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Tyrosyl Kinase Activity Is Inversely Related to Prostatic Acid Phosphatase Activity in Two Human Prostate Carcinoma Cell Lines

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Alterations in prostatic acid phosphatase (PAcP), a phosphotyrosyl phosphatase, corresponded to changes in overall tyrosyl kinase activity. PAcP added to extracts of prostate carcinoma cells with a low endogenous level of PAcP activity and elevated tyrosyl kinase activity decreased the tyrosyl kinase activity. On the other hand, when PAcP activity was decreased by the addition of androgens to cells, there was a corresponding increase in tyrosyl kinase activity.

Tyrosine phosphorylation of proteins is believed to play a major role in the control of cell proliferation. Evidence to support this belief includes the finding that a number of viral oncogenes and growth factor receptors encode tyrosine-specific protein kinases (1, 8, 22).

Phosphotyrosine levels in cells are regulated by tyrosyl kinases and by phosphotyrosyl phosphatases. The phosphatases may act on the phosphotyrosine-containing substrate proteins and may also directly regulate the activity of the tyrosyl kinases. The activity of tyrosyl kinases has been shown, in several cases, to be regulated by autophosphorylation and dephosphorylation of tyrosines (2, 4, 6, 20, 21, 26, 29).

Several phosphatases, including acid, neutral, and alkaline phosphatases, have been shown to dephosphorylate phosphotyrosine residues in various protein substrates (7, 13, 14, 19, 23, 24). One of these phosphatases is the major isoenzyme of prostatic acid phosphatase (PAcP) (13, 14), which is synthesized primarily in prostate tissue and is the most abundant phosphatase in that tissue (12, 28). The major species of PAcP preferentially dephosphorylates phosphotyrosine residues in proteins (13, 14) and has a very high affinity (K_m, 10^{-9} M) for phosphotyrosine-containing proteins (14).

Although several phosphatases have been observed to have phosphotyrosyl phosphatase activity in vitro, there is as yet no information to indicate which phosphatase may function to regulate phosphotyrosine levels in cells. In this study, we examined the tyrosyl kinase activity in prostate carcinoma cells under conditions where PAcP activity was increased by the addition of exogenous enzyme or decreased by the addition of dihydrotestosterone (DHT). The evidence presented here suggests an inverse correspondence between PAcP and tyrosyl kinase activity.

PAcP activity in prostate carcinoma cell lines. Because malignant prostate tissue has been reported to contain reduced levels of PAcP (18, 28), we chose to examine prostate carcinoma cell lines (DU145 and LNCaP [9, 10, 25]) for acid phosphatase activity, tyrosyl kinase activity, and phosphotyrosine levels. The acid phosphatase was measured in 30 μg of cell extract protein in 0.4 ml of 50 mM citrate at pH 6.0 with *p*-nitrophenyl phosphate as the substrate. The reaction was terminated by the addition of 2.0 ml of 0.1 N NaOH. The released *p*-nitrophenol was measured spectrophotometrically at 410 nm (14). The reaction rate was linear for at least 15 min at 34°C when PNPP was at a concentration of 3 mM (data not shown). We therefore compared the acid phosphatase levels from the two cell lines under these conditions of substrate saturation. LNCaP cells had about twice as much total cellular acid phosphatase activity as did the DU145 cells (Table 1). Tartrate sensitivity was used to distinguish between PAcP and other phosphatases, since tartrate is a specific inhibitor of PAcP which does not affect alkaline or most other acid phosphatase activities (12, 28). The differences in acid phosphatase activities in these cells were mainly from acid phosphatase activity inhibited by 10 mM tartrate (Table 1). To ensure that tartrate sensitivity reflected PAcP activity, we also measured the acid phosphatase activity that reacted with antibodies to PAcP (14). For both cell lines, the tartrate-sensitive activity was similar to the anti-PAcP activity (Table 1). LNCaP cells, therefore, had approximately 4.5-fold-higher PAcP activity than DU145 cells, while the tartrate-insensitive acid phosphatase activities were about the same.

