Tyrosine Protein Kinase Activity of Human Hyperplastic Prostate and Carcinoma Cell Lines PC3 and DU145

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ABSTRACT

Using the substrate poly[Glu^8Na,Tyr^20] (poly[GT]) and the autoradiographic detection of alkali-resistant phosphoproteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis, tyrosine protein kinase (TPK) has been evidenced in human hyperplastic prostates (BPH) and the prostatic carcinoma cell lines PC3 and DU145. The enzyme was mainly found in the soluble fractions from hyperplastic tissues and in Triton extracts from the cell lines. However, its specific activity in tissues was 1.5- to 4.5-fold times higher in particularly in soluble fractions and it was of the same order of magnitude as that of neoplastic cells. Under these conditions, no activity was detected in human seminal plasma and in sera from normal adult males or patients with BPH and/or prostatic carcinoma. On the other hand, some TPK activity was associated with human spermatozoa, with a specific activity 4- to 6-fold lower than in BPH tissue fractions and a total activity, per 10^9 cells, 5- to 20-fold lower than that in prostatic carcinoma cells. The activity of prostatic TPK was dependent upon the presence of the divalent cations Mn^2+ or Mg^2+ and it was completely abolished by heat denaturation. Angiotensin II, casein, and histone H2B were poor substrates compared to poly[GT]. The TPK activities towards poly[GT] as well as endogenous proteins were not stimulated by epidermal growth factor and insulin or by dihydrotestosterone and estradiol. The autoradiography of alkali-resistant phosphoproteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed several bands in both BPH tissues and neoplastic cells (molecular weight ranging from 17,000 to 200,000). Preliminary characterization of TPK by gel filtration on Sephacryl S-300 showed that the soluble enzyme from BPH tissues had a molecular weight of 50,000, while the particulate-associated TPK, when assayed on poly[GT], eluted with proteins of M, 210,000. When these peak fractions were used for endogenous phosphorylation, several major alkali-resistant phosphoproteins in the range of M, 40,000-60,000 were evidenced, together with a M, 110,000 band phosphorylated by the particulate TPK of M, 210,000. In similar conditions, the TPK solubilized from rat liver membranes and partially purified by gel filtration was associated with a M, 170,000 alkali-resistant phosphoprotein. Thus, TPKs are expressed in BPH tissues and carcinoma cell lines in BPH tissues, two forms of TPK are expressed and the predominant enzyme is soluble and of low molecular weight (M, 40,000-60,000).

INTRODUCTION

In humans, the prostate gland has the propensity to undergo hyperplastic and neoplastic changes with advancing age. Even though testicular function is necessary for the development of these diseases (1), we have demonstrated that the proliferation of cultured epithelial cells from normal and/or hyperplastic human prostates is not stimulated by steroids but rather by nonsteroidal GF present in serum and prostatic extracts (2). Furthermore, EGF, insulin, and acidic fibroblast growth factor have been shown to modulate the growth of human prostatic epithelial cells in culture (3-5).

It has been reported that the regulation of cell division by GF, as well as cell transformation induced by several viral oncopogenes, involves the activation of tyrosine protein kinases (EC 2.7.1.37) (6). Indeed, most GF receptors possess, in addition to their hormone-binding capacity, an intrinsic TPK activity capable of autophosphorylation (6). Sex steroid receptors also appear to be phosphorylated by different kinases, namely by TPK (7). Furthermore, E2 stimulates tyrosine phosphorylation of its receptor in a cell-free system (8).

A TPK activity has been measured in the human prostatic carcinoma cells LNCaP and DU145 (9) but not in human BPH. The aim of the present investigation was to verify whether TPK is also expressed in human BPH tissues and, if so, to compare its activity with that of human prostatic carcinoma cells, DU145 and PC3, as well as spermatozoa, seminal plasma, and serum. Its activity was also measured in the presence of EGF, insulin, and steroids and its molecular weight was estimated by gel filtration, using subcellular fractions from BPH tissues.

