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Enzymatic and Immunological Studies of Prostatic Acid Phosphatase

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Levels of serum acid phosphatase are elevated in prostatic cancer patients as compared with those of normal human. Prostatic cancer patients with bone metastasis show about 2 to 5 fold increased activities of serum acid phosphatase while patients without bone metastasis show slight increase (about 20%) of the activity. When we analysed serum acid phosphatase isozyme pattern with DEAE cellulose column chromatography, profiles of these enzymes of normal and prostatic cancer patients differ greatly.

In normal human, three distinct isozymes I, II and III of serum acid phosphatase were observed. Acid phosphatase of prostatic adenoma tissue surgically removed from prostatic hypertrophy patients consists of two isozymes II and III which are chromatographically corresponding to those isozymes of serum acid phosphatase. Serological analyses and inhibitor-enzyme specificities led us to conclude that serum isozymes II and III are derived from prostatic gland.

In cancer patients without bone metastasis, a greatly increased activity of isozyme III and normal level of isozyme II in serum acid phosphatase were observed. However in prostatic cancer patient with advanced bone metastasis, a huge increase of serum isozyme II activity was observed whereas isozyme III is at an undertectable level. These isozyme variations in serum acid phosphatase suggest that assay of each isozyme is a valuable approach for diagnosis and judgement of prognosis of prostatic cancer.

INTRODUCTION

It has long been known that prostatic gland contains large amount of acid phosphatase[1], however role of this enzyme is yet unknown.

Angeletti et al.[2] reported that serum acid phosphatase has two distinct isozyme

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patterns by DEAE cellulose column chromatography. They showed an increased activity of the slow moving peak in prostatic cancer patients. Kondo and Saito[3] reported purification, electrophoresis and enzymologic properties of the acid phosphatase in adenoma tissue of prostatic hypertrophy. We subsequently analysed DEAE column chromatography patterns of normal serum acid phosphatase, and showed three distinct isozymes, I, II, and III. We, furthermore, studied the serological and enzymatic specificities and the origin of these isozymes to understand correlation between serum and prostatic acid phosphatases. This type of study should add much informations to our understanding diagnostic and prognostic approach to prostatic hypertrophy and cancer.

In this communication, we report the characterization of each isozyme of serum and prostatic acid phosphatases in prostatic diseases and discuss the origins of these isozymes.

MATERIAL AND METHOD

Prostatic acid phosphatase was isolated from sliced tissue of adenoma removed from the hypertrophy patient by the methods of London[4] and Ostrowski[5]. Sliced tissues of adenoma were suspended in 0.2M acetate buffer, pH 5.0 and incubated at 4°C. Twenty-four hours later, tissue fragments were removed by filtering through gauge. With this procedure, the majority of prostatic acid phosphatase is released into the buffer. The enzymes were precipitated by 55-68% saturated ammonium sulfate, resuspended in acetate buffer and dialysed against the same buffer for 24 hrs. at 4°C. This preparation was applied to DEAE-cellulose (Sigma Chemical Co., Saint Louis) column chromatography. The detailed diagrammatic procedure was shown in Figure 1. Serum acid phosphatase was isolated and purified by Ostrowski's method from normal humans and patients with prostatic hypertrophy and carcinoma. Serum acid phosphatase and prostatic acid phosphatase activities were determined quantitatively by the methods of Bessey, Lowry and Brock[6] and Fishman and Lerner[7], respectively. p-Nitrophenyl phosphate(Wako Pure Chem. Co., Osaka) was used as the substrate and 1-tartaric acid (Sigma Chemical Co., Saint Louis) as inhibitor of prostatic acid phosphatase. Rabbit antiserum against human prostatic acid phosphatase was prepared by inoculating 2 mg proteins of enzyme with

