Comparative Study of Three Male Genital Acid Phosphatases.* (20529)

RONALD R. NOVALES AND HOWARD A. BERN. (Introduced by R. M. Eakin)
From the Department of Zoology, University of California, Berkeley.

Kutscher and Wolbergs(1) demonstrated that the high acid phosphatase content of human seminal fluid is derived almost completely from the prostate. Since that time the biochemical properties of the human prostatic enzyme have been the subject of several investigations(2-6); in addition, monkey(7) and dog(8) prostates have been shown to possess considerable acid phosphatase activity, with moderate activity present in the rabbit prostate(9). A similar enzyme occurs in the preputial gland of the male rat(10), and high acid phosphatase activity has also been reported in the seminal vesicle of the guinea pig(9).

Because of the use of rat and guinea pig tissues in the study of steroid hormone-phosphatase relations somewhat comparable to those pertaining to the human prostate, a comparative study was undertaken of acid phosphatases from 3 male genital tract sources: human seminal plasma, rat preputial gland, and guinea pig seminal vesicle. The relations of activity to pH and to substrate concentration, as well as the effects of a series of ions and organic substances, were studied. Special attention was paid to the ability of bovine serum albumin to activate all preparations. In general, a notable degree of similarity was found.

Materials and methods. Enzyme preparations of approximately equal activity were obtained as follows: (a) prostatic acid phosphatase by dilution of human seminal plasma 1:2000 with glass-distilled water; (b) preputial acid phosphatase as the supernatant after centrifugation for 10 min. at 2000 RPM of a 0.5% aqueous homogenate of secretion-free glands from mature male Long-Evans rats; (c) vesicular acid phosphatase as a similarly prepared supernatant from the secretion-free seminal vesicles of mature male guinea pigs. Studies were made immediately after preparation of the enzyme solutions, as well as occasionally after storage at 4°C for 2-3 days. Refrigeration for this period did not result in any significant change in activity. Phosphatase activity was determined by the method of Huggins and Talalay(11), using sodium phenolphthalein phosphate as substrate and their definition of 10 units of phosphatase activity as the amount liberating one mg of phenolphthalein during one hour at 37°C. Although phenolphthalein phosphate is not a naturally occurring substrate for phosphatases, it has been demonstrated that the perfused canine prostate dephosphorylates it *in vivo*(4). Unitage curves were constructed for each of the 3 enzymes, owing to the parabolic relation between enzyme concentration and the colorimetric equivalent of the liberated phenolphthalein. 0.05 M sodium acetate-sodium cacodylate (dimethylarsenate) buffers(12) were employed in the determination of pH-activity curves from pH 4.3 to 7.4; 0.08 M sodium barbital provided the buffer at more alkaline pH's, where activity was found to be insignificant. Substrate concentration effects were studied both at pH 5.4 and at the optimum pH with 0.08 M acetate and 0.05 M phthalate buffers. Efficacy of the buffer solutions was checked during the course of incubation by means of a Beckman pH meter with portable electrodes. The effects of the following ions and organic substances were determined at pH 5.4 using a standard substrate concentration of 8.3×10^{-4} M in 0.08 M acetate buffer: Mg^{++}, Ca^{++}, Mn^{++}, Zn^{++}, F^{-}, and oxalate at a concentration of 0.017 M; molybdate, tungstate, L-tartaric acid, and bovine serum albumin (Armour) in a series of concentrations; 0.5% formaldehyde. Enzyme preparations were also tested after pretreatment with 40% ethanol for 30 min. at room temperature. Each substance

