Phospholipase activity in *Candida* isolated from Patients in Nassiria City

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Abstract

Infection caused by the fungus *candida* is potentially fatal, A highly active of phospholipase activities was identifide , Analysis of products hydrolysis showed that the enzyme activities were phospholipase using TLC technique to know the producing fatty acids by using standard material from this acids was studied, some kinetic function for this enzyme was studied.

الخلاصة

ان الاصابات التي تتسبب بواسطة الخميرة *Candida* تعتبر قاتلة ، لذلك امكن التعرف على فعالية عالية للانزيم فوسفولايبيز وتم تحديد نواتج التحلل المائية لهذا الانزيم بواسطة تقنية (TLC) لمعرفة الاحماض الدهنية الناتجة وذلك بمقارنتها مع احماض دهنية قياسية ايضاً تم التعرف على بعض الصفات الحركية لهذا الانزيم .

Introduction

***Candida* is commonly isolated human apportunintic pathogn capable of causing both superficial and systemic candidoses [5] its ability to produce phospholipase is considered to be an important pathogenic feature [3] and several workers have suggested that this property could be effectively exploited as a criterion for biotyping *candida* [6].**

The enzymes involved in the metabolism of phosphatidylcoline, which is the major phospholipid of *candida* [2] , are summarized in Fig. (1) phospholipase A_2 (PLA_2) cleaves an acyl chain of phosphatidylcholin (PC) to produce lysophosphatidylcholin (lyso-PC), while phospholipase B (PLB) simultaneously removes two fatty acids (FA) to produce glycerylphosphatidylcholin (GPC) whitout formation of a lysocompound . Lysophospholipase (LP) removes an acylchain from lyso-PC formed by PLA_2 , thus producing GPC . Lysophospholipase-transacylase (LTAC) catalyses the reaction involving transacylation between two molecules of lyso-PC and is independent of CoA and ATP , LPTA hydrolysis one lyso-PC molecule to GPC and forms the fatty acid-enzyme complex (LPTA-FA) .

This complex is then hydrolysed to yield FA (hydrolytic reaction) and its fatty acid moiety is transferred to the other molecule of lyso-PC (transacylation) – Thus the enzyme responsible for these two reactions is termed lysophospholipase-transacylase [4].

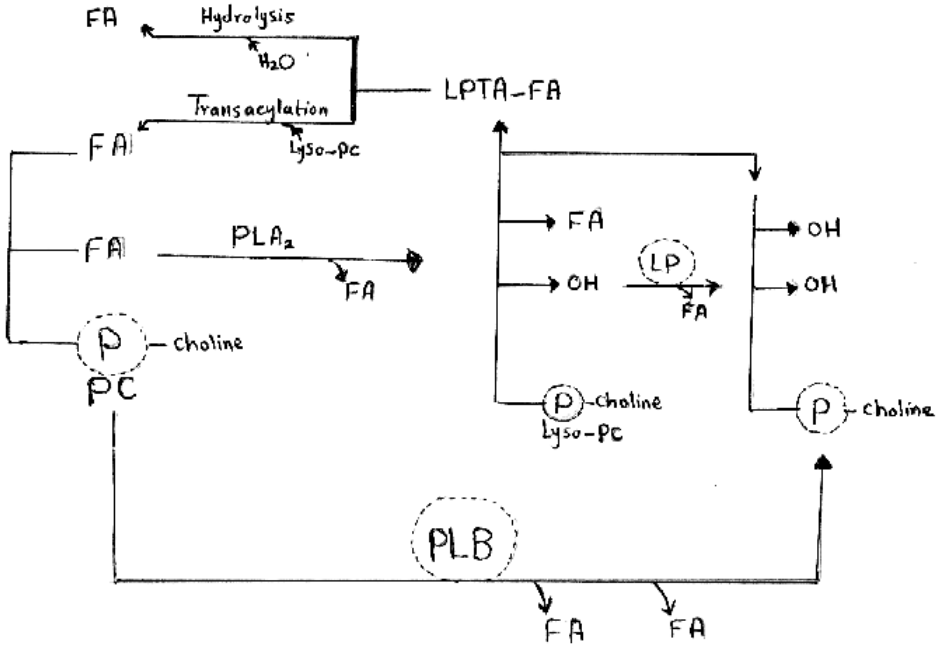

Fig.(1) phospholipase in *Candida*. PC= phosphatidylcholine; Lyso-PC = lysophosphatidylcholine ; FA = fatty acid ; GPC = glycerylphosphorylcholine ; PLA_2 = phospholipase A_2 ; LP = Lysophospholipase , PLB = phospholipase B ; LPTA = lysophospholipase-transacylase ; LPTA-FA = lysophospholipase-transacylase and fatty acid complex .