MATERIALS AND METHODS

Tissues

Human hyperplastic prostates were obtained from cadavers (age, 50-80 years) within 8 h postmortem; they were dissected and frozen at −80°C until used. After thawing and mincing, the tissue was homogenized with a Polytron in ice-cold buffer A (20 mM Tris-HCl, pH 7.4; 0.08% 2-mercaptoethanol; 10 mM ethyleneglycol-bis-(β-aminethyl ether)-N,N,N',N' ‐tetraacetic acid; 5 mM EDTA; 1 mM phenylmethylsulfonyl fluoride; 10 mM benzamidine; 10 μg/ml leupeptin; and 10 μg/ml trypsin inhibitor units/ml of aprotinin). The nuclei and cell debris were removed by low speed centrifugation (3,000 x g, 30 min, 4°C) followed by centrifugation at 105,000 g for 90 min (4°C) to allow the separation of a soluble and a particulate fraction, which was resuspended in buffer A. Membranes from Sprague-Dawley rat liver were also prepared according to the method of Blackshear et al. (10). Protein concentrations were determined (11) and preparations were aliquoted and frozen at −80°C until assayed.

Cells

Human prostatic carcinoma cell lines DU145 and PC3 (generous gift of Dr. Patel, Centre Hospitalier de l'Université Laval, Quebec, Canada) were cultured at confluency in RPMI 1640 medium that was supplemented with 10% fetal calf serum, 100 μg/ml streptomycin, and 100 units penicillin/ml (Gibco Canada). Following their detachment from flasks with 1.5 mM EDTA in phosphate-buffered saline (Mg2+ and Ca2+-free), the cells were sedimented and frozen at −80°C until used. After thawing, they were resuspended in buffer A that contained 0.5% Triton X-100 and their proteins were extracted by gentle mixing for 2 min at room temperature, followed by a low speed centrifugation. The TPK activity was determined in Triton-soluble and -insoluble fractions.

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2 To whom requests for reprints should be addressed, at Maisonneuve-Rosemont Research Center, 5415 l'Assomption Blvd., Montreal, Quebec, Canada H1T 2M4.

3 The abbreviations used are: GF, growth factor; Poly(GT), poly[Glu^8Na,Tyr^20]; TPK, tyrosine protein kinase; BPH, benign prostatic hyperplasia; DHT, dihydrotestosterone; EGF, epidermal growth factor; PAP, prostatic acid phosphatase; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; E2, estradiol.
Serum, Spermatozoa, and Seminal Plasma

Human semen samples from fertile donors were sedimented at 1200 \( \times g \) (10 min) and the seminal plasma was recovered. The pellet was washed twice in 25 mM N-2-hydroxyethylpiperazine-N\(^{-}\)2-ethanesulfonic acid buffer, pH 7.4, containing 0.9% NaCl and 10 mM benzamidine and 5% cells were counted using an hemacytometer. They were then resuspended in buffer A that contained 0.5% Triton X-100.

Sera from normal adult males, as well as sera from patients with BPH and prostatic carcinoma (elevated PAP activity), were obtained from the Biochemistry Laboratory of Maisonneuve-Rosemont Hospital.

Tyrosine Protein Kinase Assay

Exogenous Substrates. The assays were performed in the linear portion of the curves of enzyme activity versus time and protein concentration. Subcellular fractions from BPH tissues and detergent extracts from prostatic carcinoma cells, solubilized spermatozoa, and serum (25 \( \mu \)g protein) were incubated for 20 min at room temperature in phosphatization buffer, which contained 20 mM Tris-HCl, pH 7.4; 0.08% 2-mercaptoethanol; 1 mM phenylmethylsulfonyl fluoride; 10 mM benzamidine; 10 mM MnCl\(_2\); 10 mM MgCl\(_2\); 0.5% Triton X-100; 100 \( \mu \)M Na\(_2\)VO\(_4\); 50 \( \mu \)g of one of the exogenous substrates [poly(GT); histone H2B, or casein; all from Sigma]; and 20 \( \mu \)M [\(^{32}\)P]ATP (2 \( \times 10^7 \) cpm/pmol) (Dupont, Canada, Inc.), in a final volume of 100 \( \mu \)l. The reaction was stopped by the addition of unlabeled ATP (20 mM final concentration) and NaOH (0.9 \( \mu \)l final concentration), followed by heating at 90\(^\circ\)C for 10 min (12). The proteins were precipitated by the addition of TCA to attain, after neutralization, a final concentration of 10%. The precipitate was recovered on a Whatman no. 3 AH disc filter paper, and washed 3 times with 5 ml of a mixture of ice-cold 5% TCA that contained 10 mM NaH\(_2\)PO\(_4\), and 10 mM Na\(_2\)HPO\(_4\), and 3 times with 5 ml of cold 95% ethanol. The filters were then counted in a Packard scintillation fluid (Du Pont).

