Isolation and Purification of Acid Phosphatase from Human Seminal Fluid

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Abstract

The activity of acid phosphatase was measured in human seminal fluid of 25 healthy volunteers with age range (25-37) year. The results showed that the activity of the enzyme was (22.3±8.6)IU/L. ACP has been purified efficiently from human seminal fluid. The ACP purified, 3.83 fold, by different purification steps and methods, ammonium sulfate precipitation, DEAE-cellulose, ion-exchange chromatography and gel filtration chromatography (sephadex G-100). A kinetic study of the purified ACP was carried out, and it was found that the relation between disodium phenyl phosphate as a substrate and velocity of purified ACP was hyperbolic and obeyed Michaelis-Menton equation. Optimal purified enzyme concentration of disodium phenyl phosphate as a substrate was 8mM/L, while the optimal temperature and pH at which maxamium velocity obtained were 37ºC and 4.9 respectively.

Introduction

Phosphatases with optimal activity below pH of 6.0 are collectively referred to as Acid Phosphatases-ACP (E.C.3.1.3.2). ACP are a group of enzymes which catalyze hydrolysis of phosphatemonoesters with acid pH [1]. The ACP activity is present in the liver [2], plecenta [1] spleen [3], milk [4], erythrocytes, platelets [5], bone marrow [6], prostate gland [7] and seminal fluid [8] in high concentration. Normally ACP secreted from the prostatic epithelial cells into the seminal fluid, but in patients with prostate cancer they leak it into the circulation, were they can be detected as a marker for prostatic carcinoma[9].

Determination of the prostatic fraction of ACP is of clinical interest as a biomarker in the detection, staging and monitoring of carcinoma of the prostate [10]. Elevations of ACP occur in approximately 60% of men with prostate cancer, while the highest levels are encountered in patients with metastic bone involvement [11]. ACP produced in the prostate is inhibited by tartrate, a property which is useful in differentiating it from isoenzyme fractions originating elsewhere in the body [12]. More moderate elevations of ACP occur in Paget's, Gaucher's[13] and Niemann-Pick diseases, forms of hyperparathyroidism, assorted malignant cancers with bone involvement (including female breast cancer), and various hematological disorders. Additionally [14, 15], as ACP is found in very high concentrations in semen, its determination is useful in forensic medicine applications and research [8].

Usually Acid phosphatase purified from serum or an extract of prostatic tissue, but in this study deals with a purification procedure of these markers from human seminal fluid instead of prostatic tissue.

Material and Methods

Human seminal fluid has been collected from 25 healthy male. All the procedures were carried out at 4ºC. The specimen was centrifuged at 15,000 rpm for 30 min and and the supernatant was precipitate with 50% saturated with solid ammonium sulfate and centrifuged. The ACP derived from the supernatant.

I. Activity of ACP

The ACP activity of the seminal fluid eluted was determined colorimetrically by Kind and King Method [16].

I.I. Purification

The supernatant containing crude ACP was brougt to 80% saturation with ammonium sulfate and then centrifuged at 10,000 rpm for

30 min. The precipitate was dissolved in a minimal amount of 0.1 M acetate buffer(pH =5.0) containing 1mM of CaCl2, MgCl2 and MgCl2 as activater and 0.1M NaCl, dialyzed against the same buffer, and then centrifuged at 10,000 rpm for 30min.

The supernatant was applied to DEAE-cellulose column (2.3 X 50 cm) , pre-equilibrated with a 0.02M phosphate buffer(pH=7.0). The column was washed with the same buffer, followed by elution with a linear gradient of 0 to 0.5 M NaCl in a 0.02M phophate buffer (pH=7 to 6.0).The eluted achieed ACP was concentrated and further purificationwas done by passing it through a sephadex G-100 column (2.5 X 75 cm) using citrate buffer (pH=5) (0.01 M) as eluent..

The protein concentration was measured using Lowry et al method[17], and bovine serum albumin was used as a standard.The chromatographic elution profiles were determined by the absorbance at 280 nm [18].

I.2. Kinitic Studies of Isolated ACP and Partial Purification from Seminal Fluid

This study includes:

1- Effectitive concentration of substrate upon the activity of purified ACP.