Tyrosyl kinase activity in prostate carcinoma cell lines. Tyrosyl kinases are the only enzymes yet described that are phosphorylated on tyrosine residues in vivo and are affected in activity by their phosphotyrosine levels. We therefore chose to investigate whether differences in the levels of PAcP correspond to altered tyrosyl kinase activity. Because the tyrosyl kinase species in prostate cells have not yet been identified and different species may respond differently to tyrosine dephosphorylation, we began our investigations by measuring overall tyrosyl kinase activity in the cells with different PAcP levels. Extracts were prepared by suspending cells into 10 mM Tris (pH 7.0)–0.5% Nonidet P-40–0.5 mM dithiothreitol containing protease inhibitors 1 TIUAprotinin, 2 mM phenylmethylsulfonyl fluoride, and 4 μM leupeptin; all from Sigma Chemical Co., St. Louis, Mo.) and an inhibitor of PAcP and other phosphotyrosyl phosphatases, sodium orthovanadate at 25 μM (Fisher Scientific Co., Pittsburgh, Pa.). The overall tyrosyl kinase activity was determined with saturating amounts of angiotensin, a substrate that is phosphorylated exclusively at a single tyrosine residue by several different tyrosyl kinases (17, 27). Cell extract protein (12 μg) was added to a kinase reaction mixture containing 10 mM Tris (pH 7.0), 10 mM $MnCl_2$, 0.5 mM dithiothreitol, 0.5% Nonidet P-40, 20 μM ATP, 10 μCi

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TABLE 1. Acid phosphatase levels in human prostate carcinoma cell lines

Type of cell line	Phosphatase activity (nmol/mg per min)[a] in:	
	LNCaP	DU145
Total	59.5	34.4
Tartrate sensitive	28.8	6.3
Anti-PAcP sensitive	26.0	5.5

[a] Phosphatase activity was determined in 30 μg of cell extract protein with PNPP as a substrate as previously described (14). Tartrate-sensitive phosphatase activity was calculated by subtracting the activity remaining in the presence of 10 mM tartrate from the total activity. Cellular PAcP was precipitated by incubation of 30 μg of cell extract protein with rabbit anti-PAcP serum and control preimmune serum at 4°C for 2 h. After centrifugation of the immune complexes, the acid phosphatase activities in the supernatant were determined and were subtracted from the total to determine anti-PAcP-sensitive activity. Less than 3% of the total activity was precipitated from either cell extract with the control preimmune serum.

of [γ-^{32}P]ATP (New England Nuclear Corp., Boston, Mass.), 25 μM sodium orthovanadate, and 200 μg of angiotensin II (Sigma). To quantitate phosphorylated angiotensin, the reaction mixture was electrophoresed on paper at high voltage at pH 4.4 (17), and the ^{32}P-labeled angiotensin was quantitated by Cerenkov counting.

We consistently observed a two- to fourfold-higher overall tyrosyl kinase activity in DU145 cells compared with LNCaP cells. Figure 1 illustrates the results of a typical tyrosyl kinase assay in which DU145 cell extracts contained threefold-higher activity than the LNCaP cell extracts at the initial rate of the reaction (1.5 min of incubation). Similar results were obtained when endogenous cellular proteins

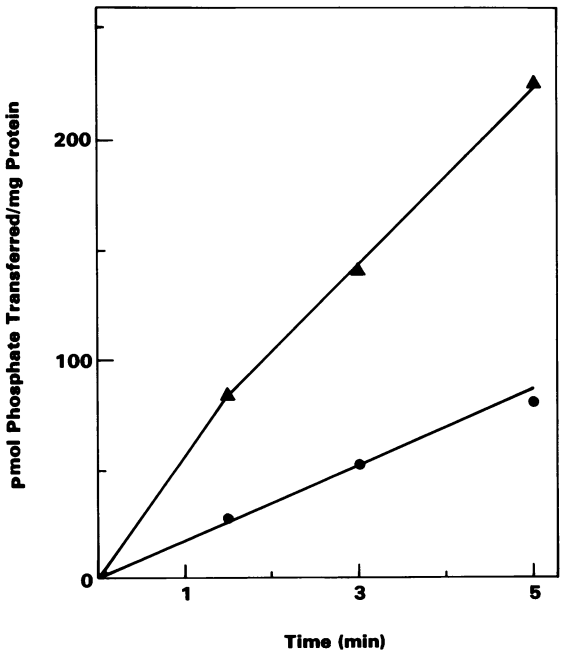

FIG. 1. Tyrosyl kinase activities in human prostate carcinoma cell extracts. Tyrosyl kinase activity was measured in LNCaP cells (●) and DU145 cells (▲), with angiotensin as the tyrosine-specific substrate. A 12-μg sample of protein from each cell lysate was added to the kinase reaction mixture, which was incubated at 34°C. The ^{32}P-labeled angiotensin produced in the kinase assays was isolated by high-voltage paper electrophoresis at pH 4.4 and was quantitated by Cerenkov counting.

