

## Study of some hormones and Partial purification of prolidase from serum of women with polycystic ovary syndrome

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### ABSTRACT

This study was done by partially purification of prolidase from serum of patients with polycystic ovary syndrome by Gel filtration technique, and using sephadex G100 gel as a stationary phase. The degree of purification (15.1) fold, enzyme yield (95.5%) and specific activity (0.00176 IU/I), were carried out. Kinetics studies for the partial purified enzyme technique showed optimal concentration of substrate which was 5 mmol/l  $K_m = 0.66ng$  and  $V_{max} = 0.80$  mM, while optimum Temperature was (35°C) and optimum pH was (8). The molecular weight of the partial purified enzyme has been determined by gel electrophoresis method, in presence of polyacrylamide gel and sodium dodecyl sulphate (SDS\_PAGE) which showed that the approximate molecular weight was (54KD), we found high level of prolactin in the patient with polycystic ovary syndrome which was (24.03) when in the control was (10.09), the value of TSH in the patient was (17.08) which is high value and in the control was (1.49), the value of T4 in the patient was (100.2) and in control was (118.4), the value of T3 in the patient was (0.3) and in control was (1.3). Testosterone in patient was (0.391) and in control was (0.206).

### Introduction

Poly Cystic ovary syndrome (PCOS) is the most common endocrine disorder amongst women of reproductive age and is associated with various metabolic perturbations and There is strong evidence that it classifies a genetic disease that affects about 10-15% of women [1], this syndrome occur with high prevalence. it is a multifactorial, heterogeneous, complex genetic, endocrine, diagnostically characterized by chronic anovulation, poly cystic ovaries and biochemical and clinical manifestations of hyper androgenism [2], PCOS is correlation with insulin resistance (IR) [3], The etiology of PCOS is unclear and decisive clinical studies are limited by ethical and logistic constraints. there is a need for early diagnosis and treatment that can help to relieve the symptoms and prevent health related problems [4].

Many women have the risk of diabetes and possibly cardiovascular disease and breast cancer with polycystic ovary syndrome [5]. The obesity or over weight effect most of the patients with PCOS, suggesting adipose tissue dysfunction [6], PCOS primarily characterized by: (1) Menstrual dysfunction, (2) cutaneous signs of

hyperandrogenism, (3) obesity, (4) disordered gonadotropin (LH) and (FSH) thyroid dysfunction(5), hyperprolactinemia(6) [7,8] Clinical practice in the assessment and management of PCOS is inconsistent, with key evidence practice gaps, whilst women internationally have highlighted delayed diagnosis and dissatisfaction with care [9,10] suggesting developmental aspect to its etiology. Human prolidase (EC.2.4.13.9) or proline dipeptides, in proline or hydroxyproline residue is located at the C-terminal position [11] The enzyme apparently contributes to the conservation of amino acids from endogenous and exogenous protein sources mainly collagen, the enzyme plays an important role in the recycling of proline for collagen synthesis and cell growth, prolidase gene (PEPE) is located in chromosome 19 and encodes a polypeptide of 493 amino acid [12]. prolidase activity was found in various organs, such as heart, brain, thymus, kidney, lungs, spleen, plasma, leukocytes, erythrocytes, and dermal fibroblasts, Enzyme replacement therapy using recombinant prolidase has been considered as a possible treatment for prolidase deficit. prolidase have many applications and have been investigated

not only as a possible treatment for Skin infections, but also as a part of anti-cancer strategies and in the dairy industry [13].

#### - Experimental

- **Collection of sample:** the number of the samples from the patients were (60), which that collected from polycystic ovary syndrome patients. blood was drawn from the vein using 5ml plastic disposable syringe. The blood was placed in clean and free anticoagulant tubes, let coagulate at room temperature .The blood serum was then separated by centrifuge at a velocity of 3000 G for 15 minutes to ensure adequate serum red blood cell extraction .The effectiveness Enzyme was measured directly and the study done invitro .

- **Diagnosis test:** Hormones include (Tryodothyronine(T3), Thyroxine(T4), Thyroid-stimulating hormone (TSH), Testosterone (T.)) were measured by using (Kit Monobind \_U.S.A)[14] and PRL and Total protein are measured used (Kit Biomerieux Franc)[15] in serum. prolidas activity in serum was measured by using Elisa Kit supplied from Cloud clon company –USA[16].

