PURIFICATION AND PROPERTIES OF ASPARTYL PROTEINASE FROM *CANDIDA ALBICANS*

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Abstract

Background: Aspartly proteinase, EC.3.4.23 isolated from leukemic patients with candidermia.

Objective: Purification and properties of proteinase from Candida albicans.

Methods: The enzyme was purified from Candida albicans by Ion exchange chromatography and gel filtration Sephacryl S-200 column.

Results: Different strains of ***C.albicans*** isolated from leukemic patients with candidemia were used for production of proteinase enzyme. Wheat bran incorporated in Sabouraud s broth to increase production of proteinase by the organism. The specific activity of purified enzyme was 400 unit/mg, fold of 19.68, and a yield of 8.48 % .The molecular weight of enzyme is 57676 daltons when estimated by using Sephacryl S-200. Stability of proteinase enzyme and its activity at different pH and temperature were studied in details.

Conclusion: Consecutive elevation of the enzymes specific activity values with the respective steps of purification. A more virulent strains the more reproducible of proteinase enzyme

Keywords: Aspartyl proteinase ; C. *albicans'*. Purification; Sephacryl S-200.

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Introduction

The extracellular proteolytic activity is one of several hydrolytic enzymes produced by C. albicans[1]; aspartyl proteinase is one of these enzymes. Aspartyl proteinase plays an important role as a virulent factor[2]. The potential virulence factor of C. albicans have been studied by a number of investigators. The proteolytic activity is associated with a 42-45 KDa; acid carboxyl enzyme has broad substrate specificity. It is active in the range of ph 2.0-7.0 with pH optimum varying from 2.5 to 5 depending on the substrate[3].

The enzyme is secreted in vitro, when the organism is cultured in the presence of exogenous proteins, usually bovie serum albumin[3,4].

Different methods of purification of C. albicans proteinase enzyme were used such as DEAE-cellulose and Sephadex G75. These are used for removing the majority of contaminating mannproteins and other proteins[5].

Aspartyl proteinase, which appears to be glycoprotein consisting of single polypeptide chain with glutamine at the N-terminus. Its molecular weight is about 45KDa, and its isoelectro point is pi 4.6. At pH 5.0, the proteinase is stable at 45°C for at least 15 min[6].

Materials and methods

Strains of *C.albicans* enzyme prepared: isolated strains from leukemic patients admitted to different hospitals in Baghdad. Wheat bran/Sabouraud s broth (1:5) was used to culture ***C,albicans.*** Inoculated cultures were incubated at 37C for 72 hrs.

Cultures were harvested with 0. IM of KH2PC[4] buffer, pH 7.2 by centrifugation.

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The activity of enzyme was tested according to Murachi[7] Protein concentration was measured according to Biuret method[8].

Crude enzyme was precipitated with 0-50% ammonium sulphate saturated, centrifugation at 3000xg for 15 min. The enzymes activity and protein concentration were estimated from broth precipitate and supernatant. Further precipitation with 50-75% ammonium sulphate was done at the same protocol. Purification of the enzyme was done by Ion-exchange chromatography with CM-cellulose which packed in column (32xl.5cm), the column was equilibrated with 0.1 M acetate buffer pH 5.0 with a flow rate of 60 ml/hr. A 20 ml of previous dialyzed crude enzyme was loaded into the column carefully until passed the exchange. A 50 ml of O.I M acetate buffer pH 5.0 was added. A 200 ml of gradient of O.I M acetate buffer pH 8.0 to 0.5M of NaCI was passed through the exchange at a flow rate of 60 ml/hr. Fractions of 5 ml were collected and the absorbance was monitored at 280 nm. The activity of the enzyme and its concentrations were estimated.

Gel filtration chromatography was used for the enzyme purification. Sephacryl S-200 column (75x2.0cm) was packed. The column was equilibrated overnight with 0.1 M acetate buffer pH 5.0 with a flow rate of 60 ml/hr. The void volume of the column was calculated by using 2 ml of blue dextrane 2000 solution. The elution volume of the enzyme was calculated. The enzyme was carefully passed through the column which equilibrated with 0.IM acetate buffer pH 5.0 with a flow rate of 60 ml/hr. Fractions of 5ml were collected. The enzymes activity and protein concentration were estimated.