In order to study the phosphorylation of angiotensin II (Sigma), prostatic fractions were incubated as described above with 200 \( \mu \)g of substrate. After protein precipitation with TCA, an aliquot of the soluble fraction was hydrolyzed in the presence of 1 N HCl for 25 min at 100\(^\circ\)C and the radioactive phosphate released from ATP was extracted with isobutanol-benzene in the presence of ammonium molybdate (13). The radioactivity remaining in the aqueous phase (peptide fraction) was determined by liquid scintillation in a Packard 963 fluid.

Endogenous Proteins. Subcellular fractions from BPH tissues and detergent extracts from neoplastic cells (100 \( \mu \)g protein) were incubated for 20 min at room temperature in the same phosphatization buffer that contained 2 \( \times 10^7 \) cpm/pmol [\(^{32}\)P]ATP but no exogenous substrate. The reaction was stopped by the addition of 20 mM unlabeled ATP and precipitation with TCA. The proteins in the pellet fraction were then neutralized with saturated Tris, solubilized in 100 mM Tris-HCl, pH 6.8, that contained 2% SDS, 5% 2-mercaptoethanol, 15% glycerol, and 0.01% bromphenol blue, and resolved by SDS-PAGE on a gradient of 7–15% polyacrylamide (14). After fixation with glutaraldehyde (15), the gels were soaked in 1 N KOH at 56\(^\circ\)C for 2 h, stained with Coomassie blue, dried, and submitted to autoradiography to detect proteins enriched in phosphate linked to their tyrosine residues (alkali-resistant phosphoproteins) (16–18).

The TPK activity of prostatic fractions from BPH tissues and cell lines was also measured in the presence of mouse EGF (50 ng) (Sigma), human insulin (50 nm) (Eli Lilly and Co., Indianapolis, IN), DHT (10 nm), and E\(_2\) (10 nm) (Steraloids). Fractions were preincubated for 15 min at 24\(^\circ\)C with the hormones; the phosphorylation buffer and [\(^{32}\)P]ATP were then added (10) and the phosphorylation reactions of both poly(GT) and endogenous proteins were allowed to proceed as described above.

Phosphoamino Acid Analysis

Because of the covalent cross-linking of alkali-resistant phosphoproteins in glutaraldehyde-fixed, KOH-treated gels, phosphoamino acid analysis was performed on selected proteins extracted from untreated gels. The soluble fraction from BPH tissues was phosphorylated and processed through SDS-PAGE as described above. Following fixation in 10% methanol-7% acetic acid, the gel was sectioned longitudinally and a portion was further processed through glutaraldehyde cross-linking, KOH treatment, Coomassie blue staining, and autoradiography. This allowed the calculation of the R\(_f\) values of alkali-resistant phosphoproteins. This portion of the gel was then matched with the untreated gel, also stained with Coomassie blue, to select bands of R\(_f\) corresponding to those of the alkali-resistant phosphoproteins. These bands were cut, homogenized in 1% SDS, heated for 30 min at 90\(^\circ\)C, and precipitated overnight in 5 volumes of acetone (–20\(^\circ\)C). The pelletted proteins were solubilized in 1 ml of 6 N HCl and then partially hydrolyzed at 110\(^\circ\)C for 90 min to minimize hydrolysis of phosphotyrosine, which is highly labile in acid medium (19). After evaporation of HCl under nitrogen, the hydrolysates were solubilized in a solution of formic acid, acetic acid, and water (25:78:879). Following the addition of internal phosphoamino acid standards, they were separated by twodimensional thin layer electrophoresis on cellulose plates with a mixture of (a) formic acid:acetic acid:water (25:78:879, v/v; 1700 V, 50 min), pH 1.9, and (b) pyridine:acetic acid:water (5:50:945, v/v; 1500 V, 45 min), pH 3.5 (18). The plates were then stained with ninhydrin (0.2% in acetone) to localize the standards and were submitted to autoradiography at –80\(^\circ\)C in the presence of intensifying screens to detect radiolabeled phosphoamino acids.