Methods

Candida **was isolated from patients with candidiasis who attended to Nassiria hospital , cells grown on sabouraud glucose (2% , w:v) agar were used as the inoculum and were grown in modified sabourand broth (medium A ; 30 gm glucose , 10 gm polypeptone and 10 gm yeast extract (w:v) in 1 Liter distilled water) at 37 ^{o}C with shaking , at an initial concentration of 1.5 x 10^5 cells ml^{-1} [4] .**

Preparation and concentration of culture filtrate

Candida **cells (2 * 10^7 cells) was inoculated into 200 ml of medium A and grown at 37 ^{o}C for 10 h with shaking . After removing cells at the stationary phase by centerfugation at 1500 g**

for 10 min , the supernatant was collected by filtration (0.45 µm pore diameter filter) and concentrated approximately 10 ml in freez dryer for assay of phospholipase activity

Enzyme assay :

Phospholipase activity was assayed using lecithin (phosphatidylcholin) [10] , The standerd incubation mixture for determination phospholipase activity contained lecithin (10 n mole) and 0.5 ml of enzyme fraction made up to a final volume of 1 ml with 0.1 M sodium citrate buffer (pH= 4) Incubation was usually carrid out at 37 ^{o}C for 30 min. After stopping the reaction by adding 2 ml of chloroform-methanol solution (1 : 2 v : v) , reaction products were extracted by the procedure of Bligh & Dyer [1] , To analyses the reaction products , the extracted was evaporated and the residue was redissolved in an appropriate volume of chlorofom-methanol (6 :1 , v : v) .

A sample of the solution was applied to a silica gel thin layer plate and developed with chloroform-methanol-water (65 : 25 : 4) (silica gel 60 : Merck , Darmstade , Germany) standard material of some fatty acid such as palmetic , Linolic , oleic and stearic was used.

Kinetic characteristics of enzyme activities

To examine the kinetic characteristics of the phospholipase activity as a function of substrate concentration , pH , incubation time and enzyme concentration , Incubation were performed in a shaking water bath at 37 ^{o}C for 30 min.

