (LasA) and B (LasB) [5], phospholipase C [6] and lipases [7], [8]. These enzymes are causing cell death, severe tissue damage and necrosis in the human host [9]. Lipase is an enzyme that catalyzes the hydrolysis of lipids. Most lipases act at a specific position on the glycerol backbone of lipid substrate. Some lipases are expressed and secreted by pathogenic organisms during the infection. Microbial lipase has industrial applications and used in detergents, cosmetics, pharmaceuticals and leather processing [10].

Many microorganisms such as bacteria, yeast and fungi are known to secret lipases. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc. [11].

As results any agents that inhibit lipase activity may be important for medicine and industry. For example, the inhibition of lipase may useful for the persons who are annoyed from the obesity. Orlistat is an inhibitor of pancreatic lipase which is used for decreasing lipid absorption by the obese person [12], [13]. Most drugs were designed to interact with specific receptors or enzymes but in some case drugs may bind to other enzyme which causes the side effect of enzyme. It has been shown that cimetidine which designs to block histamine H2 receptor, could also strongly inhibit alkaline phosphatase and catalase [14], [15]. Codeine, scopolamine and ranitidine were showed to inhibit sucrase activity [16], [17].

II. Objective

The aim of this research was to find a common drug which can inhibit bacterial lipase. In this study a muscarinic antagonist was found to be a potent lipase inhibitor and the kinetics of binding was investigated.

III. Materials and Methods

A. Culture Medium and Cell Harvesting

The salt medium was used by adding 2.5 g KH_2PO_4, 2.5 g Na_2HPO_4, 1 g NH_4NO_3, 0.2 g $MgSO_4$, and 0.01 g $CaCO_3$ to 1 lit of distilled water and the pH was adjusted to 7.0. Olive oil was used as the carbon source with the final concentration of 1%. The culture medium was aerated in a rotary shaker at 30°C for 72 h. The cells were precipitated by centrifugation and the supernatant was used for enzyme assay.

B. Enzyme Assay

Working buffer was prepared by adding different concentrations (0.06 to 0.5mM) of Para nitrophenyl palmitate (PNPP) as the substrate to 0.1 M Tris buffer pH 8. The reaction was started by adding 20 ul of supernatant to the working buffer in the test tube. The catalytic activity of lipase was continuously monitored by following the increased absorption at 410 nm for 10 min. The enzyme assay was detected in the absence and presence of the muscarinic antagonist with concentration 0.3 to 1.5 mM. The activity of enzyme was also detected at different pH, temperature and ionic strength. The results were the average of at least three separate experiments which were expressed as mean ± standard deviation (SD). Biuret method was used for protein determination.

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IV. Result and Discussion

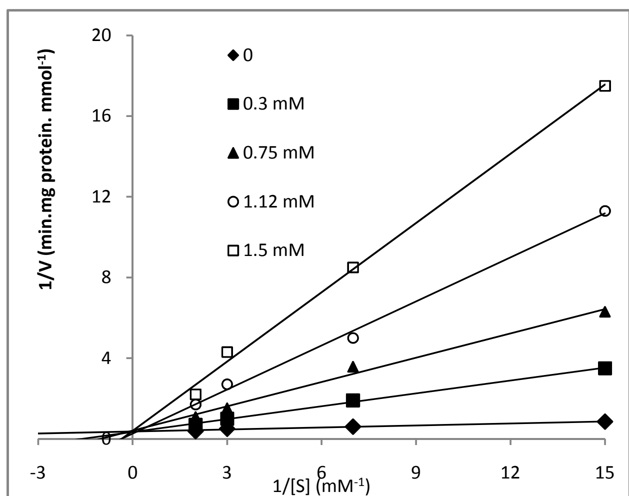

Fig. 1 Competitive inhibition resolved by Lineweaver-Burk plot

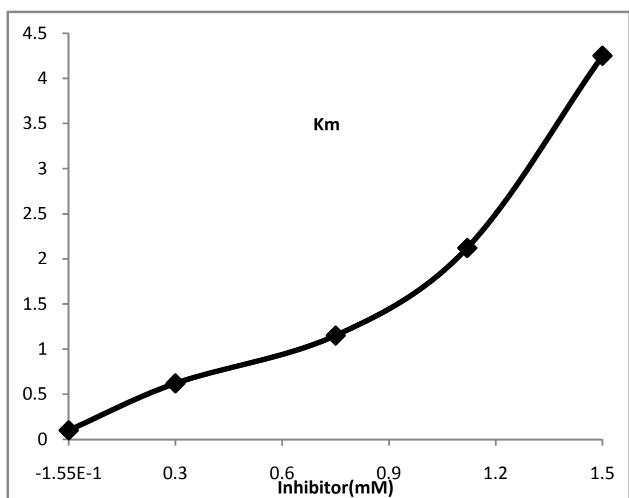

Fig. 2 The Km of enzyme increased sharply in the presence of low concentration of the drug which revealed that the affinity of lipase for substrate decreased roughly in the presence of the drug

