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PRESENCE AND ANDROGEN CONTROL OF AN ALKALINE PHOSPHATASE IN THE NUCLEUS OF RAT VENTRAL PROSTATE

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Summary

The presence of alkaline phosphatase (EC 3.1.3.1) activity has been demonstrated in nuclei of rat ventral prostate. This enzyme activity remained after washing of isolated nuclei with 0.5% Triton X-100; an acid phosphatase initially present with the nuclear fraction was removed by this treatment. The nuclear alkaline phosphatase, examined by utilizing *p*-nitrophenyl phosphate as substrate, had a pH optimum of 9.5—10.3, and a broad substrate specificity: *p*-nitrophenyl phosphate > phosphothreonine > β-glycerophosphate > phosphoserine. The nuclear phosphatase was sensitive to denaturation by heat or urea treatments and was also inhibited by P_i, L-phenylalanine, homoarginine, dithiothreitol, and EDTA. The EDTA-inhibited enzyme was maximally reactivated by Zn^{2+}, although Mg^{2+}, or Ca^{2+} were also effective at somewhat higher concentrations.

Orchiectomy of adult rats resulted in an increase in the nuclear alkaline phosphatase activity (2—3-fold at 24 or 48 h postorchiectomy). A decline in the protein: DNA ratio also occurred following orchiectomy, but the increase in phosphatase specific activity was evident whether expressed per unit of protein or per unit of DNA. Testosterone replacement following orchiectomy abolished the increase in nuclear phosphatase activity. The results suggest that the prostatic nuclear alkaline phosphatase may be involved in events related to inactivation of the prostate nucleus following androgen deprivation.

Introduction

Mammalian alkaline phosphatases (EC 3.1.3.1) have been purified from a number of tissues [1—5] but at the present time it is not possible to assign specific functions to these enzymes. In general, these phosphatases have been localized to various portions of the cell surface and are considered to function

in transport phenomena, since the enzyme is abundant in secretory organs and developing tissues. However, cytochemical studies have also indicated the presence of alkaline phosphatase activity in nucleoli and chromatin granules of interphase nuclei, metaphase chromosomes of mitotic cells [6,8,10], and in nuclei of tumor cells [6—9] and in cells undergoing rapid division, such as embryonic [10] or regenerating tissues [6]. The functions of these nuclear alkaline phosphatases have not been established but it has been suggested that they include chromatin condensation. The localization of alkaline phosphatase activity in nuclear pores of human salivary gland tumor cells [9] has been taken to support the concept that the nuclear enzyme may also function in transport. The validity of histochemical demonstration of nuclear alkaline phosphatase, however, is controversial, the dispute being centered around possible artifacts in the specificity of lead precipitation procedures (e.g. refs. see 11—14). In the present investigation, we demonstrate, using biochemical means, that the prostate nucleus does contain a phosphatase activity optimal in the alkaline pH range. The data suggest that the prostatic nuclear alkaline phosphatase functions in the inactivation of the nucleus since its activity increases following orchiectomy (a time in which prostatic function is declining) and is suppressed by testosterone replacement.

Materials and Methods

Animals. Male Sprague-Dawley rats (300—350 g) were maintained on a standard laboratory diet and water ad libitum, and were kept on a 12.5 h light and 11.5 h dark cycle. Orchiectomy was performed under light ether anesthesia via the scrotal route. Testosterone-treated animals received a daily dose of testosterone propionate (1 mg/100 g body weight) in 0.2 ml sesame oil. Control animals received an equivalent volume of sesame oil.

Preparation of nuclear sonicate. Nuclei from pooled ventral prostates of 12—24 rats were prepared according to the procedure detailed previously [15]. The isolated nuclei were suspended in 0.34 M sucrose (1.5 ml/g wet weight of prostate) maintained in an ice bath and were sonicated for a total of 2 min (15-s sonication followed by a 15-s pause) using a Biosonik IV sonicator at a setting of 35. With this procedure, more than 99% of the nuclei were disrupted, as judged by light microscopy. The nuclear sonicate was stored frozen at −20°C.