Gel Filtration

For gel filtration studies, particulate fractions from BPH tissues, as well as membrane fractions from rat liver, were resuspended with a Potter homogenizer in buffer A that contained 0.5% Triton X-100, 8 mM 3-[3-(cholaminopropyl)dimethylamino]-1-propanesulfonate, and 200 mM KCl and were extracted for 30 min on ice (with occasional stirring). After centrifugation at 105,000 \( \times g \) for 60 min (4\(^\circ\)C), aliquots of these extracts (recycling of 70% of TPK activity), as well as soluble fractions from BPH tissues, were chromatographed at 4\(^\circ\)C on a Sephacryl S-300 column (1.5 x 50 cm) equilibrated in 25 mM Tris buffer, pH 7.4, that contained 10% glycerol, 100 mM NaCl, 0.5% Triton X-100, 0.5 mM dithiothreitol, and 100 mM Na\(_2\)VO\(_4\). Fractions (1 ml) were collected and their TPK activity was assayed on poly(GT). Peak fractions were then allowed to undergo endogenous phosphorylation by incubation with [\(^{32}\)P]ATP and the resulting alkali-resistant phosphoproteins were analyzed after SDS-PAGE, as described above. Thyroglobulin (M\(_r\), 670,000), \( \gamma \)-globulin (M\(_r\), 158,000), ovalbumin (M\(_r\), 44,000), myoglobin (M\(_r\), 17,000), and vitamin B\(_12\) (M\(_r\), 1,350) (Bio-Rad) were used as standards for column calibration. The void volume of the column, \( V_v \), was determined with blue dextran while the total volume (\( V_t \)) was calculated by the dimensions of the column.

RESULTS

Phosphorylation of Exogenous Substrates. The TPK activities of four BPH tissues and the cell lines DU145 and PC3 towards the exogenous substrate poly(GT) are shown in Table 1. The specific activities of the kinase present in particulate fractions were 1.5- to 4.5-fold higher than those determined for the corresponding soluble fractions. The total TPK activity, defined as the sum of the activity found in the two fractions, varied between 73 and 383 pmol/min/g of tissue and was principally found in the soluble fraction (60–89%). Table 1 also shows that the TPK activity of DU145 and PC3 cells was mainly recovered in Triton extracts, which contained over 90% of the total TPK activity and 77–85% of the proteins. In both cell lines, the specific activity of TPK was higher in this fraction, as compared to that found in the Triton-insoluble material. On a cell basis, PC3 cells contained 1.9-fold more TPK activity compared to DU145 cells. By comparison to prostatic fractions, human seminal plasma, as well as sera from normal adult males and from patients with BPH or prostatic cancer (elevated PAP activity), was devoid of TPK activity (Fig. 1). On the other hand, human spermatozoa were slightly active, the specific activity of their TPK representing 12% of the activity of the particulate fractions from BPH tissues. On a cell basis, the
were very active, as compared to insoluble material. Indeed, TPK activity per spermatozoa (1-2 pmol/min/10^6 cells) was sera. Activity in soluble (Lane 3) and particulate (Lanes 1, 2, and 4) fractions from BPH tissues; Lane 1 shows the effect of a preincubation at 90°C for 10 min; Lane 2 represents TPK activity in the absence of divalent cations: Lane 4 is the standard assay. TPK activities in spermatozoa (Lane 5), seminal plasma (Lane 6), and sera from normal males (Lane 7) and patients with BPH (Lane 8) or prostatic carcinoma with elevated PAP (Lane 9) are also shown.