#### - separation and purification of Prolidase from serum of polycystic ovary syndrome patients

Prolidase was purified from the serum of women with polycystic ovary syndrome using the following steps:1-Addition of ammonium sulphate (80%) 2-Dialysis 3-Gel Filtration Chromatography (using Sephadex G100). The basic principle is to equalize the charges on the surface of the protein ( enzyme) , degradation of the water layer surrounding the protein and reduce the degree of watering, the Molecular weight of the enzyme determined by polyacrylamide gel electrophoresis (PAGE), describes a technique widely , forensics, genetics molecular biology .(SDS-PAGE is a technique) based on ability of protein to move within an electrical current that used for separation which is a function of the length of there polypeptide chain or of there molecular weight. this is achieved by adding sodium dodecyl sulphate (SDS) detergent to remove secondary and tertiary protein structures and to maintain the proteins as

polypeptide chains. the SDS coats the protein, mostly proportional o their molecular weight

#### - Kinetics of Prolidas.

The Kinetic study of Prolidase included:

1-Effect of the substrate concentration: by using different concentration of substrate (0.5,1, 2,3,4,5) mmol/l

2-Effect of pH: The pH effect of the prolidase reaction. Different pH solution (4.5 , 5.5 , 6.5 , 7.5 , 8.5 . 9.5 ) .

3-Effect of temperature: using to measure the effectiveness of Prolidase . The reaction was conducted at different temperatures (35,40,50,60,70,80)C°.

#### -Sodium dodecyl sulfate – polyacrylamide gel electrophoresis to Measurement molecular weight of purified Enzyme (SDS –PAGE)

Followed the way to the researcher leamli [19] to prepare polyacrylamide gel with some modification .

#### -Results and Discussion

##### -Determination the Molecular Weight of Prolidase Enzyme by Electrophoresis on Polyacrylamide Gel (SDS-PAGE) SDS

Determination of molecular weight of the enzyme by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE). This enzyme with SDS to break down the protein giving chains of variable sizes surrounded by molecules of SDS negative charge where they remove the original charge of the protein and these chains removed by electrical migration based on the molecular weights of the molecule. Surrounded by SDS molecules, they moved towards the positive electrode based on the charge-to-mass ratio and when migration is done on polyacrylamide gel using SDS It moves towards the positive electrode based on charge-to-mass ratio and when migration to polyacrylamide gel using SDS it also moves based on its size [27]. The movement of proteins as mentioned above depends on several factors, including molecular weight, by determining other factors such as charge and electric current.

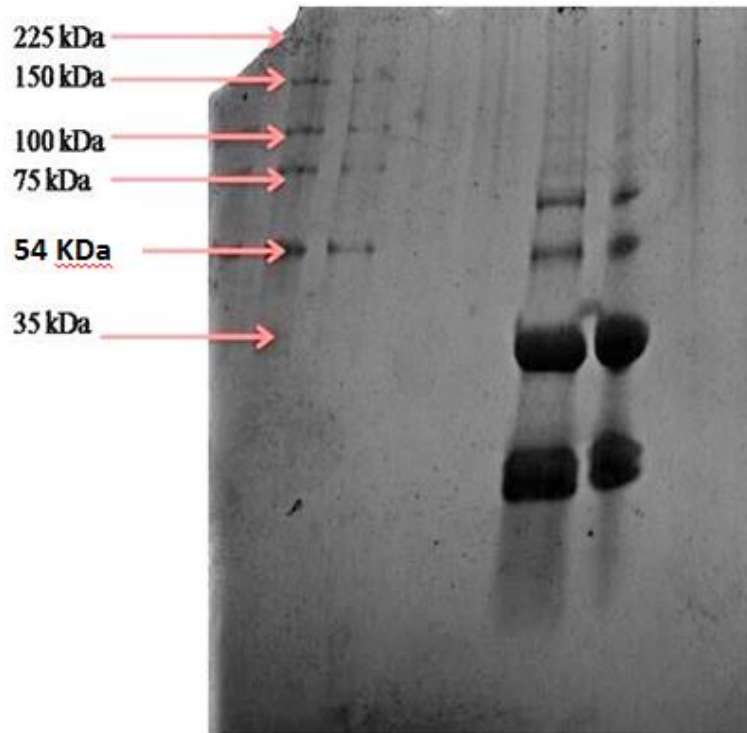


Fig. 1: electrophoresis of prolidase to determined molecular Wight compared with standard protein

In table (1) showed the concentration of hormones (T3,T4,TSH,T.) for the patient of polycystic ovary syndrome comparison with control ,When the Average account by prolactin for the patient was(24.03ng/ml) and the control was (10.09ng/ml).