Different concentrations of the substrate (disodium phenyl phosphate) (1,2,4,6,8,10,12,14)mM/L were used to study the relation between disodium phenyl phosphate and activity of purified ACP.

2- Effectitive temperature of incubation upon the activity of purified ACP

Different incubation temperatures (4, 10, 15, 20, 28, 33, 37, 39, 42, and 45) ºC were used to find the optimal temperature at which the enzyme have the maxamium velocity.

3- The effect of pH upon the activity of purified ACP.

Different buffer solutions (3.0, 4.0, 4.5, 5.0, 5.25, 5.5, 6.0, 6.5 pH) were used to find the optimal pH at which the enzyme have the maxamium velocity

Results Discussion

Acid phosphatase, normal component of the seminal fluid found in large quantities is secreted from the epithelial cells of prostatic glands. It has been reported that the purified ACP obtained from an extract of prostatic tissue was useful as diagnostic marker for prostatic cancer.we hae purified these markers were puriied from human seminal fluid as a new approuch instead of obtaing it from prostatic tissue.

The ctivity of acid phosphatase was measured in seminal fluid of healthy volunteers before the purification. The results showed that the activity of enzyme was (22.3±8.6)IU/L [Table 1], the results were good agreement with the results reported by of Pech et al, (19) , who found that the median enzyme activities of ACP was 30.00 IU/L.

Table 1
Activity of acid phosphatase in seminal fluid of healthy person

No. of specimen	Range of age (years)	ACP activity	
		Range	Mean±S.D
25	25-37	11.8-29.1	22.3±8.6

The elution profile of purified ACP obtained from human seminal fluid I shown in [fig.1]. This figure shows many peaks of protein and just one peak of ACP activity fractions (Tubes 52-58), which purified 3.68 fold. Fractions (Tubes 52-58) collected and concentrated and then used for further purification.