TPK activity per spermatozoa (1-2 pmol/min/10^6 cells) was also low, compared to that of human prostatic DU145 and PC3 cells (10-19 pmol/min/10^6 cells). As illustrated in Fig. 1, a 10-min preincubation of the particulate fraction from BPH tissues at 90°C (Fig. 1, Lane 1), as well as the deletion of Mn^2+ and Mg^2+ from the incubation mixture (Fig. 1, Lane 2), completely abolished prostatic TPK activity (compare to Fig. 1, Lane 4). When casein, histone H2B, or angiotensin II were used as substrates with the prostatic subcellular fractions, no phosphorylation was detected (data not shown). The addition of EGF and insulin, as well as DHT and E2, to TPK assays did not significantly alter the phosphorylation of poly(GT) by hyperplastic tissue fractions and neoplastic cell extracts (P < 0.05, Student’s t test) (not shown).

Endogenous Protein Phosphorylation. The TPK activity of soluble and particulate fractions from four BPH tissues (Fig. 2A), as well as Triton extracts and insoluble fractions from DU145 and PC3 cells (Fig. 2B), was also studied using the autoradiographical detection of alkali-resistant prostatic phosphoproteins. The soluble fractions from hyperplastic prostates share at least five or six major alkali-resistant phosphoproteins of M, 200,000, 84,000, 55,000, 46,000–43,000, and 17,000. With their corresponding particulate fractions, the phosphoprotein bands of M, 84,000–82,000 and 46,000 were detected in all tissues. An increasing order of phosphorylation from tissues c, b, d, and a could be observed with either poly(GT) or the endogenous proteins as substrates.

Fig. 2B shows the alkali-resistant phosphoproteins of the neoplastic cell lines. The Triton extracts of PC3 and DU145 were very active, as compared to insoluble material. Indeed, two major phosphorylated bands of M, 93,000 and 55,000 were detected with Triton extracts, while only minor phosphorylated bands were observed with Triton-insoluble material.

As for the phosphorylation of poly(GT), endogenous alkali-resistant protein phosphorylation with soluble and particulate fractions of BPH tissues as well as with Triton extracts of both cell lines was not altered when performed in the presence of EGF, insulin, DHT, and E2.

To determine whether phosphotyrosine was present in prostatic phosphoproteins, the soluble fraction, which contains the majority of TPK activity in BPH tissues, was used for phosphorylation. The four bands corresponding to major alkali-resistant phosphoproteins (pp200, pp84, pp55, and pp45) were extracted from untreated gels, hydrolyzed in HCl, and submitted to phosphoamino acid analysis. Fig. 3 shows that, for identical times of exposure to X-ray films, pp55 and pp45 contained the highest levels of phosphotyrosine. Due to the extraction of phosphoproteins from untreated gels, important quantities of phosphoserine and phosphothreonine were also detected.

Partial Characterization of TPK from BPH Tissues. As illustrated in Fig. 4A, gel filtration on Sephacryl S-300 of soluble fractions from BPH tissues yielded the elution of one major peak of TPK activity [on poly (GT)] at M, 50,000 together with two minor peaks at V0 and 210,000. For the particulate fractions, the same three peaks of TPK activity were eluted from the column, except that the highest activity was associated with the M, 210,000 peak (Fig. 4B).

When the fractions corresponding to these peaks (V0 and M, 210,000 and 50,000) were used for endogenous phosphorylation, several alkali-resistant phosphoprotein bands were seen (Fig. 5). For all fractions, the relative labeling of polypeptides of M, 40,000 to 60,000 was the most important, along with a M, 110,000 band phosphorylated by the M, 210,000 peak from particulate fractions. When extracts from rat liver membranes were used as a source of EGF receptor, the active peak on poly(GT) eluted with an apparent molecular weight of 200,000 (not shown) and this fraction supported the endogenous phosphorylation of one major alkali-resistant phosphoprotein band of M, 170,000 (Fig. 5).