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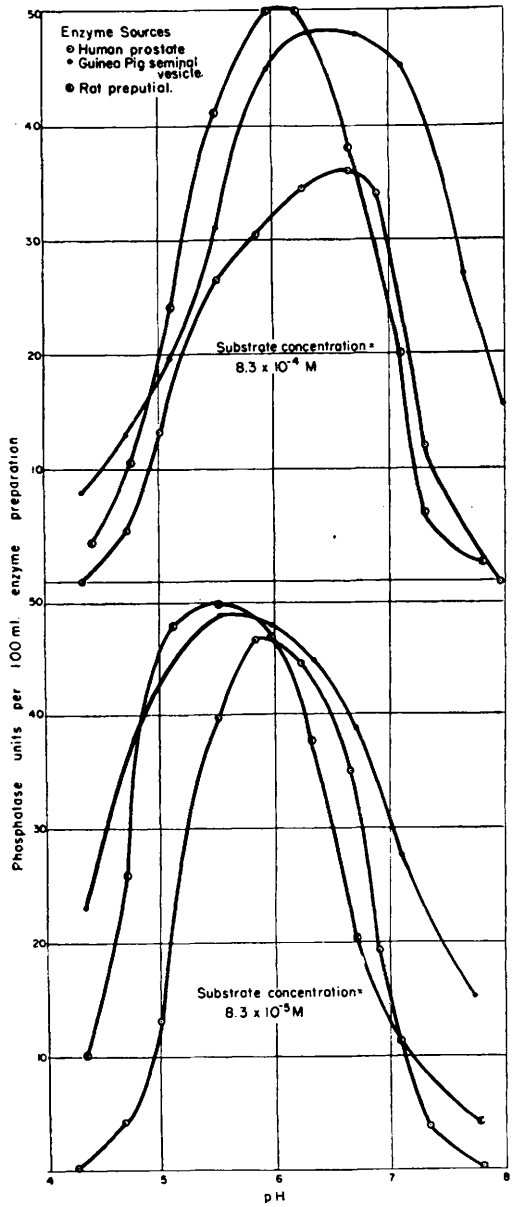

FIG. 1. pH-activity curves for human seminal, rat preputial, and guinea pig seminal vesicle acid phosphatases at 2 substrate (phenolphthalein phosphate) concentrations. Note the lower pH optima with the lower substrate concentration.

was tested several times on 2 or more preparations from each enzyme source.

Results. pH-activity curves were prepared at substrate concentrations of 8.3 x 10^{-4} M and 8.3 x 10^{-5} M. Fig. 1 shows that maximal activity occurs at a lower pH with the lower substrate concentration. In general, pH optima (5.5-6.5) are similar for the 3 enzymes. The limiting substrate concentration was around 3 x 10^{-4} M for all 3 enzymes; substrate inhibition was evident at higher concentrations. Michaelis constants were estimated from curves and were also calculated according to the method of Lineweaver and Burk(13). They were found to fall in the range of 1 x 10^{-5} to 5 x 10^{-5}; no attempt was made to determine the constants more accurately in view of the impurity of the preparations.

The metallic cations tested (Mg^{++}, Mn^{++}, Ca^{++}, Zn^{++}) inhibited the 3 enzymes to some extent; however, the results were seldom reproducible with several preparations of the same enzyme. Molybdate, tungstate, fluoride, L-tartrate, and oxalate all had appreciable inhibitory effects at the concentrations employed, except possibly at the lowest concentration of tartrate. The similar responses of the 3 enzymes to these substances are striking (Table I).

Incubation of the enzyme preparations in 40% ethanol for 30 min. at room temperature before enzyme assay resulted in total inactivation, whereas 0.5% formaldehyde had a very limited effect.

Bovine serum albumin had an activating effect over a range of concentrations. Human seminal enzyme and guinea pig vesicular enzyme were similarly activated, but male rat preputial phosphatase showed considerably less response. Thus, 0.01% albumin activates the human enzyme 235% and the guinea pig enzyme 350%, but has no effect on the rat enzyme. Only with concentrations of albumin as high as 0.1% is there an appreciable effect on preputial phosphatase (30% activation). On the other hand, 0.005% albumin still significantly activates the former (35% and 85%, respectively). A linear relation was found to exist between concentration of seminal phosphatase and the amount of activation with 0.02% albumin in the presence of excess substrate at both pH 5.4 and 6.0.