Table (1) The value of ( PRL,T3,T4,TSH ,T.) in patient with poly cystic ovary syndrome

Groups	T. ns mg/dl	TSH ** mmol/l	T4 * mmol/l	T3 * nmol/l	Prolactin ** ng/ml
Patient N=60	0.391 ±0.097	17.08 ±4.15	100.2 ±22.5	0.3 ±0.062	24.03 ±4.25
Control N=60	0.206 ±0.051	1.49 ±0.261	118.4 ±26.3	1.3 ±0.315	10.09 ±2.24
P value	0. 2	0.0029	0.0977	0.0272	0.004

\* Significant P<0.05

\*\* Highly significant p<0.01

ns Non significant

we found high level of prolactin in the patient with polycystic ovary syndrome which was Highly significant P<0.01 the high value of this hormones can be accuse of interruption of menstruation [17], the highly significant of TSH and low value of T3 and T4 may be happen because of the low thyroid function this causes the pituitary gland to secrete TSH [18] and The hypothalamus at the base of the brain produces the thyronin-releasing hormone (TRH), which in turn stimulates the pituitary gland to produce thyroid stimulating hormone (TSH) and the hypothalamus produces somatostatin from the

pituitary gland which has the opposite effect on the production of (TSH) and reducing its secretion[21], Symptoms associated with hypothyroidism include irregular menstrual dysfunction due to ovulation problems and prolactin hormone levels with an increased risk of PCOS[22].

and testosterone hormone was no moral difference between the patient and control It can be explained that there are no significant differences between healthy women with polycystic ovary syndrome because they have been taking treatment with metformin pills for a period of not less than three months, which indicates their response to treatment and thus reduce the difference in the level of this hormone between women infected and control[23] addition, the absence of significant differences between the two groups can be explained by a positive correlation between the mRNA levels of the androgen receptor and FSH receptors[24] [25] .

**-partially purification of prolidase from serum of polycystic ovary syndrome patients**

From our study we found that the degree of purification of prolidase was (3.30) folds and yield of enzyme (85.1), specific activity 0.0024 IU/mg then we can showing the result in table (2) the steps of purification was complete by using gel filtration using Sephadex G100 which showed a single peak in fraction five when the fold was 15.1 and the enzyme yield was (95.5%) while specific activity was (0.00176) IU/mg) .

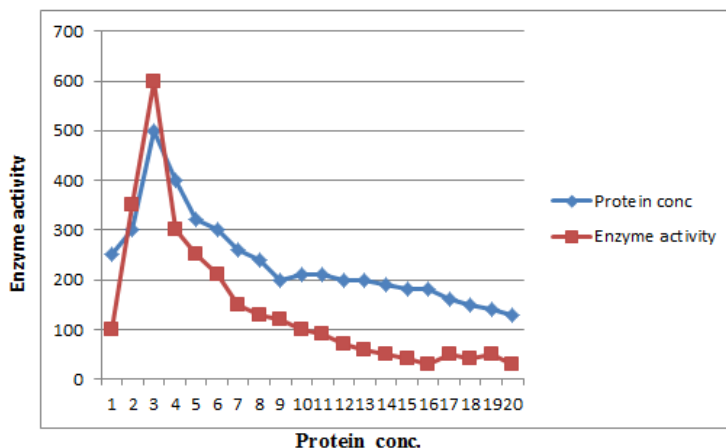


Fig. 2: purification of prolidase with gel filtration on sephadex G-100 (elution curve)

table (2) steps of purifications of prolidase

Steps of purification	Volume ml	Activity IU/l	Total activity	Protein conc mg/ml	Specific Activity IU/l	Yield %	Folds	Total Protein Mg
Crude serum	5	136.32	0.681	46.2	0.0029	100	1	171.5
Ammonium sulphate	4.5	100.47	0.45	32.0	0.00165	73.6	1.49	137.2
Dialysis	5	81.01	0.405	10.1	0.0024	85.1	3.30	50.8
Gel filtration	5	55.11	0.275	2.44	0.00176	95.5	15.1	16.65

3.3. Kinetic study of partially purified prolidase

- Concentration of substrate

The optimum concentration of the substrate was 5mmol/l and the constant value of the Michaels –

menten of substrate was (0.66mM) and the maximum velocity was (0.80mM) when the other studies showed the Km for the Patient was 2.90+/-0.22 and 2.88+/-0.27mM and the Vax value was 6.02 U/mg.