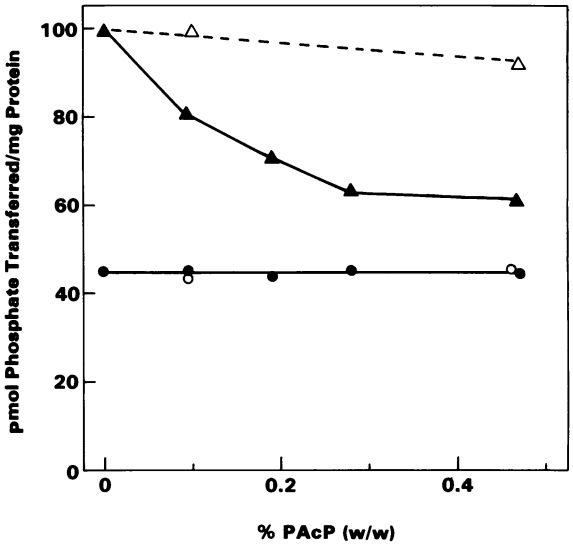

FIG. 2. Effect of PAcP on tyrosyl kinase activity in cell extracts. Tyrosyl kinase activity was assayed at 34°C for 1.5 min after the addition of purified active PAcP or heat-inactivated (65°C for 8 h) PAcP. The different amounts of added PAcP are expressed as the ratio of enzyme weight to cell protein weight. ▲, DU145 cells plus native PAcP; △, DU145 plus heat-inactivated PAcP; ●, LNCaP cells plus native PAcP; ○, LNCaP cells plus heat-inactivated PAcP.

were used as substrates and tyrosine phosphorylation was quantitated by phosphoamino acid analysis of the product (data not shown). It was possible that other components in the cell extracts, such as PAcP, significantly interfered with tyrosyl kinase activity determinations. To test this possibility, we incubated the product of the kinase reaction, ^{32}P-labeled angiotensin, with the DU145 as well as the LNCaP cell extracts in the kinase reaction mixture. Less than 5% of the product was lost during a 5-min incubation period (data not shown). Therefore, under these reaction conditions, loss of the product did not significantly affect tyrosyl kinase activity determinations.

Phosphotyrosine levels in cellular proteins corresponded to tyrosyl kinase activities. To compare the relative phosphotyrosine levels, the two prostate carcinoma cell lines were labeled for 17 h with $^{32}P_i$ in phosphate-free medium containing 2% dialyzed fetal calf serum. The ^{32}P-labeled proteins, obtained from about 10^6 cells for each line, were extracted with phenol followed by chloroform-methanol (5). The extracted proteins were subjected to partial acid hydrolysis (6 N HCl, 110°C, 2 h) and resolved by high-voltage paper electrophoresis (17). Quantitation of the ^{32}P-labeled phosphoserine, phosphothreonine, and phosphotyrosine residues obtained from triplicate experiments for each cell line revealed that the LNCaP cells (0.04% phosphotyrosine) had on the average a 2.5-fold-lower proportion of phosphotyrosine linkages than did the DU145 cell line (0.10% phosphotyrosine). In separate experiments, the differences between the two cell lines ranged from two- to fourfold, with DU145 cells always containing the higher levels of phosphotyrosine. Therefore, relative phosphotyrosine levels reflected the differences in tyrosyl kinase activities measured in the cell extracts (Fig. 1).