DISCUSSION

Alkali-resistant protein phosphorylation is frequently used to study TPKs and their substrates (16–18). This method, together with the phosphorylation of the substrate poly(GT), led to a clear demonstration of the presence of a tyrosine protein kinase in human BPH. The absence of phosphorylation after heat denaturation of prostatic extracts, as well as the absolute requirement of the divalent cations Mg^2+ and Mn^2+ for activity, is also in favor of an enzymatic entity. Its activity in BPH tissues was of the same order of magnitude as in PC3 and DU145 human prostatic carcinoma cell lines and was also elevated compared to that of human spermatozoa. It is therefore unlikely that intraprostatic spermatozoa (20) contribute significantly to the TPK activity of BPH tissues. The lack of activity in seminal plasma also suggests that prostatic TPK is not normally secreted. It has been reported that TPK activity might be elevated in sera of patients with different types of cancers (21), but the present data show that sera of patients with prostatic cancers (high PAP activity), as well as with BPH, were devoid of TPK activity.

In BPH tissues, the particulate fraction contained only 17 to 40% of the total TPK activity but its specific activity was 1.5- to 4.5-fold higher than the TPK present in the soluble fraction. The high recovery of TPK in the soluble fraction might partly

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Fig. 1. Tyrosine protein kinase activity of BPH tissue fractions, sperm, and sera. Activity in soluble (Lane 3) and particulate (Lanes 1, 2, and 4) fractions from BPH tissues; Lane 1 shows the effect of a preincubation at 90°C for 10 min; Lane 2 represents TPK activity in the absence of divalent cations; Lane 4 is the standard assay. TPK activities in spermatozoa (Lane 5), seminal plasma (Lane 6), and sera from normal males (Lane 7) and patients with BPH (Lane 8) or prostatic carcinoma with elevated PAP (Lane 9) are also shown.
Fig. 2. Autoradiography of alkali-resistant phosphoproteins labeled by prostatic TPK from BPH tissue fractions and prostatic carcinoma cells after SDS-PAGE. Fractions from BPH tissues and cell lines were phosphorylated (100 µg protein for each) by incubation with [γ-32P]ATP, as described in “Materials and Methods,” and processed through SDS-PAGE, glutaraldehyde cross-linking, alkali treatment, and autoradiography. A, soluble (lanes 1, 3, 5, and 7) and particulate (lanes 2, 4, 6, and 8) fractions prepared from different hyperplastic prostates (a-d) (see Table 1). B, Triton extracts (lanes 1 and 3) and insoluble material (lanes 2 and 4) from PC3 (a) and DU145 (b) prostatic carcinoma cells. The position of protein standards as well as their molecular weight are also indicated. The Kodak XAR films were exposed at —80°C for 4 h [A (a) and B (a and b)] or 17 h [A (b–d)], in the presence of Cronex intensifying screens.

Fig. 3. Autoradiographic detection of phosphoamino acids. Labeled phosphoproteins from soluble fractions of BPH tissues were processed through SDS-PAGE, identified by autoradiography on alkali-treated gels (pp200, pp84, pp55, pp45), extracted from untreated gels, and hydrolyzed in HCl, as described in “Materials and Methods.” Following thin layer electrophoresis, phosphoamino acid standards were located by ninhydrin staining and their labeling by autoradiography. The first dimension, at pH 1.9, was from left to right and the second, at pH 3.5, from bottom to top, pS, phosphoserine; pT, phosphothreonine; pY, phosphotyrosine (1 week of exposure to Kodak XAR film with Cronex intensifying screen at —80°C).