Phosphatase assay. Unless described otherwise, nuclear-associated alkaline phosphatase was assayed by the following procedure. The reaction medium in a final volume of 1.0 ml contained 50 mM sodium carbonate/bicarbonate buffer and 3 mM *p*-nitrophenyl phosphate (Tris salt), final pH 9.68 at 37°C. The reaction was started by the addition of 70—130 μg of nuclear sonicate protein and was allowed to proceed at 37°C for 40 min. It was terminated by adding 1.0 ml of 10% (w/v) ice-cold trichloroacetic acid. The mixture was centrifuged at 7710 × *g* for 5 min, a 0.6-ml aliquot of the supernatant was removed and added to 2.4 ml of 1.0 M Tris base (pH 11.8). The amount of *p*-nitrophenol released was measured spectrophotometrically at 400 nm [16]. In enzyme assays with other substrates, the amount of P_i released was determined by the method of Martin and Doty [17]. All assays were performed in duplicate or triplicate and

individual assay values were within 7% of one another. Control assays were performed as above but with the omission of either the enzyme source or substrate; the combined values of these controls were subtracted from experimental values.

Other methods. Protein content of the nuclear sonicate was assayed by the method of Lowry et al. [18] using bovine serum albumin as standard. DNA was estimated by the method of Burton [19] with calf thymus DNA as standard.

Results

Effect of time course, pH, and Triton X-100 treatment. Utilizing the model phosphatase substrate, *p*-nitrophenyl phosphate, nuclear-associated phosphatase activity was linear through a reaction time of 60 min under the standard experimental conditions. The enzyme activity was not increased by including Triton X-100 (final concentration 0.1%, v/v) in the reaction medium or by added Mg^{2+}. Two peaks of activity were found by varying the pH; one was at pH 5.6—5.8 (50 mM sodium acetate buffer) and the other was in the pH range 9.5—10.3. However, when nuclei were prepared by including a wash with Triton X-100 [20], only 4% of the acid phosphatase activity was retained whereas 70% of the alkaline phosphatase activity was recovered associated with nuclei (Table I). Since treatment of nuclei with Triton X-100 is known to remove the outer envelope of the nuclear membrane [20], this suggests that the acid phosphatase activity is associated either with the outer nuclear envelope itself, or is present in possible adhering components such as endoplasmic reticulum or lysosomes. However, nuclei prepared by the methods employed here have very low microsomal or mitochondrial contamination as judged by enzyme markers [15]; lysosomal marker enzymes were not tested. These data also strongly suggest that the alkaline phosphatase activity is an integral part of the nucleus since only about 30% of the activity was lost following washing with Triton X-100 (Table I). This loss might result from leakage of the enzyme

TABLE I

THE EFFECT OF WASHING NUCLEI WITH TRITON X-100 ON PHOSPHATASE ACTIVITY ASSOCIATED WITH NUCLEI OF RAT VENTRAL PROSTATE

Triton washed nuclei were prepared by resuspending isolated nuclei in 0.25 M sucrose containing 1 mM $MgCl_2$ and 0.5% Triton X-100 (v/v) [20] and pelleting the nuclei by centrifugation. This was followed by two washes with 0.25 M sucrose containing 1 mM $MgCl_2$ and sonication treatment (see Materials and Methods). Acid phosphatase activity was measured in a total reaction volume of 1.0 ml containing 50 mM sodium acetate buffer and 3 mM *p*-nitrophenyl phosphate, final pH 5.50 at 37°C; alkaline phosphatase was as described in Materials and Methods. The values for the Triton-washed nuclei represent the mean of two independent preparations of nuclei and the numbers in parentheses represent percent activity compared to no Triton treatment. The results are expressed as nmol *p*-nitrophenol/h per mg protein.

Treatment	Acid phosphatase	Alkaline phosphatase
No Triton	491 (100)	435 (100)
Triton	22 (4)	306 (70)

TABLE II

THE SUBSTRATE SPECIFICITY OF NUCLEUS-ASSOCIATED ALKALINE PHOSPHATASE ACTIVITY OF RAT VENTRAL PROSTATE

Phosphatase activity was assayed as described in Materials and Methods except that various substrates (3 mM) were substituted for *p*-nitrophenyl phosphate. The relative activity is expressed with respect to the specific activity with *p*-nitrophenyl phosphate as substrate being set at 1.00.

Substrate	Relative activity
p-Nitrophenyl phosphate	1.00
β-Glycerophosphate	0.40
Phosphoserine	0.19
Phosphothreonine	0.70
Glucose 1-phosphate	0
Glucose 6-phosphate	0

from the nucleus due to changes in the permeability of the nuclear membrane following the detergent treatment.