Purified PAcP inhibited tyrosyl kinase activity in cells with low phosphatase activity. Mixing experiments with lysates of the two cell lines indicated that the LNCaP cells, which had higher PAcP activity, contained a tyrosyl kinase inhibitor

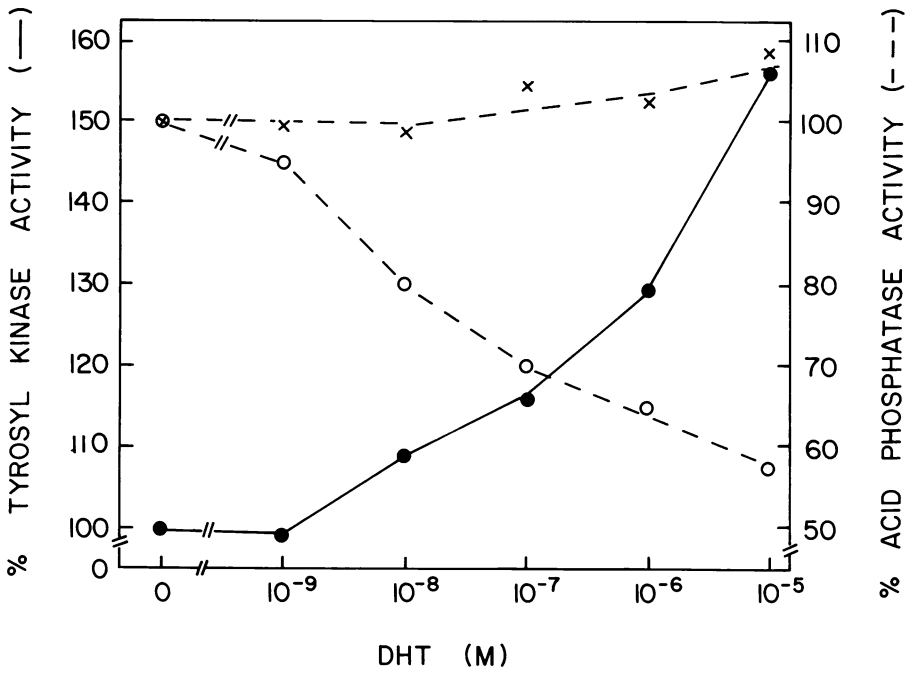

FIG. 3. Effects of DHT on tyrosyl kinase and acid phosphatase activities. Various concentrations of DHT, as indicated, were added to LNCaP cells that had been exposed to steroid-depleted media for 48 h. Cells in triplicate flasks were harvested after 48 h for quantitation of tyrosyl kinase activity (●), tartrate-sensitive acid phosphatase activity (○), and tartrate-insensitive acid phosphatase activity (×) as described in the text. The results shown are the means of triplicate assays where the range is ≤5%.

(unpublished observation). To further explore whether PAcP acts as a tyrosyl kinase inhibitor, the cell extracts, without vanadate, were incubated with PAcP. The PAcP used in this experiment was purified from human seminal plasma and was shown to have a single amino-terminal amino acid residue (14–16). After incubation on ice for 3 min with PAcP, the tyrosyl kinase activity was assayed at its initial velocity in the presence of vanadate to inhibit the PAcP. The tyrosyl kinase activity in the DU145 cell extracts was reduced by 30 to 50% by the addition of PAcP. In marked contrast, the tyrosyl kinase activity in the LNCaP cells, which contained the higher PAcP levels, was not inhibited by the addition of PAcP (Fig. 2).

We next sought to determine whether serine- and threonine-specific protein kinase activities were affected by the addition of purified PAcP or whether PAcP acted specifically on tyrosyl kinases. We measured [γ-^{32}P]ATP transfer to endogenous cellular proteins catalyzed by endogenous protein kinases in the same reaction mixture as described for the tyrosyl kinase assays, except that angiotensin was omitted. Phosphoamino acid analyses of the products revealed that more than 98% of the kinase activity was specific for phosphoserine and phosphothreonine (data not shown). Less than a 5% loss of overall kinase activity was observed during 5 min of incubation after the addition of PAcP. In the same experiment, the tyrosyl kinase activity was reduced about 30%, in agreement with the results shown in Fig. 2 (data not shown). Therefore, PAcP appeared to specifically inhibit tyrosyl kinase activity, but only in the cell extracts that contained low endogenous levels of PAcP.