Further purification was done. To determined molecular weight of proteinase enzyme, different standard proteins were used. (Bovine serum albumin (67000); Ovalbumin (43000); Chymotrypsinogen A (25000) and Ribonuclease A, (13700) Daltons , applied through Sephacryl S-200 column, then eluted with 0.1 M acetate pH 5.0 . The V(/Vo ratio was calculated for each standard protein and for the proteinase enzyme, and then standardization was done, by plotting the elution volume (Ve) of each standard proteins to the void volume (Vo) of blue dextrane.

To determine proteinase enzyme stability at 5°C and -20°C , the enzyme was kept at these temperature with 0.1 M acetate buffer pH 5.0, its activity was determined consecutively according to Murachi[7]. The optimum pH of *C.albicans* proteinase enzyme was determined using different buffers with different pH (3.5-8.0). The optimum temperature for enzyme was incubated at different temperature between(20-45)°C for 10 minutes .

Results

The activity of proteinase enzyme in the crude was 122 unit/ml, protein concentration was 6mg/ml, and the specific activity was 20.33 unit/mg (Table 1). The activity of the enzyme in the precipitate with 0-50 % saturated ammonium sulphate was 4 unit/ml; the protein concentration was 5mg/ml, while this of the supernatant was 60 unit/ml.

Table 1: Purification of protemase enzyme from C. *albicans.*

Step	Volume (ml)	Activity (unit/ml)	Protein (mg/ml)	Total activity (units)	Specific activity(unit/mg protein)	Fold	Yield %
Crude	145	122	6	17690	20.33	1	100
Precipitation by ammonium sulphate 50-75%)	20	425	2	8500	212.5	10.45	48.04
Ion exchange (CM-cellulose)	35	182	0.75	6370	242.6	11.94	36
Gel-filtration by Sephacryl S-100							
First step	20	160	0.45	3200	355.55	17.49	18.08
Second step	15	100	0.25	1500	400	19.68	8.48

In the second step of precipitation with 50-75% saturated ammonium sulphate, the activity of the enzyme was 104 unit/ml, while its activity in the dissolved precipitate was 425 unit/ml, protein concentration was 2mg/ml, and the specific activity was 212.5 unit/mg.

In the first step of purification (Ion-exchange chromatography), the activity of the enzyme was occurred in tube (4-7) which was 182 unit/ml, protein concentration was 0.75 mg/ml and the specific activity of 242.66 unit/mg. There was no enzymes activity in tubes no. (40-43) as shown in figure 1.

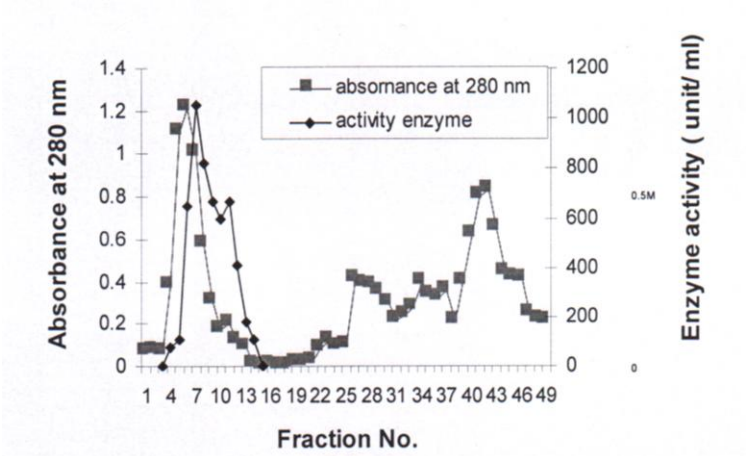

Figure 1. Purification of *C. albicans* proteinase enzyme with CM-cellulose column (32x 1.5 cm) washing with (50) ml of 0.1 M acetate buffer pH 5.0 and eluted with (200) ml of a gradient of 0.1 M acetate buffer pH 5.0 to 0.5M NaCI.

