A Comparison of Canine Prostate and Wheat Germ Oil Acid Phosphatase.* (26955)

HARRIS ROSENKRANTZ AND JAMES LANGILLE (Introduced by Z. Hadidian)
Department of Biology, Clark University and Biologics Testing Laboratory, Worcester, Mass.

The acid phosphatase of the canine prostatic fluid is a relatively stable enzyme which is little affected in the course of a week by significant concentrations of proteolytic enzymes present in the same fluid (1, 2). Evidence exists that a number of acid phosphatases occur in nature (3-8). In this study the acid phosphatase of canine prostatic fluid is compared with that of wheat germ oil for its action on natural and synthetic substrates at different pH levels.

Methods. Methods developed primarily for estimation of serum alkaline phosphatase (9) have been extended to the study of acid phosphatase simply through the employment of a buffer system with a lower pH. In the present study, monophenylphosphate (9), β-glycerophosphate (10), phenolphthalien diphosphoric acid (11), glucose-1-phosphate and o-carboxyphenylphosphate (12), were used for a comparative study at various pH levels between 4 and 7.

The details of the methods used are as follows: (1) *β-glycerophosphate*—4.5 ml of 1% sodium β-glycerophosphate buffer were incubated with 0.1 ml canine prostatic fluid for 1 hour at 37.5° C in an atmosphere of air. The incubation was carried out in 12 ml heavy-walled centrifuge tubes so that enzyme inactivation with 1 ml 30% trichloroacetic acid and centrifugation would not require further transfers. Control tubes were incubated without prostatic fluid, the latter being introduced after the protein precipitant. One ml of the supernatant was mixed with 1 ml molybdic acid and 0.4 ml of the Fiske-SubbaRow reagent. The solution was diluted to 9 ml with water and read at 660 mμ.

(2) *Phenolphthalien diphosphoric acid*—0.3 ml prostatic fluid and 1 ml 0.06 M potassium acid phthalate containing 0.72 mg phenolphthalien diphosphoric acid were permitted to stand at room temperature for 2 minutes. The reaction was stopped with addition of 8.5 ml of 0.2 M carbonate-bicarbonate buffer (pH 10.4) and the dephosphorylated phenolphthalien was measured at 550 mμ. When the release of phosphate was followed, 1 ml of 10% trichloroacetic acid was substituted for the carbonate-bicarbonate buffer.

(3) *o-Carboxyphenylphosphate*—To 2 ml of 0.15 M acetate—HCl buffer were added 0.1 ml prostatic fluid and 0.5 ml of 0.00365 M o-carboxyphenylphosphate. The reaction was followed by recording absorption at 298 mμ. every 2 min. The readings at 20 min. were used for calculations. When the release of phosphate was followed, trichloroacetic acid was used as above.

(4) *Monophenylphosphate*—2.5 ml of 0.19% monophenylphosphate were combined with 2.5 ml of a 0.2 M Tris-acetate buffer to which 0.1 ml prostatic fluid was added. The solution was incubated for 30 min. at 37.5° C and the reaction halted with the addition of 1 ml 30% trichloroacetic acid. Three ml of the supernatant were transferred to a cuvette and optical density obtained at 270 mμ. An aliquot was taken for phosphate determination by the Fiske-SubbaRow procedure.

The tris-acetate system was also utilized for comparing β-glycerophosphate with glucose-1-phosphate. Two-tenths ml of 0.064 M substrate was used and release of inorganic phosphate was determined as above.

The method for collecting and preparing the canine prostatic fluid is described elsewhere (1). Wheat germ acid phosphatase

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TABLE I. Comparison of Activities of Canine Prostatic and Wheat Germ Acid Phosphatase Using Release of Phosphate as End Point.