Androgen effects on PAcP activity and tyrosyl kinase activity. It has previously been reported that PAcP activity is regulated by androgens in LNCaP cells which have retained their androgen receptors (9, 10). To test the effects of androgens, different concentrations of DHT were added to LNCaP cells in medium containing 1% heat-inactivated, dialyzed fetal calf serum. Upon the addition of DHT, the cellular PAcP activity was found to be reduced (Fig. 3). There was an approximately 45% reduction in tartrate-sensitive acid phosphatase activity (Fig. 3) at 10^{-5} M DHT that was also observed for the anti-PAcP-sensitive phosphatase activity (data not shown). In contrast, there was a slight increase observed in the tartrate-insensitive acid phosphatase activity. Therefore, not all acid phosphatases showed a decrease in activity after androgen treatment. When the PAcP activity was decreased, there was a negative correspondence in overall tyrosyl kinase activity. An increase in tyrosyl kinase activity of more than 50% was found in cells that had been treated with 10^{-5} M DHT (Fig. 3). To test whether a compound with a structure similar to that of DHT affected tyrosyl kinase or PAcP activity, cholesterol was added to LNCaP cells. Cholesterol (10^{-5} M) did not alter the kinase or PAcP activities (data not shown).

If the increase in tyrosyl kinase activity in the hormone-treated cell extracts was related to the decrease in PAcP activity, purified PAcP added to the extracts should inhibit the tyrosyl kinase activity. To examine this possibility, purified PAcP was added to the extracts of control cells and cells which had been treated with DHT (10^{-6} and 10^{-5} M) for 48 h. The initial rate of tyrosyl kinase activity was then determined in the presence of vanadate. Approximately 40 to 50% of the incremental increase in tyrosyl kinase activity in the DHT-treated cells was inhibited by the addition of PAcP (data not shown). The tyrosyl kinase activity in control LNCaP cell extracts that had not been treated with DHT was not detectably affected, in agreement with the results shown in Fig. 2.

These results demonstrate a negative correspondence between PAcP and tyrosyl kinase activity. Increased PAcP resulted in the inhibition of tyrosyl kinase activity, while

decreased PAcP corresponded to increased tyrosyl kinase activity. Since PAcP preferentially dephosphorylates phosphotyrosine (13, 14), one possibility is that the inhibition in tyrosyl kinase activity is caused by dephosphorylation of phosphotyrosine residues in tyrosyl kinases themselves. However, the possibility has not been ruled out that there are alternative mechanisms for the inhibition of tyrosyl kinase activity by PAcP.

Although PAcP appears to inhibit the overall tyrosyl kinase activity from some prostate carcinoma cells, regulation of tyrosyl kinase activity is undoubtedly very complex. The tyrosyl kinases may contain multiple tyrosine phosphorylation sites, some of which exert negative and some of which exert positive effects on the tyrosyl kinase activity. Moreover, there are serine and threonine residues in tyrosyl kinases whose phosphorylation as well as dephosphorylation may affect phosphotransferase activity (3). Multiple phosphotyrosyl phosphatases may also be interacting with the tyrosyl kinases. Indeed, vanadate, a phosphotyrosyl phosphatase inhibitor, has been found to increase phosphotyrosine levels in several different cell types (2, 11) as well as the phosphotransferase activity of Rous sarcoma virus-encoded pp60^{src} (2), presumably by the inhibition of phosphotyrosyl phosphatases. Because of the complexity of their regulation, it is of importance to determine which specific tyrosyl kinase in the prostate carcinoma cells may be altered in activity by PAcP and to determine the precise mechanism whereby PAcP inhibits their activity.

There are several isoenzymes of PAcP in prostate cells (15, 16, 28). One form of the isoenzyme is in secretory vesicles and is subsequently secreted. Another form is not secreted and is present in the cytoplasm as well as at the plasma membrane (28; Lee, unpublished observations). It is unclear which form of PAcP may interact with the cellular tyrosyl kinases.

The biological role of PAcP in prostate tissue has not been previously determined. PAcP levels have been found to be greatly reduced in prostate tumor tissue (18, 28). It is interesting that the LNCaP cells, which contain the higher PAcP activity and lower tyrosyl kinase activity, grow at less than half the rate of the DU145 cells. Future studies are aimed at determining whether PAcP, by regulating tyrosine phosphorylation levels, may play a role in cell growth control in prostate tissue. The prostate carcinoma cells may provide a useful experimental system to examine the relationships between cell growth, tyrosyl kinases, and phosphotyrosyl phosphatases.

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