Effect of various substrates. Nuclear alkaline phosphatase was active toward several other substrates, although the activity was considerably lower than with *p*-nitrophenyl phosphate. These substrates, in the order of reducing efficacy were phosphothreonine, β-glycerophosphate, and phosphoserine (Table II). No detectable activity was observed with glucose 1-phosphate or glucose 6-phosphate as the substrate.

Effect of various inhibitors. The effect of various phosphatase inhibitors on the nuclear alkaline phosphatase activity was determined. The enzyme was inhibited by P_i, L-phenylalanine and homoarginine (Table III). These compounds are known to inhibit alkaline phosphatases from other sources [3]. No inhibitory effect was found with NaF or sodium tartrate, whereas about 30% inhibition was observed in the presence of 30 mM sodium oxalate. These latter compounds are known to inhibit acid phosphatases [21]. The nuclear alkaline

TABLE III

THE EFFECTS OF VARIOUS PHOSPHATASE INHIBITORS ON THE ALKALINE PHOSPHATASE ACTIVITY OF RAT VENTRAL PROSTATE NUCLEI

The alkaline phosphatase activity was measured as described in Materials and Methods except that various inhibitors were included in the reaction medium as indicated. Aliquots of nuclear sonicate were preincubated in the presence of the inhibitor for 5 min at 37°C and the reaction initiated by the addition of *p*-nitrophenyl phosphate.

Inhibitor	Relative activity			
	30 mM	25 mM	10 mM	1 mM
None	1.00	1.00	1.00	1.00
Inorganic phosphate	0.10	—	—	0.93
NaF	0.93	—	—	1.02
L-Phenylalanine	—	0.32	0.61	1.01
Homoarginine	—	0.07	0.18	0.68
Sodium tartrate	0.98	—	—	1.02
Sodium oxalate	0.73	—	—	0.98

TABLE IV

THE EFFECT OF EDTA AND DITHIOTHREITOL ON THE ALKALINE PHOSPHATASE ACTIVITY OF RAT VENTRAL PROSTATE NUCLEI

Assays in the presence of EDTA were initiated by the addition of *p*-nitrophenyl phosphate (0.01 ml) to assay mixtures containing nuclear sonicate, 50 μmol carbonate/bicarbonate buffer, and the appropriate amount of EDTA (final pH 9.6) which had been preincubated 10 min at 25°C and 5 min at 37°C. The final reaction volume was 1.0 ml. Assays in the presence of dithiothreitol were performed as described in Materials and Methods except that the indicated amount of dithiothreitol was present.

Addition (mM)	Relative activity
None	1.00
EDTA	
0.005	0.92
0.025	0.67
0.100	0.06
Dithiothreitol	
0.50	0.87
1.00	0.70
4.00	0.09

phosphatase activity was not significantly altered with increased ionic strength (up to 300 mM NaCl in the reaction). However, it was inhibited by dithiothreitol (Table IV), suggesting that disulfide linkages may be important structural features of the enzyme in its active form. Rat liver alkaline phosphatase is a dimer whose monomers are linked covalently by disulfide bridges [22].

Effect of divalent cations. The enzyme activity was almost completely in-

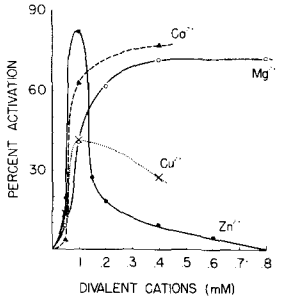

Fig. 1. The effect of varied divalent cations on alkaline phosphatase activity of nuclear sonicate of rat ventral prostate. Aliquots of nuclear sonicate were incubated for 10 min at 25°C and 5 min at 37°C in a medium which would be 50 mM with respect to Tris/glycine buffer (pH 9.68 at 37°C) and 0.1 mM sodium EDTA in a final reaction volume of 1.0 ml (0.1 mM EDTA gives >90% inhibition of alkaline phosphatase activity). The appropriate divalent cation was added to the reaction mixture and allowed to incubate with the enzyme source for 5 min at 37°C. The reaction was started by the addition of *p*-nitrophenyl phosphate and was terminated after 40 min by the addition of 1.0 ml 10% trichloroacetic acid. The *p*-nitrophenol released was assayed as described in Materials and Methods. The alkaline phosphatase activity was determined as the activity in the full reaction mixture minus the activity of controls in which substrate or enzyme were absent. Percent activation was determined with respect to alkaline phosphatase activity assayed under these conditions but with EDTA and exogenous cations omitted from the reaction medium.