In the second step of purification (Gel filtration chromatography), the activity of the enzyme was occurred in tube no.(49-53) which was 160 unit/ml, the protein concentration was 0.45 mg/ml and the specific activity was 355.55 unit/mg. As shown in Figure 2.

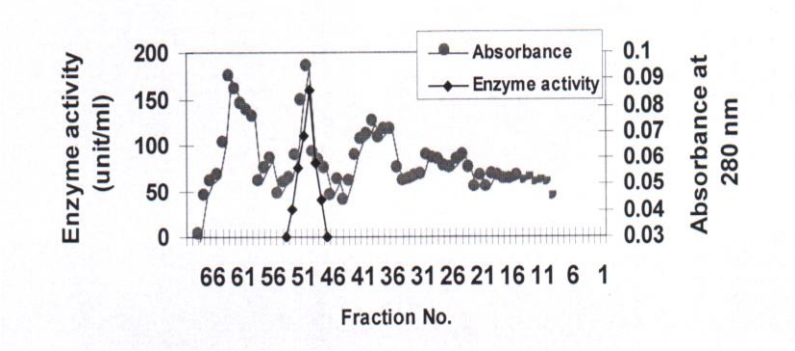

Figure 2: Purification of C. albicans proteinase enzyme with Sephacryl S-200 column (75 x 2.0 cm), eluted with 0.1 M acetate buffer pH 5.0, at a flow rate of 60 ml/hour (gel filtration chromatography-first step)

Further purification of proteinase enzyme with gel filtration chromatography was done; the activity of the enzyme was occurred in tube no.(49-52) which was 100 unit/ml, the protein concentration was 0.25 mg/ml; the specific activity was 400 unit/mg, with a fold of 19.68 and a yield of 8.48 % as shown in Figure 3.

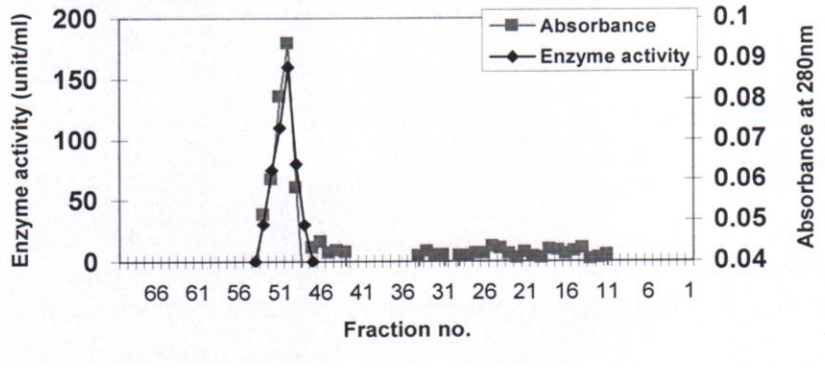

Figure 3: Purification of C. albicans proteinase enzyme with Sephacryl S-200 column (75 x 2.0 cm), eluted with 0.1 M acetate buffer pH 5.0, at a flow rate of 60 ml/hour (gel filtration chromatography-second step)

Figure 4 shows the determination of the molecular weight of proteinase enzyme against the standard proteins used for such purpose, and the molecular weight was 57676 daltons.

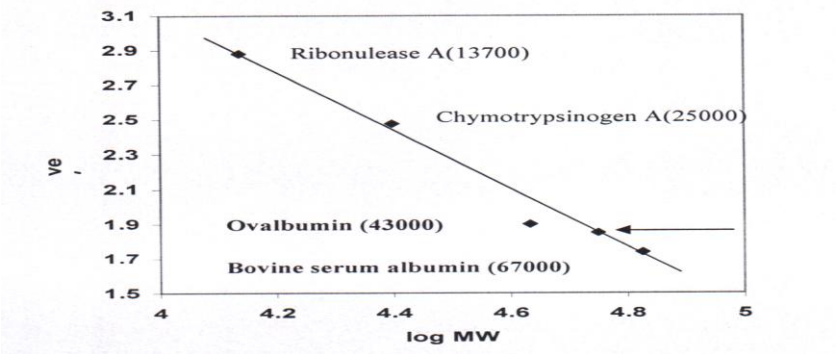

Figure 4: The molecular weight of C. albicans proteinase enzyme by gel filtration, using Sephacryl S-200 (75 x 2.0 cm)

The stability of proteinase enzyme at each of 5°C (refrigerator) and -20°C (freezer) was studied, as shown in Figure 5. The remaining activity of the enzyme at 5°C at the first day of purification was 100%; 90% and the activity was decreased to reach 9% at the eighth day. But at the same time the remaining activity of the enzyme at -20oC at the first day was 100% and reached 50% after 25 days.