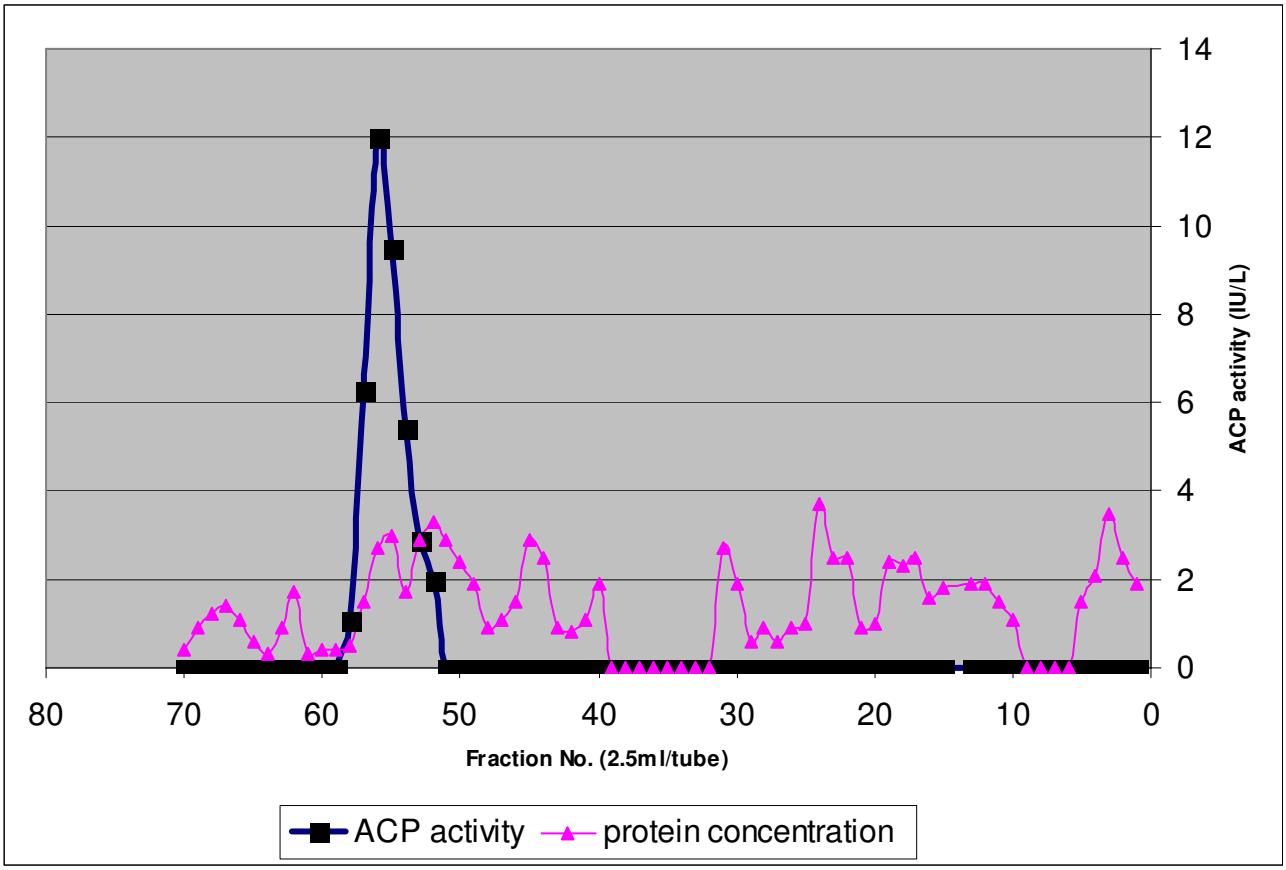

Fig. 1
DEAE-cellulose ion exchange column chromatography of ACP fraction after 80% saturation of ammonium sulfate precipitation .The column was equilibrated in 0.02M sodium phosphate pH=7.0 and elution was performed with alinear gradient of this buffer to 0.02M sodium phosphate pH=6.0 containing different concentration of NaCl (0.1-0.5)M.

The elution pattern of a sephadex G-100 column chromatography is shown in [Fig.2]. ACP activity was detected on the fractions (tubes 8-11), 6 peaks of protein almost overlapping with ACP activity were obtained by sephadex G-100 gel filtration.