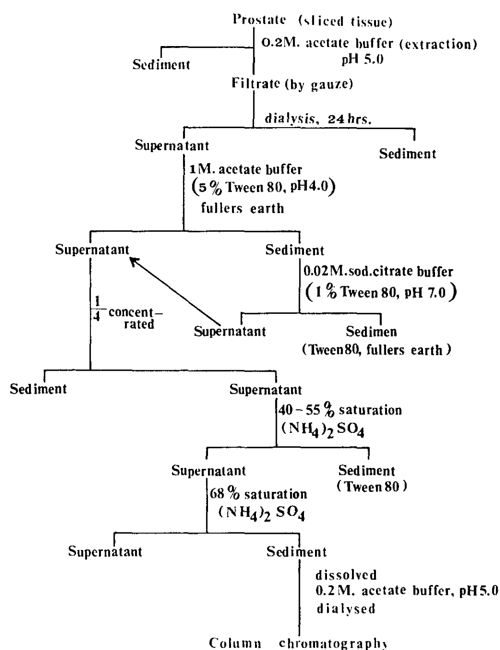

Fig. 1 Isolation Procedure of Prostatic Acid Phosphatase

Freund's complete adjuvant (Difco, Detroit) once a week a total of 4 times. The specificity of this antiserum against prostatic acid phosphatase was assayed by serological absorption method. Agar (Sigma Chemical Co., Saint Louis) gel electrophoresis was carried out on microscopic slide glass at 6V per cm for 90 min. in a barbital buffer, pH 8.2, at an ionic strength of 0.05 M and then immunoelectrophoresis was done according to the methods of Scheidegger[8].

RESULTS

Comparative chromatographic analyses of acid phosphatase of serum and prostatic adenoma tissue

A typical chromatographic profile of serum acid phosphatase of normal male humans is shown in Fig. 2. Two major peaks Is and IIs and one minor peak IIIs were observed. Relative acid phosphatase activities of Is, IIs and IIIs are about 73, 27 and 1% respectively. Thus, serum acid phosphatase consists of three isozymes. Acid phosphatase isolated from adenoma tissue of hypertrophic patients shows two major peaks IIp and IIIp corresponding to the IIs and IIIs isozymes of serum acid phosphatase (Fig. 3). Although, the level of serum acid phosphatase isozyme IIIs activity is a barely detectable level (about 1 % of the total activity), the relative activity of the prostatic acid phosphatase peak IIIp is about 40% of the total prostatic acid phosphatase. Occasionally an additional minor isozyme peak Ip in front of peak IIp was found in extracts from prostate tissue. Since this minor isozyme Ip appears occasionally, it is possible that the Ip isozyme is a decomposed