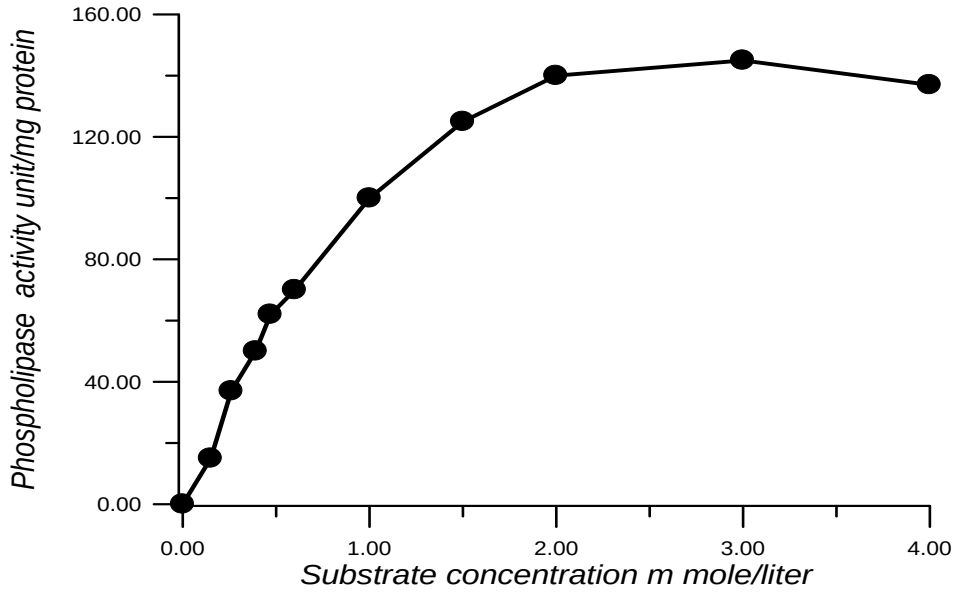

Fig.(2) phospholipase activity as a function of substrate concentration

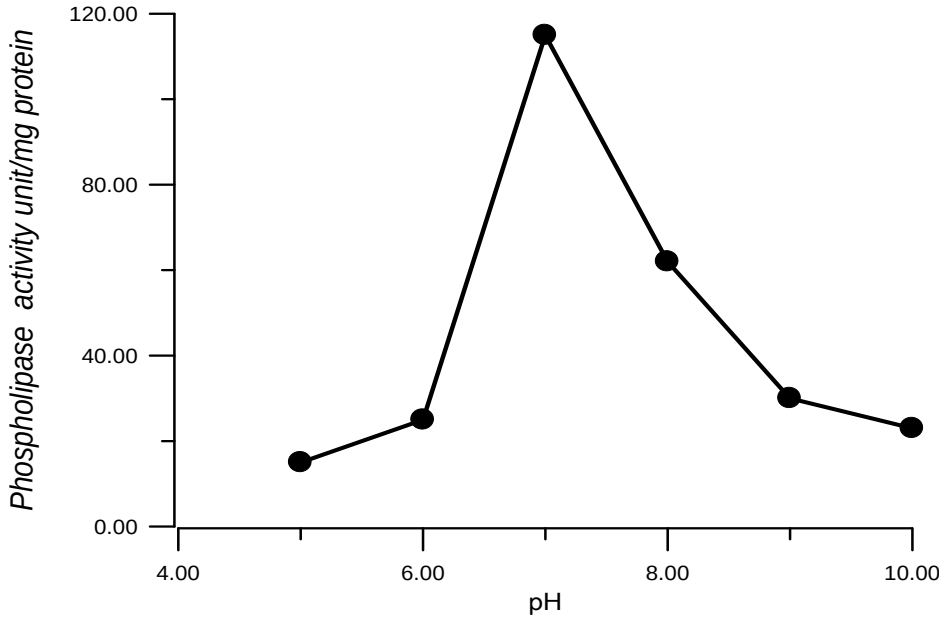

Fig.(3) phospholipase activity as a function of pH

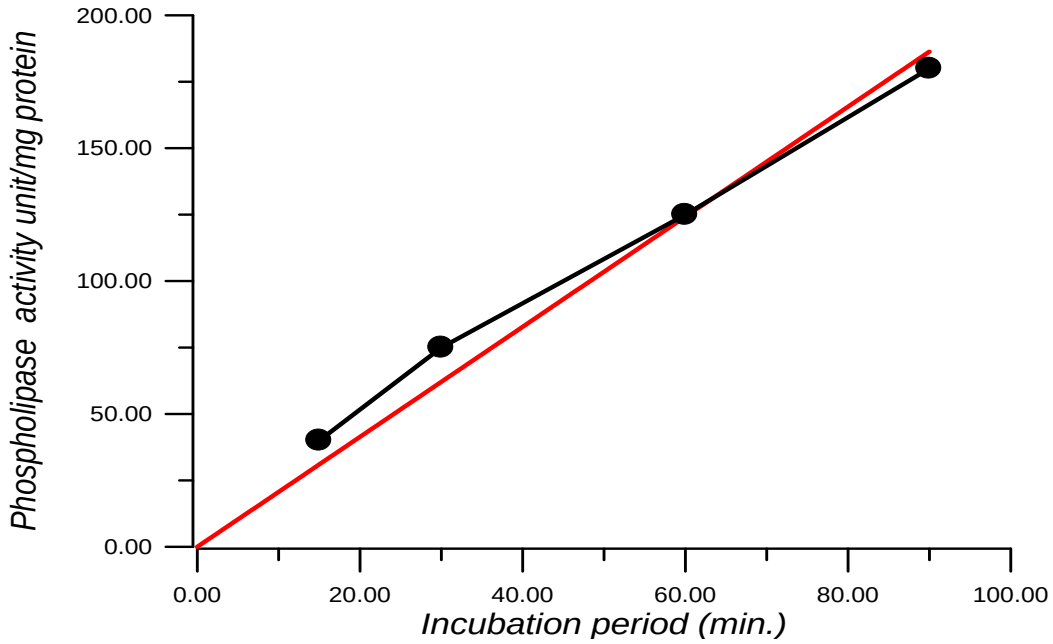

Fig.(4) phospholipase activity as a function of Incubation time

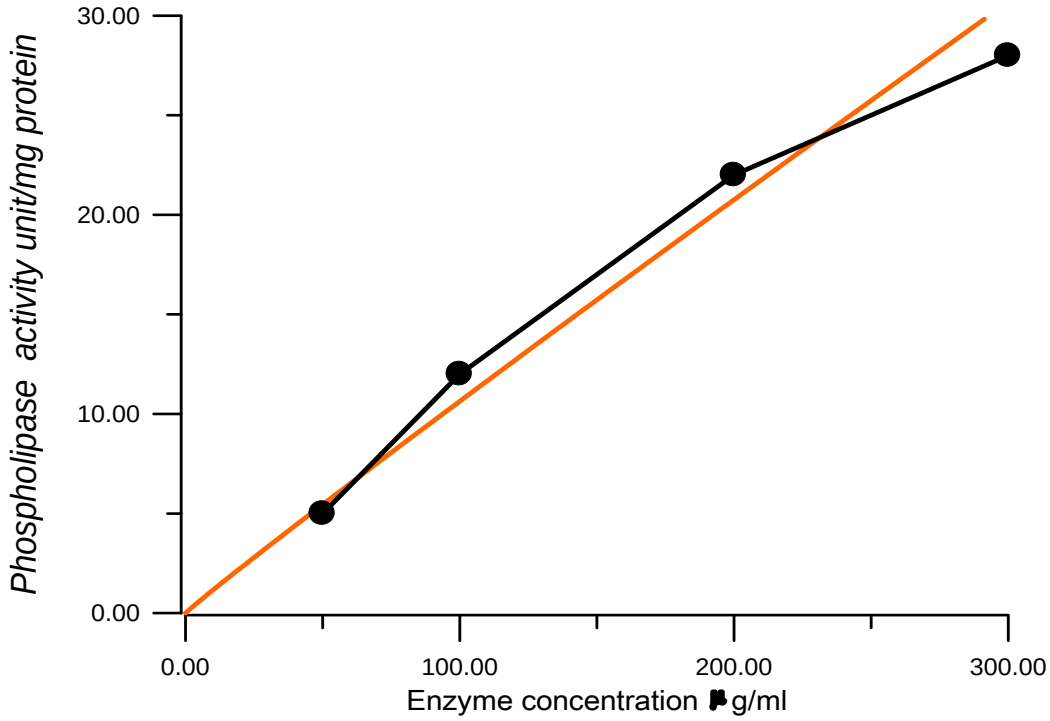

Fig.(5) phospholipase activity as a function of enzyme concentration

Results & Discussions

Identification of phospholipase activity :-

The lipid soluble products from phosphatidyl cholin breakdown by phospholipase were analyzed , nonesterified fatty acids are detected by TLC technique . Such as palmetic acid by comparism of the result with standard fatty acids find that extracted products contain the same value of (R_f) for palmetic acid that agrees with the result of sharon *et al* [8].

Fig.(2) illusterates the activity of phospholipase as a function of substrate concentration the amount of substrate was not rate limiting at the concentraction (2 mmol/l) used in this assay

Enzyme activity increases until it reaches to 2 m mole /liter concentration when the enzyme is being saturated with substrate and enzyme activity is nearly stable . There is no any

effect whatever increasing in concentration , the case which agrees with many results detected by a lot of researchers ; such as Vidotto *et al* [9].

Fig.(3) explains effects of pH values as a function of phospholipase activity , we noticed that optimum pH for activity was 7 , which means that this enzyme is effective in a neutral medium and decreases in alkali medium , this disagrees with results of some researchers on the same enzymes, Sharon and others [8] explained that optimum pH for phospholipase extracted from *cryptococcus neoformance* was 4 which means that this medium is week acids indicating different pH for enzyme activity according to the difference of its source.

Fig.(4) shows the effect of incubation period on enzyme activity , in other terms , the activity's increasing is proportionally parallel with the period from (30-90) mint, and the more time is increasing , the more chances are there for enzyme reaction with substrate , which this comes also to agree to the same result in [3].

Finally , Fig.(5) explains the effect of enzyme concentration on its activity , it is clear that the activity is increasing in parallel with enzyme concentration's increasing with stability of the concentration of substrate , Serve and others [7] Detected that the rate of reaction is proportionally parallel with enzyme concentration within a wide range when a stable concentration of substrate is used with much quantity unneeded by enzymes , we can use this relation to measure a certain value in a tissue extracted or any biological liquid.

References

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