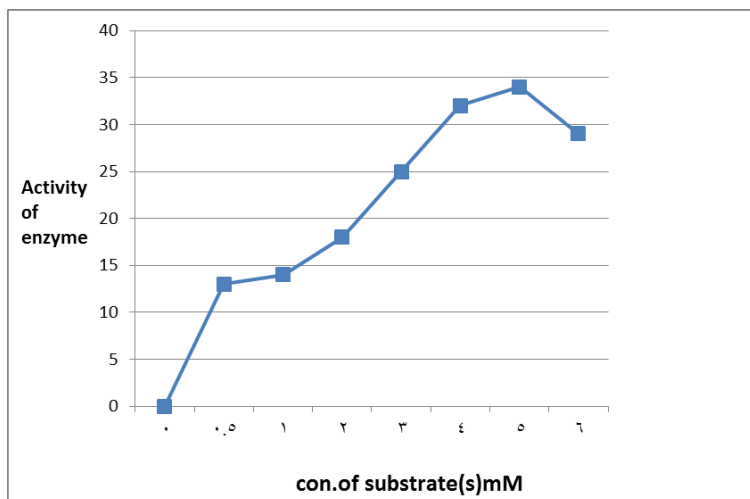


Fig. 3: effect of Concentration of substrate on the activity of prolidase

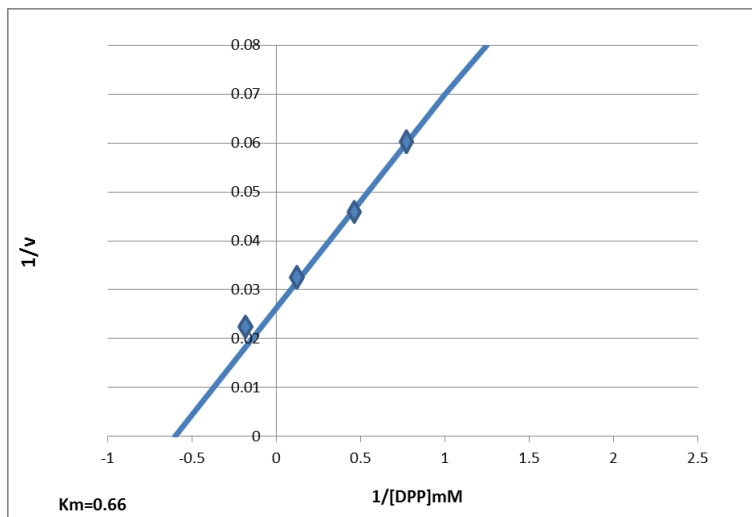


Fig. 4: Kinetic study of prolidase enzyme .

**-Effect of temperature**

The optimum temperature of the enzyme activity was 35C°, the results varied with the other studies may be happened because of partial glycyyl proline hydrolysis

at higher temperatures the effect of several divalent cations on prolidase activity. This modified assay is agree with study that done by Mr. wilk and Mr. Kalms (2017) for the optimum temperature [20].