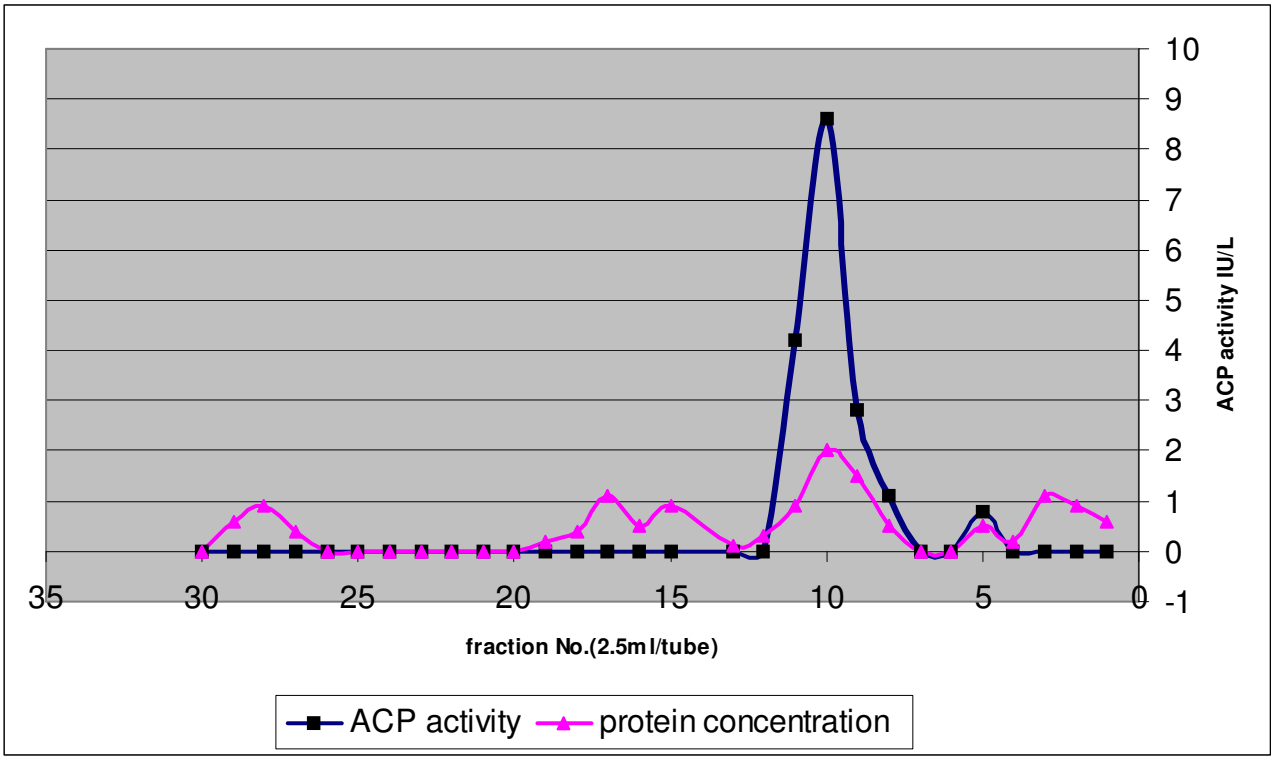

Fig. 2
Sephaex G-100 gel filtration column chromatography of ACP fraction after DEAE-cellulose chromatography. Elution was performed with 0.01M citrate buffer pH=6.0.

Acid phosphatase was successfully isolated and purified from human seminal fluid 3.83 fold [Table 2], by using different purification steps and methods ammonium sulfate precipitation, ion-exchange chromatography and gel filtration chromatography (sephadex G-100) . The degree of purification of ACP [Table 2] obtained by us was higher than that reported by Aoki at el. (8), purified the enzyme 3.2 fold from seminal fluid by using affinity chromatographic method.

Table 2

Purification degree of ACP purified from seminal fluid

Steps	Elute (ml)	Protein conc. (mg/dl)	ACP Activity (IU/L)	Specific activity (IU/L)/mg	Degree of purification (fold)
Crude seminal fluid	5	14.51	16.3	1.123	1
DEAE-cellulose	3.5	2.9	12	4.14	3.68
Sephadex G-100	2.5	2.0	8.6	4.3	3.83

Fig.3 shows the effect of substrate concentration (disodium phenyl phosphate) upon initial velocity of purified ACP. The relation between (disodium phenyl phosphate) and velocity for purified ACP was hyperbolic and obeyed Michaelis-Menton equation. While the purified ACP from liver tissue obeyed Hill equation (20) in which the liver enzyme form differ significantly in size and the composition of their carbohydrate components to prostatic isoenzyme (20).

The optimal concentration at which maximum velocity of purified ACP was 8mM/L, while the maximum velocity of total ACP in serum was 10mM/L (16) and 8mM/l to prostatic isoenzyme (21).

At relatively higher substrate concentration the velocity of the reaction declined with increasing substrate concentration.This indicates the binding of more than one substrate molecule at the active centere of the enzyme with substate formation of inactive substrate enzyme complex (22).