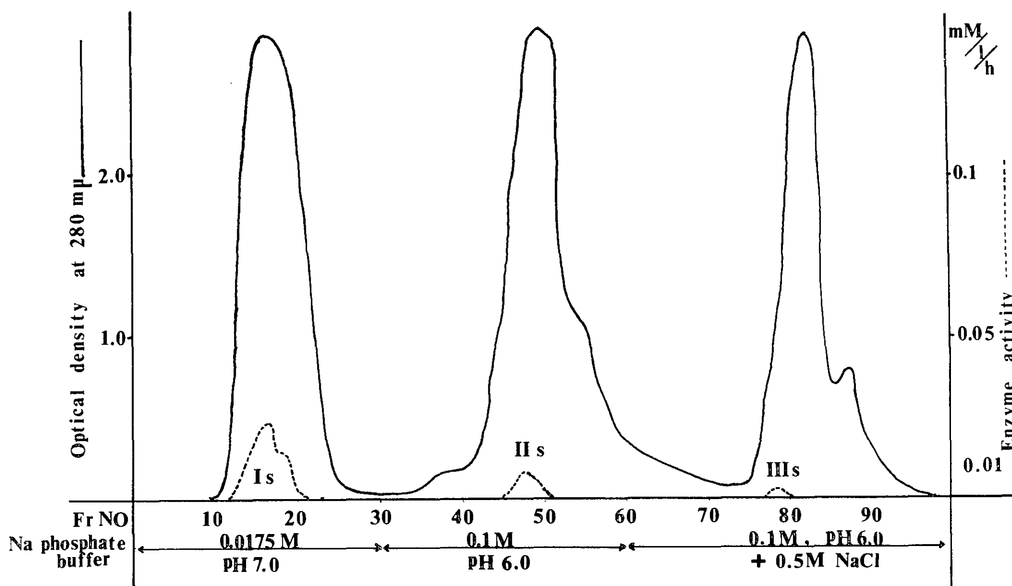

Fig. 2 Column chromatography of normal serum

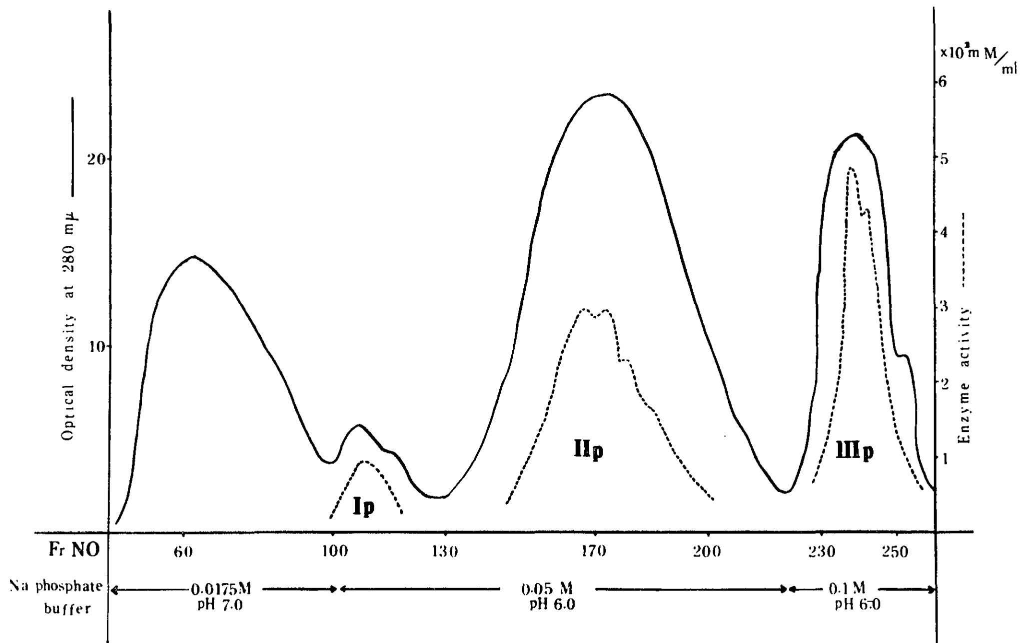

Fig. 3 DEAE cellulose column chromatography of acid Phosphatase from prostatic hypertrophy

product of other isozymes.

In serum acid phosphatase of prostatic cancer patients without bone metastasis, an elevated isozyme IIIs activity was observed as shown in Table 1, whereas isozyme IIs activity is about the same as those of normal and of hypertrophy patient. Serum acid phosphatase of cancer patient with advanced bone metastasis shows isozyme IIs exclusively. The level of isozyme IIs activity was about 20-fold higher than that of normal human serum. Two other peaks were almost undetected (Fig. 4). These observations indicate that formation of cancer accompanies with change in pattern of serum acid phosphatase. Therefore it may be suggested that these two isozymes IIs and IIIs are prostate specific acid phosphatase.

Table 1. Acid phosphatase activity of sera

sera		total activity (mM/l/hr)	isozyme activity (% of total activity)		
			Is	IIs	IIIs
normal male serum		0.592±0.097	72.7±4.4	26.7±4.8	0.7±1.1
BPH serum		0.778±0.386	74.2±6.0	24.3±7.5	1.5±1.8
sera of prostatic carcinoma with	no bone metastasis	0.698±0.267	60.4±6.7	27.0±4.7	12.6±4.2
	advanced bone metastasis	30.150	0.1	99.9	0