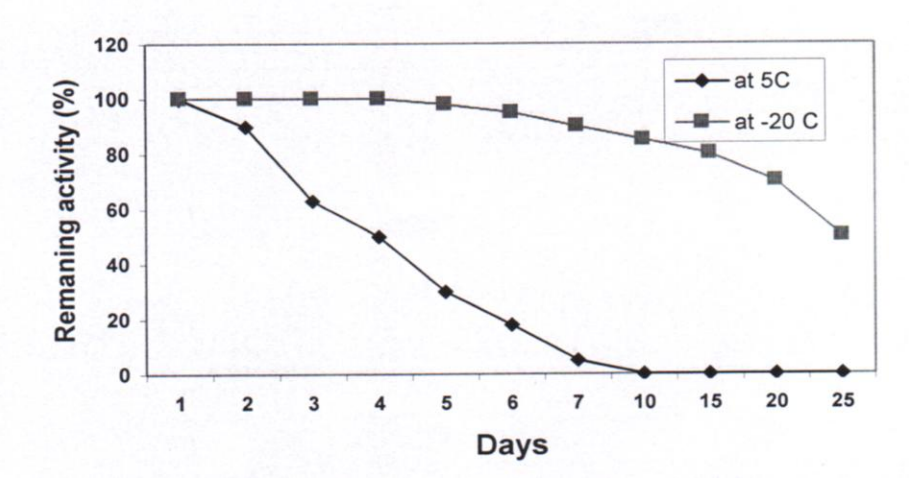

Figure 5: The stability of C. albicans proteinase enzyme at different temperature

The optimum pH of enzyme was determined, as shown in Figure 6, whereas the highest enzyme's activity were occurred in the pH (5-5.5). which were 100 and 92 unit /ml. And the enzyme lost its activity at pH (7-8) .

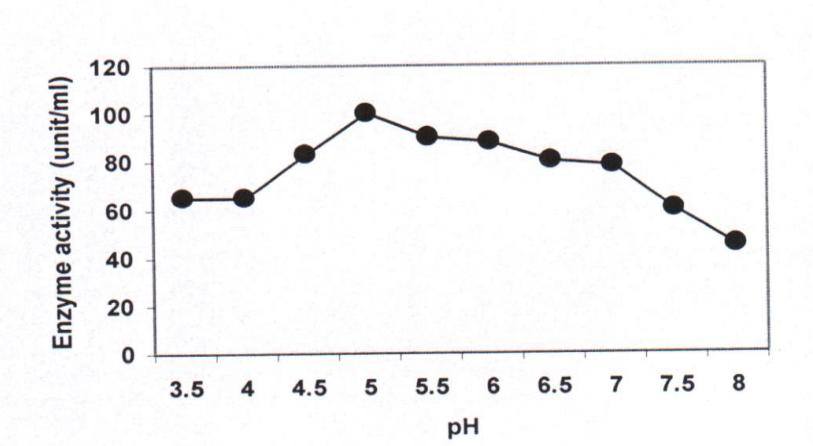

Figure 6: The optimum pH of C. albicans proteinase enzyme

Figure 7 shows the optimum temperature of proteinase enzyme was noticed at 35°C which was gave 100 unit/ml, while the lowest one was at 20°C which was 40 unit/ml.