Fig. 4. Gel filtration on Sephacryl S-300 of subcellular fractions from BPH tissues. Soluble (5.3 mg protein) fractions and extracts from particulate fractions (3.2 mg protein) were chromatographed on S-300 columns, as described in “Materials and Methods.” The TPK activity from soluble (A) and particulate extract (B) fractions was assayed by the phosphorylation of poly(GT). In A, the position of protein standards used for column calibration is also indicated, as well as $V_c$ and $V_e$. The TPK activity would correlate with the degree of cell activation in proliferative processes remains to be established. Interestingly, in human breast cancer the soluble TPK activity is significantly higher than in normal or benign breast tissues (22). On the other hand,
the localization of TPK at the plasma membrane is usually associated with the transmembrane mechanism of action of GF leading to cell proliferation (6).

Our data from gel filtration indicate that the majority of TPK activity in BPH tissues (soluble fractions) can be attributed to one peak of enzyme with apparent molecular weight of 50,000. This fraction catalyzed the alkali-resistant phosphorylation of multiple peptides extending from M, 40,000 to 60,000 and, principally, M, 55,000. This protein was also shown to contain phosphotyrosine and was highly phosphorylated by the two minor peaks (Vt and M, 210,000) of soluble fractions. The particulate TPK was mainly associated with proteins of molecular weight 210,000, leading to the phosphorylation of a major alkali-resistant band of M, 50,000, along with a M, 110,000 band that was less intensively labeled. The M, 50,000 alkali-resistant phosphoprotein band was also labeled when the two minor peaks of particulate TPK, eluted at Vt and at M, 50,000, were used. Because of the autopolyphosphorylation properties of TPKs (6), it may be suggested that TPKs are present among these alkali-resistant phosphoproteins that correspond to the peak fractions from S-300. It is noteworthy that, when extracts from rat liver membranes were used as a source of EGF receptor, the major alkali-resistant phosphoprotein detected after autoradiography was of M, 170,000, a value in the range of that reported for its TPK (6). It may, therefore, be concluded that, if EGF receptors are present in BPH membranes, their TPK activity is likely to be low. This is reinforced by our data on the measurement of TPK activity of BPH tissue fractions and cell lines in the presence of EGF, together with those of substrate specificity. A similar situation also applies to the TPK of the insulin receptor (M, 95,000) (6). Indeed in both systems, their autopolyphosphorylation and the phosphorylation of poly(GT) are stimulated by their corresponding growth factor (10, 23) and these enzymes can phosphorylate casein (12, 24) and histones (25). Under our experimental conditions, BPH tissue fractions were unable to phosphorylate angiotensin II, a substrate reported to be phosphorylated by extracts of LNCaP and DU145 human prostatic carcinoma cell lines (9). Since the presence of EGF receptors has been demonstrated in the prostatic carcinoma cell line LNCaP (26), investigations are in progress to determine whether TPKs from BPH tissues differ from that of cultured prostatic carcinoma cells and prostatic cancer tissues. The possibility that the low molecular weight soluble TPK would be due to pp60vic appears unlikely, since this TPK phosphorylates both angiotensin II (13, 27) and casein (12). However, it remains to be established whether these low molecular weight TPKs could be interrelated.

From several studies on the growth of human prostatic epithelial cells in culture (2–5, 28, 29), it appears that the relation between steroids and growth factors is still unclear. On the other hand, steroid receptors can be phosphorylated by protein kinases, including TPKs (7), and E2 can stimulate the phosphorylation of its own receptor on tyrosyl residues (8). The addition of DHT and E2 in both types of assay for prostatic TPK did not alter its activity in fractions obtained from BPH tissues or cell lines.

In conclusion, TPKs are present in human BPH tissues and prostatic carcinoma DU145 and PC3 cells and their activities in these tissues and cells are elevated compared to human spermatozoa, seminal plasma, and sera and do not appear to be related to EGF or insulin receptors. The major TPK in BPH tissues is soluble and of low molecular weight. Since the expression of tyrosine protein kinase appears to be a prerequisite to cell proliferation (6), it is important to characterize these enzymes in normal, BPH, and cancerous prostate in order to evaluate their role in the development of these diseases, as well as to evaluate similarities or differences in their expression in these tissues.

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