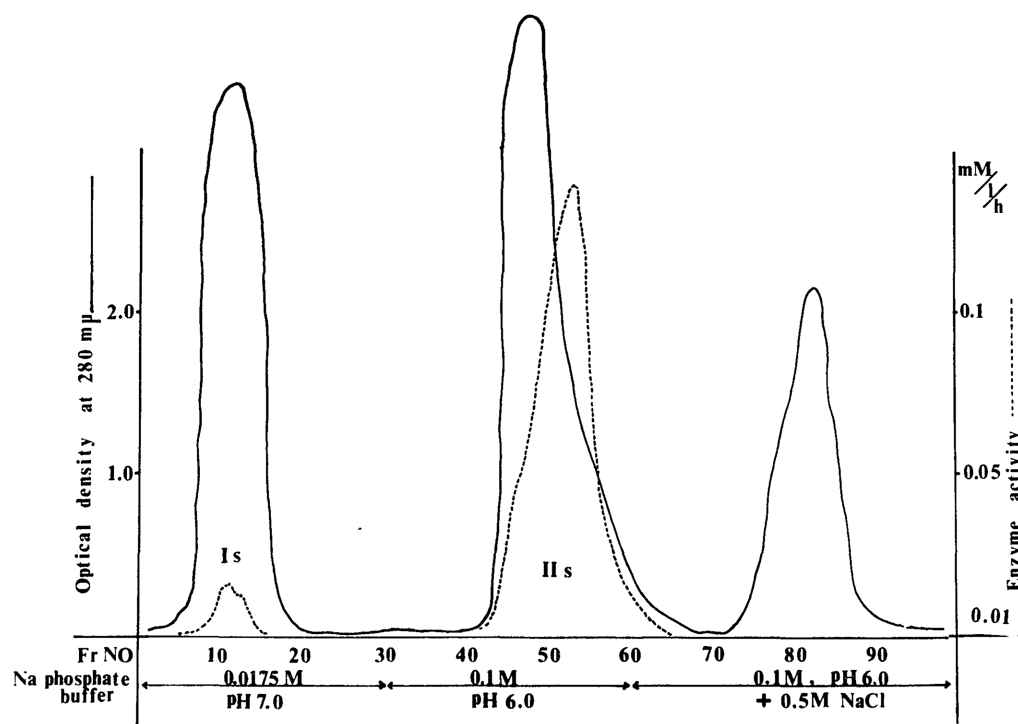

Fig. 4 Column chromatography of the patient with metastatic cancer of Prostate

Rechromatography of the prostatic isozyme IIp

Since a drastic increase in the IIs isozyme activity of serum acid phosphatase was found in prostatic cancer patients with advanced bone metastasis, the detailed characterization of the isozymes IIs and IIp may provide valuable informations for disease processes. We, thus, rechromatographed the peak IIp fraction of prostatic acid phosphatase. As shown in Fig. 5, we obtained a single peak (designated as IIpr hereafter) without contamination of IIIp and minor peak Ip isozymes. This result indicates that isozyme IIp can be purified by DEAE cellulose column chromatography. Thus, the isozyme Ip may probably be an independent isozyme, although the possibility that the isozyme Ip is a decomposed product of isozyme IIp may not be excluded.

Serological identification of prostatic acid phosphatase

In recent years, immunological techniques became available for crucial identification of homologous and non homologous enzymes[9)10)]. Therefore, it was of great interest to study antigenicity of these acid phosphatase isozymes. We immunized rabbits with IIpr isozyme by injecting 2mg IIpr protein preparation per a week for a total of four injections. The resulting anti-IIpr serum was mixed with the IIpr isozyme preparation, incubated for one hour at 37°C and centrifuged at 8,000 rpm for 30 min. Enzyme activity of supernatant