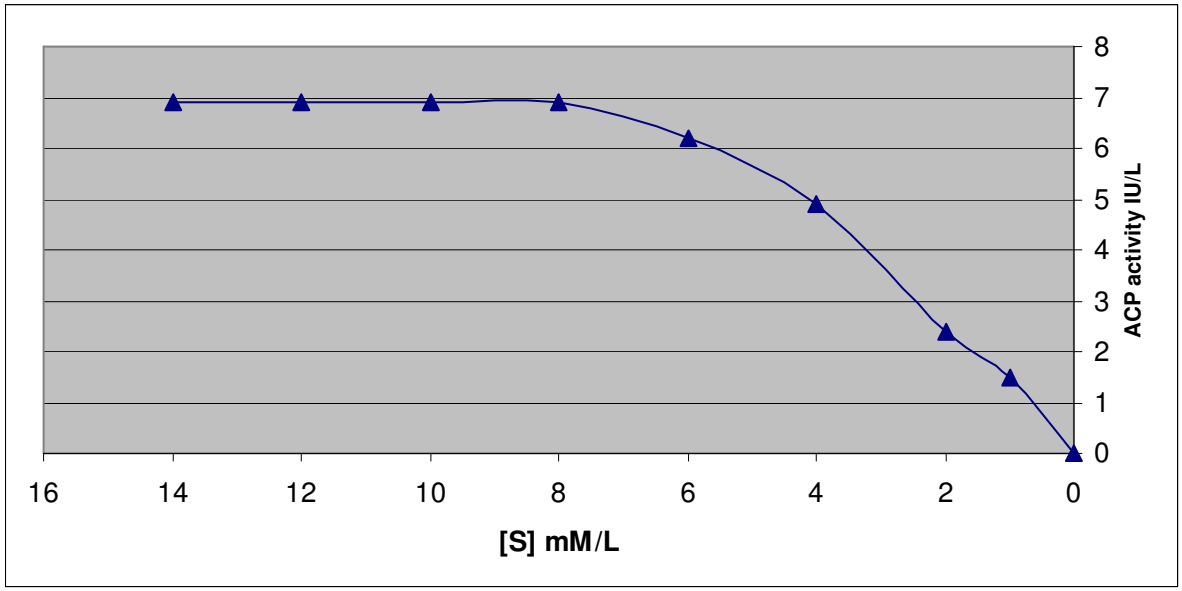

Fig.3
Effect of substrate (disodium phenyl phosphate) concentration upon reaction rate (velocity) of purified ACP. The reaction was carried out in citrate buffer pH=4.9 at 37ºC.

Temperature effect on purified ACP was shown in [Fig.4], where optimal temperature at which maximum velocity obtained for purified ACP was 37ºC. At higher temperatures the rate of enzyme reaction slowed down due to protein denaturation. The liver isonzyme was more sensitive to thermal denaturation, and have maximum velocity at 36ºC [(20)]. Purified ACP obtained from mature soybean seeds showed high activities at temperatures above 80ºC, using p-nitrophenylphosphate as substrate [(23)].

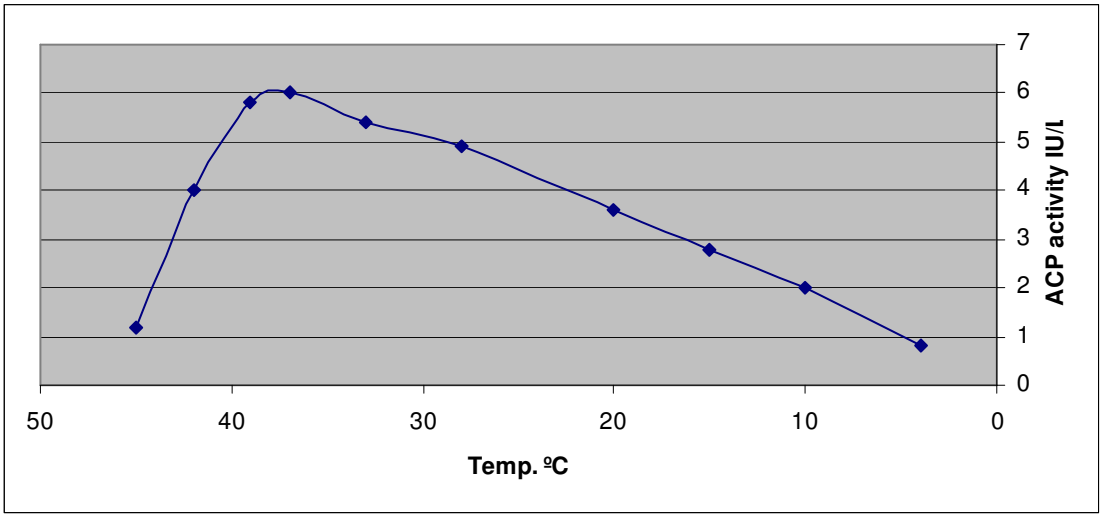

Fig. 4
Effect of incubation temperature upon the activity of purified ACP. The activity was determined at different incubation temperature at pH=4.9

Figure.5 shows the effect of pH on the activity of purified ACP. The optimum pH was 4.9, and the pH and the temperature were similar to that of prostatic isoenzyme and total ACP [16, 21].