Enzyme inhibition is an important subject in the word of enzymes. Many reports have been published in concern of common drugs as inhibitor of enzymes. In this case it can be noted to the ranitidine, scopolamine which inhibited yeast sucrase and cimetidine and tramadol which inhibited alkaline phosphatase and catalase. Inhibitors of lipase may be not useful for its industrial application but they are useful for intestinal lipase to prevent the absorption of lipids in obese persons. Few drugs have been identified as lipase inhibitor. Recently, orlistat has been introduced as an inhibitor of lipase and widely used in obesity to prevent lipid absorption. This study, for the first time introduces another common drug as an inhibitor of lipase. The aim of this research was to investigate the kinetic parameters and the kind of inhibition of this drug. Our results showed that the muscarinic antagonist could interact with *Pseudomonas aeruginosa* lipase. Lineweaver-burk plot determined that the drug could inhibit the lipase by competitive manner (Fig. 1).

Ki of the drug (0.03mM) was also resolved by using secondary plot from the primary plot (Fig. 3). The small amounts of IC_{50} and Ki of the drug verified that the drug has been bound to lipase by high affinity. Previous studies reported that drugs bound to enzyme with different affinity.

Determination of IC_{50} and Ki of ranitidine for sucrase and tramadol for alkaline phosphatase showed that they bound with moderate affinity [18], [19] while cimetidine bound to catalase with high affinity [15].

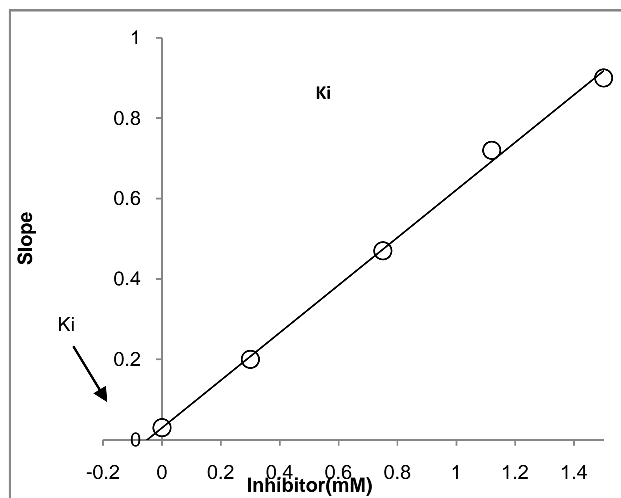

Fig. 3 Secondary plot derived from Lineweaver-burk Ki was determined to be about 0.03mM

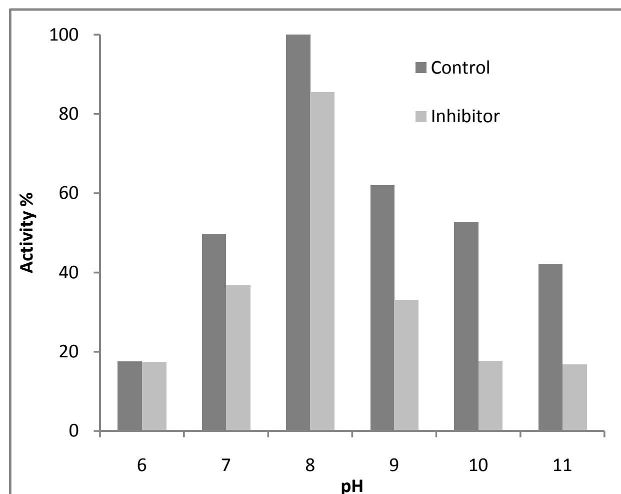

Fig. 4 Effect of different pH on the activity of enzyme in the absence and presence of the inhibitor. The pH profile of the enzyme was the same in the presence and the absence of drug (control)

Fig. 4 shows the effect of different pH on the activity of the lipase in the presence and absence of the drug. Maximum activity of the enzyme was observed at pH 8 in both cases. The pH profile obeys from the same pattern in the presence

and absence of drug. This suggests that variation of pH could not prevent the drug to bind the enzyme; on the other hand, the drug did not use ionic interaction for linking to the enzyme. The enzyme activity was also determined in various temperatures (Fig. 4). Optimum temperature of activity was detected to be at 60°C and the pattern of temperature profiles were also the same in both cases. High temperature could not refuse the linking of the drug to the enzyme. This proposes that the drug has not used hydrogen bond to link the enzyme.