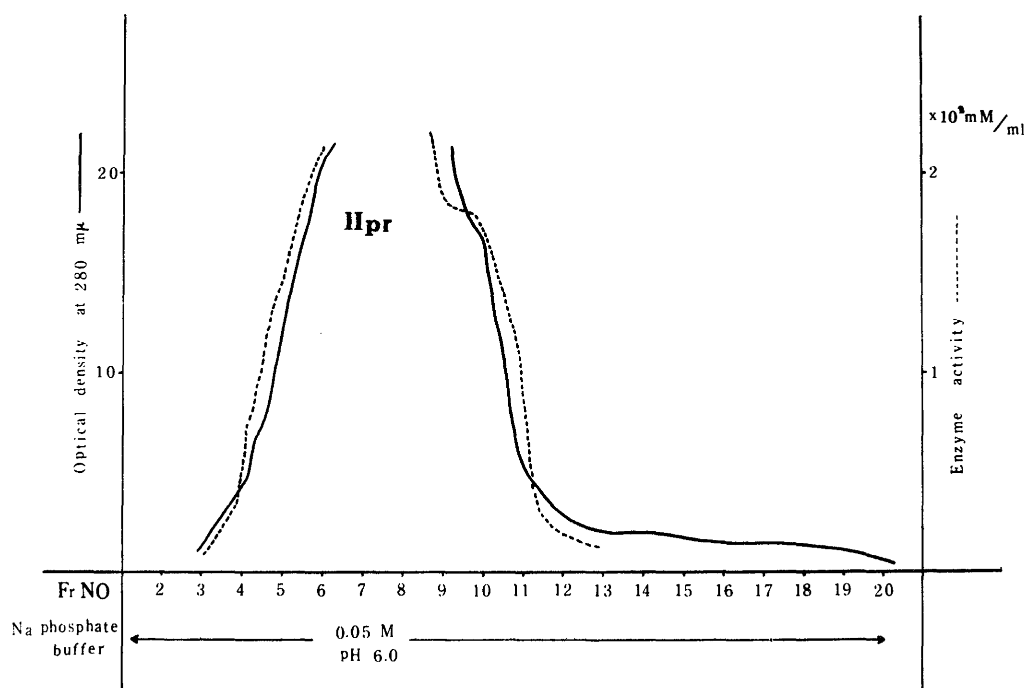

Fig. 5 Rechromatography of III Fr on DEAE-cellulose column

was about 3% of untreated IIpr isozyme activity (Table 2). In considering small amounts of non precipitable enzyme-antibody complex, We believe that 100% of the enzyme was neutralized by the antibodies.

When anti-IIpr serum was mixed with serum acid phosphatase isozyme IIs of normal human followed by one hour incubation and centrifugation, the residual enzyme activity in supernatant was about 90% of the untreated IIs isozyme activity (Table 2). This observation suggests that the peak IIs of serum acid phosphatase consists of at least two different isozyme origins. One of them should be derived from prostatic gland (about 10%) and the other is unknown. Since a drastically elevated activity of serum acid phosphatase IIs isozyme was observed in prostatic cancer patients with advanced bone metastasis, the antigenecity of this isozyme (designated IIsm hereafter) was examined with anti-IIpr serum. About 95% of the enzyme activity was neutralized (Table 2). This result indicates that the enzyme activity of the peak IIsm consists of mainly prostatic acid phosphatase IIp and small amounts (less than 5%) of acid phosphatase of other origin.

Characterization of prostatic acid phosphatase

a) *pH dependency*

The pH dependency of enzyme activity of the rechromatographed IIpr was studied. As shown in Fig. 6, two optimal peaks at pH 3.8 and 5.5 were observed. In contrast, the optimal pH for the activity of normal serum acid phosphatase isozyme IIs is about 5.0.

Table 2 Absorption and inhibition of enzyme

acid phosphatase				% activity of enzyme: absorption by anti-IIpr serum	% activity of enzyme: inhibition by 1-tartrate
normal serum acid phosphatase		total			30
		isozymes	Is	50	51
			IIs	10	35
			IIIs	—	20
serum acid phosphatase of prostatic carcinoma with	no bone metastasis	total			36
		isozymes	Is	27	27
			IIs	75	75
			IIIs	33	43
	advanced bone metastasis	total			84
		isozymes	Is	26	26
			IIs*	95	95
			IIIs	—	—
acid phosphatase of prostatic adenoma tissue		total			94
		isozymes	Ip	100	90
			IIp	100	95
			IIIp	100	93

— indicates undetectable activity
* this particular isozyme IIs of adenocarcinoma with bone metastasis is designated IIsm for frequent use in the text.

These results led us to conclude that prostatic acid phosphatase isozyme IIpr is distinguishable from serum acid phosphatase isozyme IIs by pH dependency. As can be seen in this figure, however, the pH dependency of the serum acid phosphatase isozyme IIs activity showed a quite broad peak. The serum IIs activities corresponding to the optimal pHs (3.8 and 5.5) of prostatic acid phosphatase isozyme IIpr are relatively high, indicating that the normal serum acid phosphatase isozyme IIs may be composed of prostatic acid phosphatase and those of other origins. These observation with pH dependency and the previously mentioned serological test suggest that perhaps only 10% of the serum acid phosphatase isozyme IIs is derived from prostatic gland. The remaining 90% is originated from other organs.

b) *Inhibition by 1-tartrate*

Fishman and Lerner[7] reported that 1-tartrate inhibits prostate specific acid phosphatase in serum. Thus, it became desirable to study specific inhibition of serum and prostatic acid phosphatase isozymes. 1-Tartrate inhibits 30% of whole serum enzyme activity of normal male whereas it inhibits about 94% of whole prostatic acid phosphatase activity extracted from prostatic adenoma tissue, as shown in Table 2. Subsequently, we studied inhibition of each prostatic and serum acid phosphatase isozyme. 1-Tartrate inhibitions of