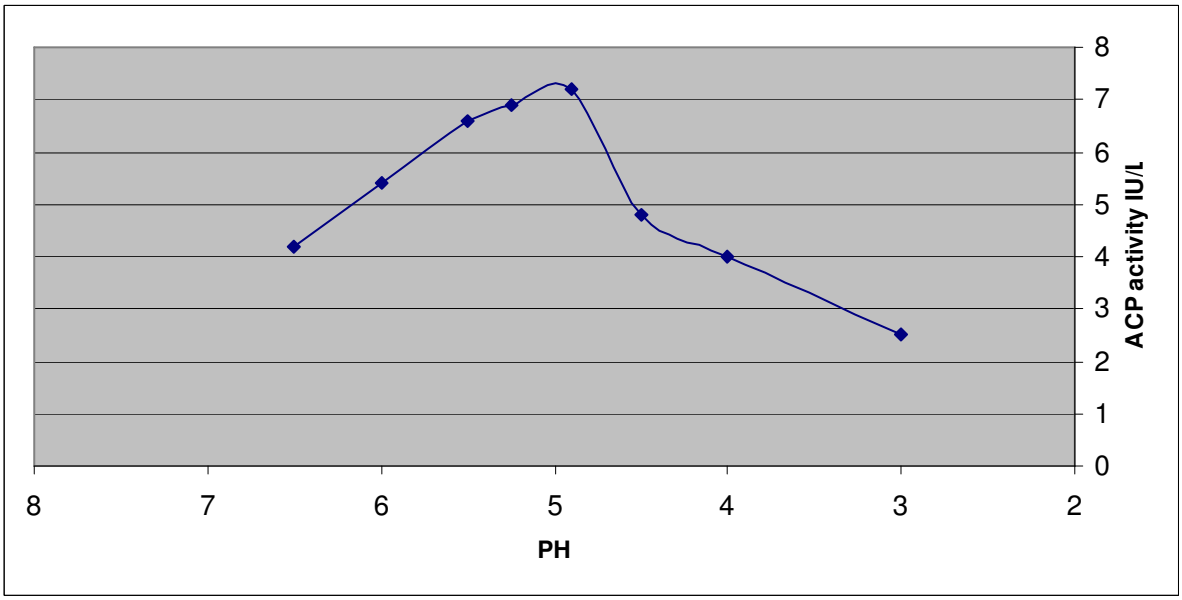

Fig. 5
Effect of pH upon the activity of purified ACP. The activity was determined at different pH at 37ºC

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عزل وتنقية الفوسفاتيز الحامضي من السائل المنوي لأشخاص أصحاء

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الخلاصة :

تم قياس مستوى نشاط الفوسفاتيز الحامضي في ٢٥ عينة من السائل المنوي لأشخاص أصحاء تتراوح أعمارهم بين (٢٥-٣٧) سنة. وقد أظهرت النتائج أن معدل فعالية الفوسفاتيز الحامضي في السائل المنوي (22.3±8.6) وحدة عالمية /لتر.تم تنقية أنزيم الفوسفاتيز الحامضي ٣,٨٣ مرة باستعمال طرق تنقية مختلفة ، الترسيب بكبريتات الأمونيوم , وكروماتوغرافيا التبادل الايوني باستعمال الهلام DEAE-Cellulose وتقنية الترشيح الهلامي باستعمال Sephadex G-100. تم إجراء بعض الدراسات الحركية لأنزيم الفوسفاتيز الحامضي المنقى وقد أظهرت النتائج أن الأنزيم يخضع لمعادلة ميكايلس-منتن وان التركيز الأمثل للمادة الأساس المستعملة Disodium phenyl phosphate كانت ٨ ملي مول /لتر.ودرجة الحرارة المثلى هي ٣٧ °م, في حين كانت قمة الأس الهيدروجيني (PH) الأمثل للفوسفاتيز الحامضي المنقى هي ٤,٩ .