Discussion. The similarity of the pH optima (around 6.0) and of the Michaelis constants of the 3 genital acid phosphatases studied furnish some evidence for their pos-

TABLE I. Effect of Various Substances on Male Genital Tract Acid Phosphatases.*

Substance	Concentration	% inhibition of enzyme: Human seminal	Guinea pig vesicular	Male rat preputial
Sodium fluoride	1.7×10^{-2} M	95	100	100
" molybdate	10^{-4} M	93	96	95
	10^{-5} M	81	87	81
	10^{-6} M	44	58	52
" tungstate	10^{-5} M	97	95	91
	10^{-6} M	68	81	80
L-tartaric acid	10^{-2} M	88	96	96
	10^{-3} M	31	25	42
	10^{-4} M	12	13	8
Sodium oxalate	10^{-2} M	39	44	20
Formaldehyde	0.5%	17	28	16
Ethanol incubation†	40%	100	100	100

* Substrate: 8.3×10^{-4} M phenolphthalein phosphate at pH 5.4 (acetate).
† 30 min. pretreatment at room temperature.

sible identity. However, Roche(14) has emphasized that to be reliable pH optima should be obtained on purified preparations. The pH optimum of human prostatic acid phosphatase reported herein is somewhat higher than that found by some earlier workers(6, 11). However, Lundquist's detailed study (2) demonstrated an optimum as high as pH 6.3 with certain substrates (*e.g.* calcium-phosphorylcholine). In general, all 3 genital phosphatases are characterized in their impure state by pH optima higher than those ordinarily ascribed to group II phosphatases, to which they are generally assigned(14).

The lower pH optima seen when substrate concentration is reduced are of interest in view of some observations(15) on alkaline phosphatase. It was found that lowering the substrate concentration brought the optimum pH toward neutrality, a 10-fold decrease in substrate concentration diminishing the optimum by about one pH unit. However, the view(15) that the approach of the optimum to neutrality resulting from lowered substrate concentration may reflect the condition seen *in vivo*, where substrate concentrations may be low, is difficult to reconcile with our data. With the acid phosphatases, lowered substrate concentration results in a pH optimum further removed from neutrality.

The variable results obtained with the metallic ions may possibly be due to their occurrence in the enzyme preparations themselves; appreciable calcium is found in human seminal plasma(16). An inhibition of the prostatic enzyme by Mg^{++} was repeatedly found, despite the presumed lack of effect of this ion on group II phosphatases(14).

The effects of certain anions and organic substances provide data of comparative value (Table I). The well established inhibitors of group II acid phosphatases: fluoride(14), molybdate and tungstate(17), and oxalate (5,18), had similar effects on all preparations. The human prostatic enzyme can be distinguished from isodynamic serum acid phosphatases by its sensitivity to L-tartrate (3,19-21) and to ethanol(22-24) and its resistance to formaldehyde(20,24,25). These 3 substances have similar influences upon the guinea pig and rat genital enzymes, despite their origin from sex accessories which are not homologous to the human prostate.

Acid phosphatase from the male rat preputial gland is activated by 0.1% albumin to about the same extent as β-glucuronidase(26) from the female rat preputial gland (31% as compared with 39%). In attempting to account for the considerably greater activation of human seminal and guinea pig vesicular acid phosphatases, it should be noted that the rat preputial homogenate is prepared from a very different type of tissue, consisting of holocrine acini with a high lipid content(27).

The marked increase in prostatic acid phosphatase activity in the serum generally diagnostic of metastasizing human prostatic carcinoma(28) may be related in part to the

activation of the enzyme by serum albumin. In addition, a thermostable, low molecular weight organic activator of prostatic acid phosphatase has been found in serum(29). Although alkaline hyperphosphatasemias generally reflect an actual enrichment by the enzyme, rather than an activation(30), the latter possibility in regard to prostatic acid phosphatase in human serum seems worthy of consideration.

Summary and conclusions. Three male genital tract acid phosphatases, from human seminal plasma, rat preputial gland and guinea pig seminal vesicle, show a high degree of similarity in their pH-activity curves, Michaelis constants, and reactions to certain anions and organic substances. As a group these enzymes are fluoride-, tungstate-, molybdate-, oxalate-, L-tartrate-, and ethanol-sensitive and formaldehyde-resistant. All are activated by serum albumin, the preputial enzyme less than the other two.

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