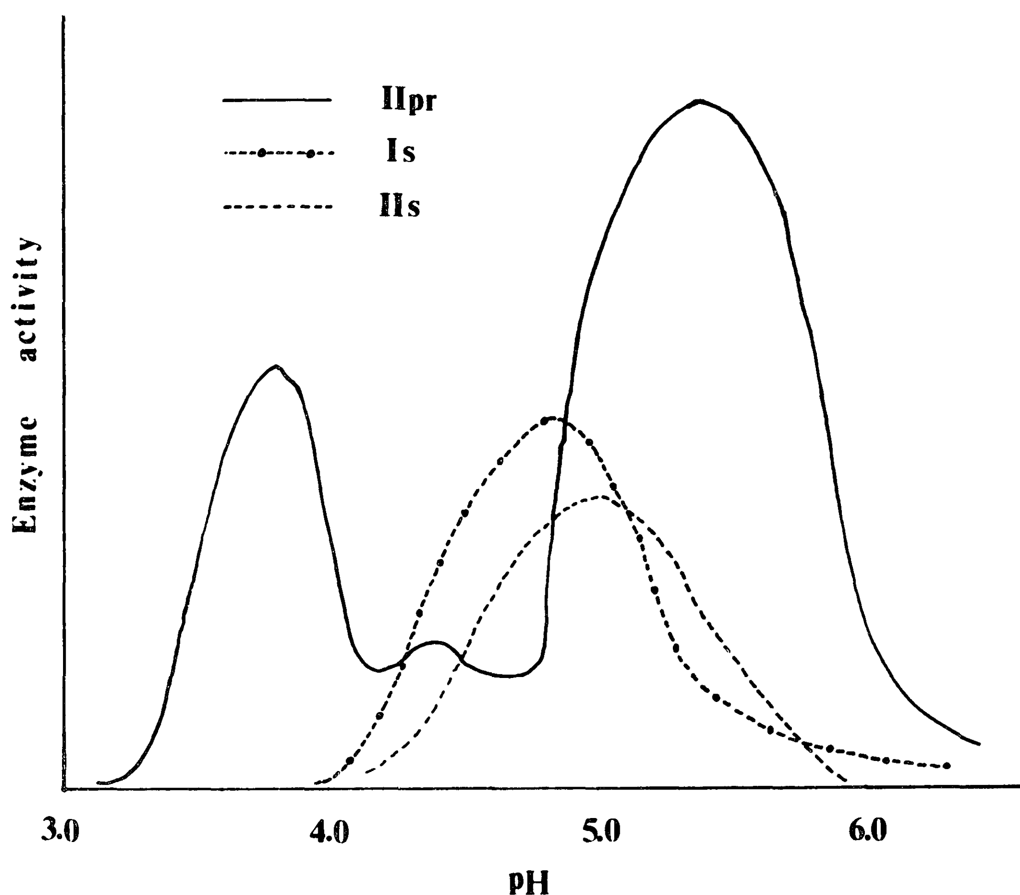

Fig. 6 Optimum pH of IIpr, Is and IIs

prostatic acid phosphatase isozymes Ip, IIp, and IIIp from prostatic adenoma tissue were 90, 95 and 93% respectively. However, 1-tartrate inhibited 51% of isozyme Is, 35% of isozyme IIs and 20% of isozyme IIIs activities of normal male human serum. On the other hand, isozymes IIs of prostatic cancer patients without bone metastasis and with bone metastasis (IIms) were inhibited by 75% and 95% respectively. These experiments suggested that 1-tartrate is not a specific inhibitor for normal serum acid phosphatase isozymes IIs and IIIs although it preferentially inhibits IIs and IIms of cancer patients. Since we suggested that serum acid phosphatase isozymes IIs and IIIs are derived from prostate, it should not be concluded that 1-tartrate specifically inhibits prostatic acid phosphatase in normal sera.