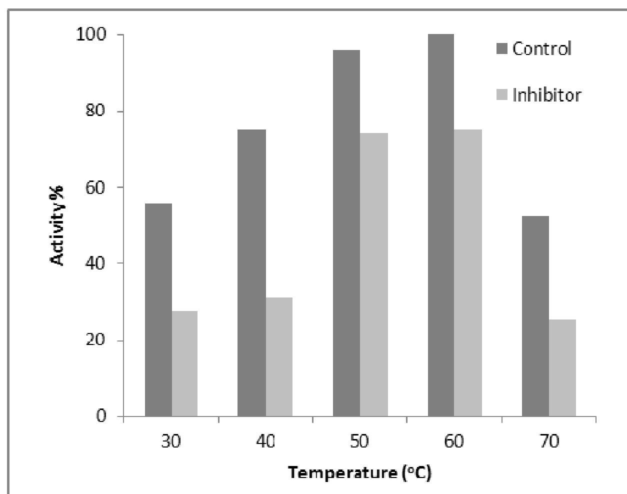

Fig. 5 The effect of temperature on the activity of enzyme in the absence (control) and presence of the muscarinic antagonist. In the presence of the drug, the activity of the enzyme was reduced. In both cases the maximum activity of enzyme was observed at 60^0C.

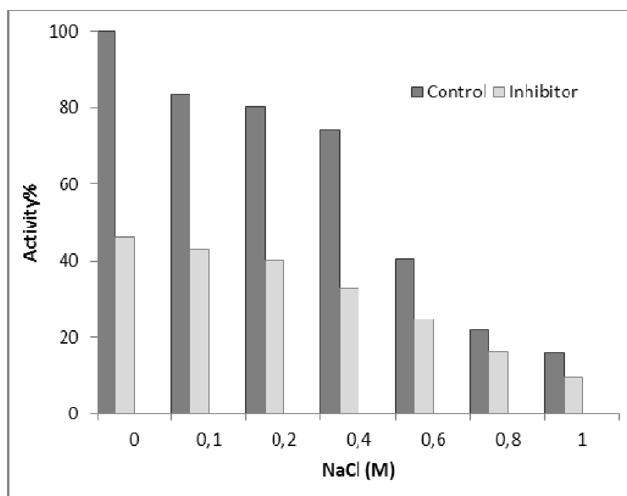

Fig. 6 The effect of ionic strength on enzyme activity. The presence of NaCl reduced the activity of the enzyme. In all concentration of NaCl, the activity of enzyme in the presence of the drug was lower than control

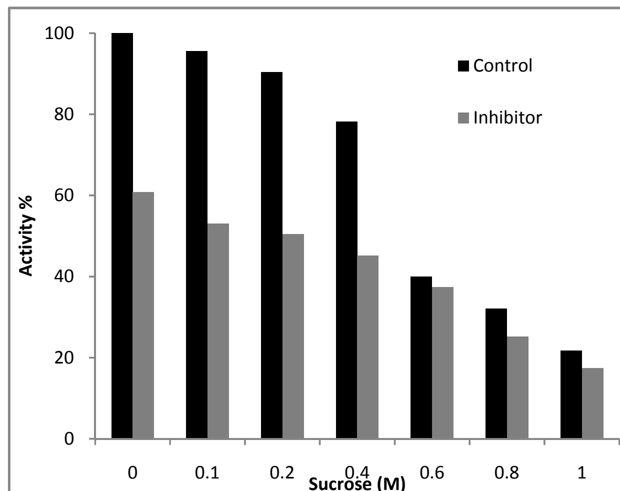

Fig. 7 Viscosity effect on enzyme activity. Increasing sucrose concentration decreased enzyme activity. Minimum activity was observed in 1M sucrose

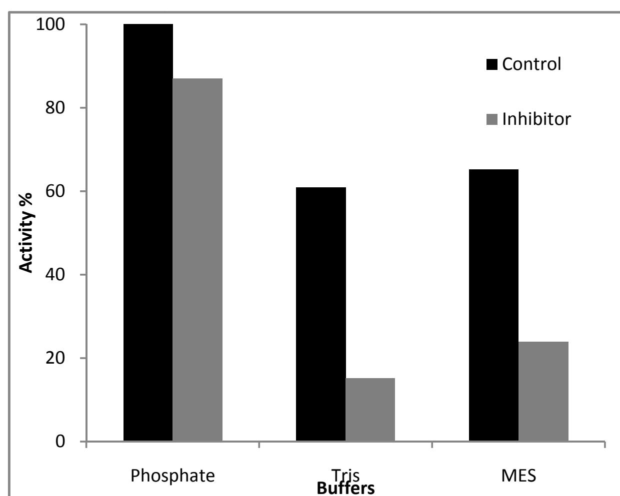

Fig. 8 Maximum activity was detected in Phosphate buffet while the activity was minimum in Tris

V. Conclusion

In conclusion, the muscarinic antagonist could competitively inhibit *Pseudomonas* lipase. It bound to the enzyme with high affinity. The drug did not use ionic interaction or hydrogen bond to link the enzyme. Also neither ionic strength nor viscosity changes could refuse binding of muscarinic antagonist to the enzyme but could decrease enzyme activity.