hibited by EDTA at a concentration of 10^{-4} M (Table IV). Zn^{2+} was the most potent divalent cation in reactivating the nuclear alkaline phosphatase previously inhibited by EDTA (Fig. 1). More than 80% of the activity was found in the presence of 0.1 mM $ZnCl_2$; however, concentrations greater than 0.1 mM were inhibitory. Mg^{2+} and Ca^{2+} could also reactivate the enzyme (about 70% at a concentration of 0.4 mM). Cu^{2+} was able to restore about 40% of the phosphatase activity but was inhibitory at concentrations above 0.1 mM. Alkaline phosphatases have been shown to be Zn^{2+}-containing metalloenzymes [22—24]; in addition a second divalent cation site, principally for Mg^{2+} has been proposed [22]. The present data would suggest that the prostatic nuclear alkaline phosphatase may bind divalent cations in a similar manner.

Effect of heat and urea treatment. The nuclear alkaline phosphatase activity was sensitive to heat and urea denaturation. Prior incubations, for various lengths of time at 56°C or in the presence of different concentrations of urea (37°C), resulted in a progressive decrease in the enzyme activity (Table V); approx. 40% of the activity was lost by subjecting the enzyme to a temperature of 56°C for 15 min, and treatment with 3.2 M urea resulted in an almost complete inactivation of the enzyme.

Effect of orchiectomy and testosterone treatment. Prostatic nuclear alkaline phosphatase activity increased by 2—3-fold following orchiectomy (Table VI). This was in marked contrast to the acid phosphatase activity which did not change under the same conditions. The protein content of the nucleus declined with castration as is shown by the reduced protein: DNA ratio; however, the rise in phosphatase activity is evident whether the specific activity of the

TABLE V

THE EFFECTS OF TEMPERATURE AND UREA ON THE ALKALINE PHOSPHATASE ACTIVITY OF NUCLEI OF RAT VENTRAL PROSTATE

Treatment	Relative activity
Minutes at 56°C *	
0	1.00
5	0.90
15	0.60
30	0.36
Initial urea concentration (M) **	
0	1.00
1.6	0.64
3.2	0.03
4.8	0

* Sonicates of rat ventral prostate nuclei were preincubated at 56°C for various lengths of time in a volume of 0.52 ml. At the end of preincubation the assay tubes were placed on ice for 3 min and sodium carbonate/bicarbonate buffer (50 μmol), pH 9.68, was added, the reaction tube was placed at 37°C for 3 min and the reaction started by the addition of 3 μmol *p*-nitrophenyl phosphate. The final volume was 1.0 ml and the incubation time was 40 min.

** Nuclear sonicates were preincubated for 20 min at 37°C in the presence of the urea concentrations given in a volume of 0.50 ml. The reaction was started by addition of 50 μmol sodium carbonate/bicarbonate buffer, pH 9.68, and 3 μmol *p*-nitrophenyl phosphate in a volume of 0.50 ml. Thus, the final reaction volume was 1.0 ml and the initial urea concentration was reduced by 50% during the assay period (40 min).

TABLE VI

THE EFFECTS OF CASTRATION AND MAINTENANCE OF CASTRATE ANIMALS BY TESTOSTERONE PROPIONATE ON THE ALKALINE PHOSPHATASE ACTIVITY OF NUCLEI OF RAT VENTRAL PROSTATE

The numbers in parentheses represent the percentage of intact controls.

	N	nmol *p*-nitrophenol/ h per mg protein	nmol *p*-nitrophenol/ h per mg DNA	Protein/ DNA
Normal	8	435 ± 26 * (100)	1348 ± 65 (100)	3.32
Castration				
24 h	2	1042 ± 88 (240)	2568 ± 189 (190)	2.46
48 h	3	1263 ± 226 (290)	3138 ± 487 (232)	2.50
48 h + oil	1	1042 (240)	2960 (220)	2.84
48 h + testosterone	1	473 (109)	1742 (129)	3.69

* ± S.E.

enzyme is expressed per unit of protein or per unit of DNA. Testosterone replacement therapy to castrated individuals prevented the increase in alkaline phosphatase activity and also elevated the protein: DNA ratio. This suggests that androgens in some manner repress the activity of the nuclear alkaline phosphatase.