Immunoelectrophoretic analysis of prostatic acid phosphatase

When acid phosphatase preparation derived from adenoma tissues of prostatic hypertrophy was submitted to immunoelectrophoretic analysis, three distinct bands were found. Isozymes IIpr and IIIp corresponding to those of DEAE cellulose column chromatography

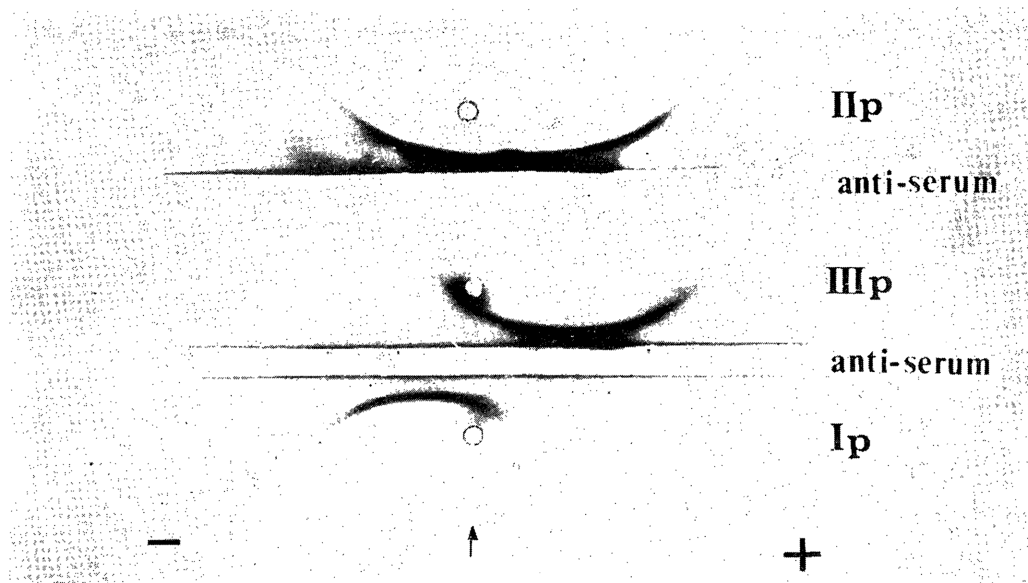

Fig. 7 Immunoelectrophoresis of Ip, IIp and IIIp.

of acid phosphatase migrate toward cathode. Isozyme IIIp moves faster than isozyme IIpr as shown in Fig. 7. The minor isozyme Ip of prostatic acid phosphatase migrate toward anode. From this observation, it is defenitely concluded that prostatic acid phosphatase has three distinct isozymes, Ip, IIp, and IIIp.

DISCUSSION

When anti-IIpr serum was mixed with prostatic isozyme IIIp and followed by absorption and precipitation procedure, more than 90% of the enzyme activity was lost, indicating that isozymes IIp and IIIp were serologically indistinguishable. This may suggest that these two isozymes are the products of a single structural gene or these isozymes share a common subunit. In addition anti-IIpr serum also crossreact with isozyme Ip, implying that it shares common antigen with the other two isozymes (IIp and IIIp). However the isozyme Ip moved opposite direction from the other two isozymes in electrophoresis.

It should be mentioned here that anti-IIpr serum can absorb about 19% activity of isozyme IIs of female serum acid phosphatase. This observation led us to hypothesize that though female does not have the prostatic gland the female serum acid phosphatase isozyme IIs share a common subunit with the prostatic isozymes. Recently, Suyama et al.[12] observed that treatment of seminal fluid acid phosphatase with neuraminidase results in retarded mobility of electrophoretic patterns of the isozymes IIp and IIIp. Therefore it seems likely that protein modification by sialic acid increases negative charge which accelerate electrophoretic mobility.

This possibility may be a formation of multiple isozymes by modification of product

of a single structural gene and may explain that the number of sialic acid on isozyme IIIp is more than that of isozyme IIp.

In our separate study[11] with various human organs, it may be suggested that isozyme Is and a portion of the isozyme IIs are derived from various other organs such as liver, kidney, red blood cells and bone marrow. The critical determination of origin and serological cross-reactivity of each isozyme of acid phosphatases should shed lights on mechanism of isozyme formation.

In the present publication, we have shown that male serum acid phosphatase isozymes IIs and IIIs are derived from prostatic gland and that isozymes IIs and IIIs activities are drastically elevated by formation of prostatic cancer with or without bone metastasis. Therefore the determination of activities of serum acid phosphatase isozymes IIs and IIIs provides powerful diagnostic tool for prostatic cancer.

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