Substrate	Source of acid phosphatase	Amt of enzyme or fluid	Buffer system	mg PO_4 = released/hr/ml fluid pH 4	pH 5	pH 6	pH 7
β-glycerophosphate	Wheat germ	.1 mg	Glycerophosphate	.43	.54	.40	.11
"	" "	2.0 "	"		14.3	10.6	5.5
"	Prostatic fluid 353*	.1 ml	"	2.1	2.6	3.4	2.5
"	" " 362	"	"	5.3	7.0	8.2	5.6
"	" " 368	"	"	12.1	13.1	14.8	11.9
"	" " 379	"	"	8.1	9.3	11.1	7.9
"	" " 385	"	"	5.3	6.8	8.4	5.8
β-glycerophosphate	Wheat germ	2.0 mg	Tris-acetate		12.6	7.5	4.3
"	Prostatic fluid 387	.1 ml	"	3.0	3.5	3.2	2.0
"	" " 386	"	"	2.9	3.7	3.4	2.4
Glucose-1-phosphate	Wheat germ	2.0 mg	"		6.0	4.4	1.7
"	Prostatic fluid 368	.1 ml	"	3.2	3.1	3.6	2.4
"	" " 386	"	"	1.9	2.1	1.7	1.1
"	" " 379	"	"	1.9	2.6	1.9	1.6
"	" " 385	"	"	1.3	3.2	1.5	.9
Phenylphosphate	Wheat germ	.5 mg	"	3.8	6.4	5.8	2.3
"	Prostatic fluid dog pool	.1 ml	"	6.6	7.8	6.8	4.9

* Number assigned to each dog.

was obtained from Worthington Biochemical Corp.

Results and discussion. Data in Table I indicate that whereas wheat germ acid phosphatase optimally releases phosphate from β-glycerophosphate near pH 5, prostatic acid phosphatase functions more actively near pH 6. However, in a tris-acetate buffer both enzymes operate maximally near pH 5. This is true for the natural substrates, glucose-1-phosphate and β-glycerophosphate as well as synthetic organic phosphate, monophenylphosphate.

A decreased release of inorganic phosphate from glucose-1-phosphate was observed with both enzymes indicating a lower affinity of this substrate for acid phosphatase whereas using synthetic substrates both enzymes showed optimum activity at pH 5 (Table II). With prostatic fluid en-

TABLE II. Comparison of Activities of Canine Prostatic and Wheat Germ Acid Phosphatase Using Release of Phenyl Group as End Point.

Substrate	Source of acid phosphatase	Amt of enzyme or fluid	Buffer system	mg phenyl group released per unit time per ml fluid* pH 4	pH 5	pH 6	pH 7
Monophenylphosphate	Wheat germ	.5 mg	Tris-acetate	1.52	2.78	2.25	.75
"	Prostatic fluid 368†	.1 ml	"	3.35	3.63	3.0	2.6
"	" " 369	"	"	2.79	3.10	2.76	1.96
o-carboxyphenylphosphate	Wheat germ	.1 mg	Acetate-HCl	.02	.03	.01	.006
"	Prostatic fluid 353	.1 ml	"	.22	.58	.54	.41
"	" " 385	"	"	.19	.43	.40	.31
Phenophthalien	Wheat germ	.6 mg	Potassium acid	.012	.022	.016	.004
Diphosphoric acid	Prostatic fluid 368	.3 ml	Phthlate	.053	.085	.099	.081
" "	Prostatic fluid pooled sample	"	"	.024	.034	.046	.033

* Time indicated in procedures.

† Number assigned to dog.

TABLE III. Stoichiometry in Release of Phenyl and Phosphate Groups by Acid Phosphatase from Synthetic Organic Phosphates.*

Substrate	pH	Phenyl group released		Phosphate group released	
		Wheat germ	Prostatic fluid	Wheat germ	Prostatic fluid
		μM			
Monophenylphosphate	4	.27	.50	.32	.56
	5	.48	.55	.55	.65
	6	.40	.49	.50	.58
	7	.13	.35	.20	.42
o-carboxylphenylphosphate	4	.08	.29	.07	.41
	5	.09	.35	.10	.44
	6	.06	.31	.04	.31
	7	.05	.24	.02	.29
Phenolphthalien diphosphoric acid	4	.04	.08	.09	.17
	5	.07	.11	.13	.24
	6	.05	.15	.13	.29
	7	.02	.11	.11	.23

* Concentrations of organic phosphates used were those recommended by the individual procedures; 0.5 mg wheat germ and 0.1 ml canine prostatic fluid acid phosphatases, respectively, were compared.

zyme phenolphthalien diphosphoric acid provided an exception with an optimum at pH 6.

The data in Table III disclose the stoichiometry of the release of phosphate and phenyl groups in each method investigated here. In the case of phenolphthalien diphosphoric acid approximately 2 molecules of phosphate were obtained for each molecule of phenolphthalien.

Summary. Canine prostatic fluid contained a potent acid phosphatase which was more active against the natural β-glycerophosphate than several synthetic organic phosphates tested. A distinction between the enzymes from prostatic fluid and wheat germ oil could be made on the basis of optimal pH requirement. Stoichiometric determinations indicated that the simplicity of phosphate determination was to be preferred to measurement of the released phenyl group derivative.

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