Discussion

The presence and stimulation by androgens of alkaline phosphatase in the prostate gland have been well established [25]. In the rat prostatic complex, alkaline phosphatase activity is concentrated predominately in the ventral lobe [26] and has been shown by histochemical techniques to be localized to the basal and luminal borders of epithelial cells [27]. The present investigations demonstrate that the nucleus of the ventral prostate is also a site of alkaline phosphatase localization. The nuclear alkaline phosphatase demonstrated two qualities which distinguish it from previously reported prostatic alkaline phosphatases: (1) it was inhibited by L-phenylalanine and (2) its activity was increased by androgen deprivation.

Inhibition of alkaline phosphatase activity by homoarginine is considered a property specifically of liver and bone type alkaline phosphatases [28] whereas inhibition by L-phenylalanine is a characteristic of intestinal and placental alkaline phosphatases [29]. The prostatic nuclear phosphatase was inhibited by both compounds but was more sensitive to homoarginine; and, in concord with this property (i.e. liver/bone type of alkaline phosphatases), the nuclear enzyme also demonstrated susceptibility to heat and urea inactivation [30, 31]. Since the nuclear phosphatase is inhibited by L-phenylalanine, it is probably different in character from the cytoplasmic enzyme. The 13% inhibition by phenylalanine of the total prostatic alkaline phosphatase activity observed by Wright et al. [32] may reflect the inhibition of the nuclear-derived portion of the phosphatase activity.

The total alkaline phosphatase activity of the rat ventral prostate is stimulated by testosterone [27,33], whereas the specific activity of the nuclear

enzyme, described in the present work, was found to increase upon orchiectomy and was suppressed by testosterone treatment. A possible explanation of this result is that alkaline phosphatases in different portions of prostatic epithelial cells have different androgen sensitivities and could be involved in different cellular processes. The bulk of prostatic alkaline phosphatase appears to be located in the basal and luminal plasma membranes [27]; these alkaline phosphatases could be involved in transport phenomena [3] and hence would conceivably be stimulated by androgens.

It would appear that a transport role is not the primary function of this enzyme in prostatic nuclei since its activity is increased following hormone deprivation, a time during which prostatic cellular activity is decreasing. Alkaline phosphatase has been localized by cytochemical means to nuclear pores of salivary gland tumor nuclei [9], suggesting a possible transport function. However, Franke et al. [34] did not find enrichment of alkaline phosphatase activity in nuclear membrane preparations (compared to whole nuclei) although nuclear pore complexes were present. On the other hand, possible physiological functions of prostatic nuclear alkaline phosphatase may include dephosphorylation of nuclear phosphoproteins or the condensation of chromatin. A role of the nuclear enzyme as a protein phosphatase would be suggested by its activity toward phosphothreonine and phosphoserine; however, prostatic nuclear phosphatase activity toward ^{32}P-labeled lysine-rich histone has a pH optimum of 7 and is not inhibited by L-phenylalanine or homoarginine (Wilson, M.J. and Ahmed, K., unpublished data). With respect to a possible function in the contraction of chromatin, it is of interest to note that the activity curve for alkaline phosphatase in developing chick brain correlates most strikingly with the change in mitotic index [10,35]. LeStourgeon et al. [36] have demonstrated an increase in the intranuclear concentrations of actin when non-proliferative states are induced which coincides with the condensation and inactivation of chromatin. In the rat ventral prostate there is a decrease in the number of nuclei within four days following orchiectomy [37]. The prostatic nuclear alkaline phosphatase may actually be the manifestation of an ATPase functioning in the chromatin condensation leading to nuclear pycnosis, since it has been shown that alkaline phosphatases in general possess ATPase activity as well [22,38]. In accord with this, the prostatic nucleus appears to contain both Mg^{2+}- and Ca^{2+}-stimulated ATPase activities (Wilson, M.J., Ahmed, K. and Fischbach, T.J., unpublished data). The function of the prostatic nuclear alkaline phosphatase and the mode of its regulation by androgens remain to be established.

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