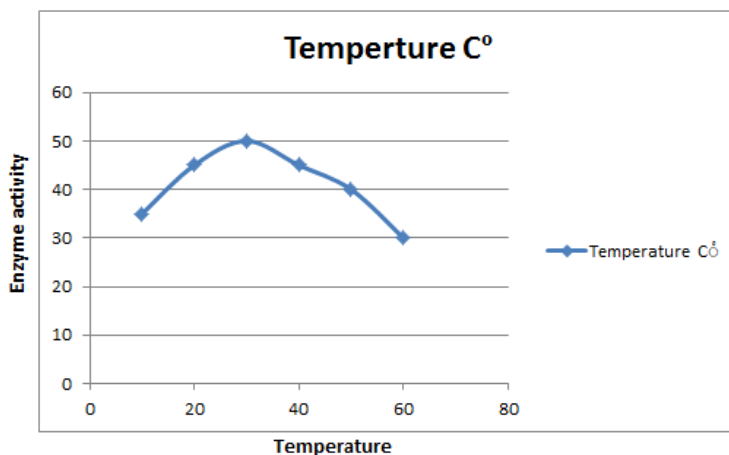


Fig. 5: effect of Temperature on Prolidase activity

**-Effect of pH**

The optimal pH of prolidase was (8.0) that found as a result of study as showing in figure (6). In this pH the glycyyl -l-proline and glycyylhydroxy -l-proline for preparation of very high activity. The result of pH

varied with other studies ,the optimal pH for erythrocyte prolidase is also at pH 8.0 [26] we are indebted to the study that done by Mr. wilk and Mr . Kalms (2017)[19] for the optimum pH by Prolidase.

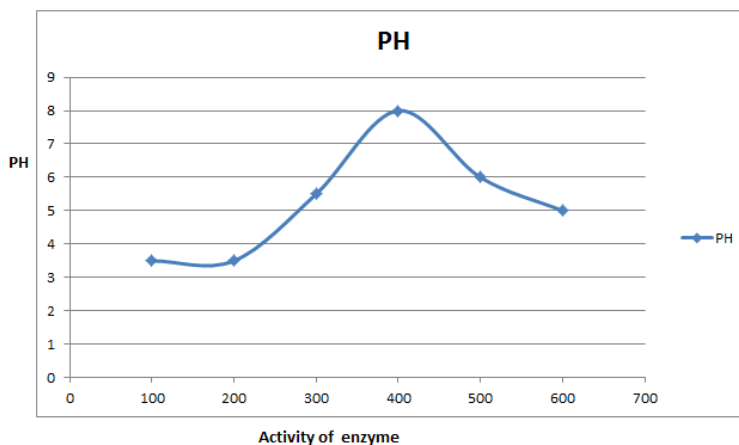


Fig. 6: effect of pH on prolidase activity

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## دراسة هرمونية وتنقية جزيئية لأنزيم البرولايديز من مصلى المصابات بمتلازمة تكيس المبايض و دراسة خواصه الحركية ووزنه الجزيئي

ريمي مروان محمد صالح ، فراس طاهر ماهر ، نغم قاسم كاظم

قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

### المخلص

اجريت الدراسة الحالية لتنقية انزيم البرولايديز جزيئياً من مصلى المصابات بمتلازمة تكيس المبايض باستخدام تقنية الترشيح الهلامي وباستخدام جل السفادكس G100 حيث بلغت درجة التنقية 15.1 والفعالية النوعية 0.00176 IU/I وحصيلتها انزيمية 95.5% واجريت الدراسة الحركية للانزيم المنقى جزيئياً حيث كان تركيز المادة الأساس الأمثل 5mmol/l وبلغت قيمة ثابت ميكالس منتن 0.66 والسرعة القصوى 0.80 mM بينما الدرجة الحرارة المثلى لعمل الأنزيم هي 35°C والأس الهيدروجيني الأمثل هو pH(8). تم تقدير الوزن الجزيئي للانزيم المنقى من المرضى بتقنية الترحيل الكهربائي على هلام متعدد الأكريل امايد بوجود كبريتات دوديكايل الصوديوم (SDS\_PAGE) حيث بلغ الوزن الجزيئي للانزيم (54) KD. وبالنسبة للدراسة الهرمونية فقد وجدنا مستويات عالية من هرمون البرولاكتين لدى النساء المصابات بمتلازمة تكيس المبايض حيث بلغ مستواه (24.03) بينما بلغ مستواه في مجموعة السيطرة (10.09) وبالنسبة للهرمون المحفز للغدة الدرقية بلغت قيمته في النساء المصابات (17.08) وفي السيطرة (1.49) اما هرمون الثايروكسين بلغت قيمته في النساء المصابات (100.2) وفي السيطرة (118.4) وبالنسبة لهرمون ثلاثي يودوثايرونين يبلغ تركيزه في النساء المصابات (0.3) وفي السيطرة بلغ (1.3) اما عن هرمون التستوستيرون فقد بلغ مستواه في النساء المصابات (0.391) وفي مجموعة السيطرة يبلغ (0.206).