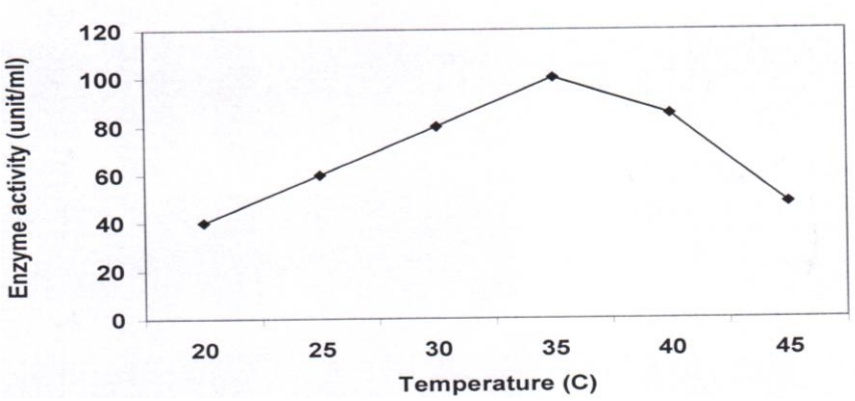

Figure 7: The optimum temperature of C. albicans proteinase enzyme

Stability of *C. albicans* proteinase enzyme according to the pH of substrate was determined , the results are shown in Figure 8, and the highest activity was seen at pH (4.5-5.0), which were (93-100)unit/ml, while the lowest ones were seen at pH (7.0-8.0) which were (60,43 and 23) unit/ml respectively.

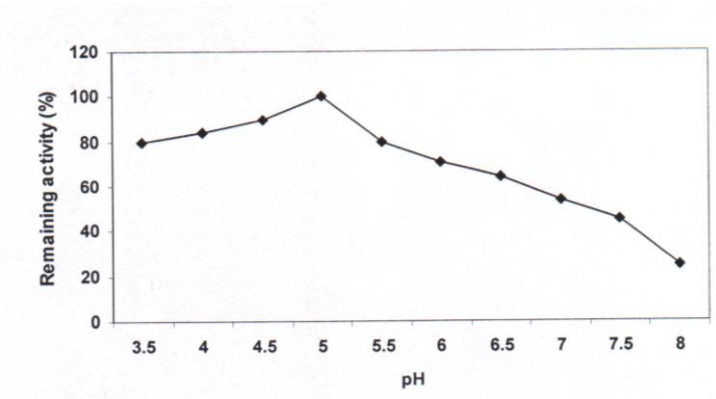

Figure 8: Determination of stability of C. albicans proteinase enzyme according to the pH of the substrate

Discussion

Different steps of purification of proteinase enzyme were used in this study: starting from crude enzyme with a specific activity of 20.33 unit/mg. In the second step which was the precipitation with ammonium sulphate (50-75 %) the specific activity was 212.50 unit/mg.

In the third step of purification with Ion-exchange chromatography the specific activity of the enzyme was 242.66 unit/mg. In the fourth step of purification which was Gel-filtration chromatography the specific activity of the enzyme was 355.55 unit/mg, while the last step of purification which was the further purification by Gel-filtration chromatography the specific activity of the enzyme was 400 unit/mg, fold of 19.68 and a yield of 8.48 % . From these results we can see the consecutive elevation of the enzymes specific activity values with the respective steps of purification.

These results disagree with that Morrison et al[5] who obtained pure enzyme

from *C.albicans*, with a specific activity of 174.9 unit/mg with a yield of 1.29 % by using different strains in both studies. Virulent strains of *C. albicans* isolated from leukemic patients were used in this study, while those used by Morrison et al[5] were standard strains carries no. AI15. This means the more virulent strains the more reproducible of proteinase enzyme[9,10].

The molecular weight of the enzyme was 57.676 Dalton and this results disagree with those obtained by other authors[4,6,11] which were ranged between 42000-45000 dalton , the differences in both results may come from: the variability among strains studied, and the techniques applied for determination of the molecular weight of the enzymes gel-filtration chromatography was used in this study, while they applied SDS-polyacrylamide gel electrophoresis for this purpose.

The optimum pH of C. *albicans* proteinase enzyme was 5.0, and this disagree with Yamamoto et al[11] who determined the optimum pH of proteinase enzyme 3.2. The optimum temperature of *C.albicans* proteinase enzyme was 35°C, and this is consistent with Bromelani[7].

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