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REFERENCES

[1] A. Mrozik, Z. Piotrowsks-Seget, S. Labuzek. "Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons." *Pol J Environ Stud* 12, 15-25, 2003.
[2] K.N. Timmis, D.H. Pieper. "Bacteria designed for bioremediation." *Trends Biotechnol* 17, 201-204, 1999.
[3] Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, et al. Complete genome sequence of pseudomonas aeruginosa PA01, an opportunistic pathogen. Nature. 2000;406:959-64
[4] E. Frimmersdorf, S. Horatzek, A. Pelnikevich, L. Wiehlmann, D. Schomburg. "How *Pseudomonas aeruginosa* adapts to various environments: a metabolomic approach." *Environ Microbiol* 12, 1734-1747, 2010.
[5] S. J. Cryz, B.H. Iglewski, "Production of alkaline protease by *Pseudomonas aeruginosa*" *J Clin Microbiol* 12(1):131-133, 1980.
[6] R. A. Bever, B. H. Iglewski, "Molecular characterization and nucleotide sequence of the *Pseudomonas aeruginosa* elastase structural gene" *J Bacteriol*, 170(9):4309-4314. 1988.
[7] R.M. Ostroff, M.L. Vasil, "Identification of a new phospholipase C activity by analysis of an insertional mutation in the hemolytic phospholipase C structural gene of Pseudomonas aeruginosa" *J Bacteriol*, 169(10), 4597-460,11987.
[8] W. Stuer, K.E. Jaeger, U.K. Winkler, "Purification of extracellular lipase from Pseudomonas aeruginosa" *J Bacteriol*, 168(3):1070-1074, 1986.
[9] A. Martinez, P. Ostrovsky, D. N. Nunn, "LipC, a second lipase of *Pseudomonas aeruginosa*, is LipB and Xcp dependent and is transcriptionally regulated by pilus biogenesis components" *Mol Microbiol,* 34(2)**:**317-326, 1999.
[10] B. König, K. E. Jaeger, A. E. Sage, M. L. Vasil, W. König, "Role of *Pseudomonas aeruginosa* lipase in inflammatory mediator release from human inflammatory effector cells (platelets, granulocytes, and monocytes" *Infect Immun*, 64(8), 3252-3258, 1996.
[11] E.W. Seitz, "Industrial application of microbial lipases: A review" *J Amer Oil Chem Soc*, 51, 12-16, 1974.
[12] Sztajer H, Maliszewska I, Wieczorek J. Production of 3. exogenous lipase by bacteria, fungi and actinomycetes *Enzyme Microb Technol* 1998; 10: 492-7.
[13] J. Zhi, A.T. Melia, H. Eggers, R. Joly, I.H. Patel, "Review of limited systemic absorption of orlistat, a lipase inhibitor, in healthy human volunteers" *J Clin Pharmacol* 35 (11): 1103–1108, 1995.
[14] M.C. Mancini, A. Halpern, "Pharmacological treatment of obesity". *Arq Bras Endocrinol Metab* 50(2), 377–89, 2006.
[15] D. Minai-Tehrani1, S. Khodai1, S. Aminnaseri, S. Minoui et al, "Inhibition of renal alkaline phosphatase by cimetidine" *Drug Metab lett*, 2011.
[16] D. Minai-Tehrani, S. Minoui, M. Sepehre, et al, "Inhibitory effect of codeine on sucrase activity" *Drug Metab lett*, 3, 58–60, 2009.
[17] D. Minai-Tehrani, N. Fooladi, S. Minoui, Z. Sobhani-Damavandifar, et al, "Structural changes and inhibition of sucrase after binding of scopolamine" *Eur J Pharmacol*, 635, 23-26, 2010.
[18] D. Minai-Tehrani, M. Eslami, N. Khazaei, E. Katebian, et al, "Inhibition and structural changes of liver alkaline phosphatase by tramadol" *Drug Metab lett*, in press.
[19] D. Minai-Tehrani, M. Ghaffari, Z. Sobhani-Damavandifar, S. Minoui, et al, "Ranitidine induces inhibition and structural changes in sucrase." *J Enzyme Inhib Med Chem*, 27(4): 553–557, 2012.