Chromatin-associated Protein Kinases in Human Normal and Benign Hyperplastic Prostate

Ahmed Rayan, Said A. Goueli, Paul Lange, and Khalil Ahmed

ABSTRACT

Nuclear phosphoproteins and protein kinases of human normal and benign hyperplastic prostate (BPH) were studied in an effort to delineate their properties and to identify any underlying differences therein. Chromatin-associated protein kinases active towards phosvitin, lysine-rich histone, and endogenous nonhistone proteins were characterized in human prostatic nuclei. The general properties of the human chromatin-associated prostatic protein kinases were similar to those of rat ventral prostate chromatin. Polyamines stimulated the phosphorylation of endogenous nonhistone proteins and phosvitin. Protein kinases active towards phosvitin and lysine-rich histones were unaltered in BPH tissue as compared with the normal prostate. However, phosphorylation of chromatin-associated nonhistone proteins was markedly enhanced (average, 123%) in BPH tissue as compared with the normal tissue. The results indicate a change in the protein kinase reaction specifically involving chromatin-associated nonhistone proteins of BPH tissue as compared with normal human prostate.

INTRODUCTION

Abnormal growth of the prostate, as reflected in the incidence of BPH and carcinoma, is a very common pathology in the aging human male (33, 34). Since androgens were implicated in the pathogenesis of these diseases (21, 22, 24), a number of parameters related to the interaction of testosterone with its target cells have been examined. These have included investigations of 5α-reductase activity, various testosterone metabolites, and androgen receptor proteins in the human and animal prostate (16, 25, 27, 31, 35).

Increased 5α-reductase activity and levels of 5α-DHT in the BPH tissue have been reported (13, 31, 35). It is generally accepted that testosterone exerts its effect on the growth and function of the prostatic gland through interaction of the 5α-DHT-receptor complex with chromatin and consequent increase in transcriptional activity (see, e.g., Refs. 23, 28, 30, 37, and 38). It has also been suggested that chromosomal proteins (especially nonhistone proteins and their phosphorylated derivatives) may play a regulatory role in the control of transcription (for reviews, see, e.g., Refs. 1, 8, 9, 11, 26, and 36). In this regard, we have provided evidence that rat ventral prostatic nuclear protein kinase reactions involved in the phosphorylation of nonhistone proteins are profoundly influenced by the androgenic status of the animals (2–6, 8, 9, 19). In particular, the rat ventral prostatic nuclear protein kinase activities (towards phosvitin and nonhistone proteins) were significantly reduced within 12 h following androgen deprivation in the adult rat. Results indicated that control of these protein kinase reactions in the prostate was mediated via the androgen-receptor complex mechanism, so that the changes in the nuclear protein kinase activity occurred in concert with the changes in the nuclear androgen receptor. Based on these studies, we have postulated that certain nuclear cyclic AMP-independent protein kinases may play a role in the mediation of androgen action in the prostate. In view of this, it would be of interest to determine the nature of the human prostatic nuclear protein kinases and to identify any changes which may occur in pathologies such as BPH and prostatic carcinoma. The present work is the first to describe the general properties of the normal human and benign hyperplastic prostate nuclear protein kinase reactions. We have found that the properties of these nuclear-associated protein kinase reactions are similar to those observed with the nuclear preparations from rat ventral prostate. However, the rate of phosphorylation of endogenous chromatin-associated proteins of human prostatic tissue was significantly higher in chromatin isolated from BPH tissue than that from normal prostate.

MATERIALS AND METHODS

Prostatic Tissue. BPH tissue was obtained from patients undergoing open prostatectomy or transurethral resection surgery, or from cadavers at autopsy (within 15 h of death). A total of 81 BPH samples was obtained (12 from open prostatectomy, 56 from transurethral resection, and 13 from autopsy). All BPH samples were from individuals varying in age from 66 to 85 years. Nine normal prostate glands (from individuals less than 40 years of age) were obtained at the time of autopsy. The tissue specimens were cut into thin pieces, frozen on dry ice, and stored frozen at −20°C until used. The evaluation of BPH was carried out by the Pathology Studies Committees of the University of Minnesota and the Minneapolis Veterans Administration Medical Center.

Preparation of Nuclei and Chromatin. The frozen tissue was thawed in 2.0 ml sucrose containing 1 ml PMSF, 1 ml MgCl₂, and 3 ml BME on ice and minced with scissors, and a 20% homogenate was prepared using a Tissuemizer (Tekmar, Cincinnati, OH) operated at a setting of 6 for a total of 1.5 min (alternating 30-s homogenization and 30-s rest periods). This was followed by homogenization (6 strokes) with a Potter-Elvehjem tissue grinder using a loose-fitting Teflon pestle (0.005- to 0.010-inch clearance) and filtration of the homogenate through 2 layers of nylon bolting cloth (110 mesh). The filtrate was adjusted to 2.18 m

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3 The abbreviations used are: BPH, benign prostatic hypertrophy or hyperplasia; DPV, partially (about 30%) dephosphorylated phosvitin; LRH, lysine-rich histone; 5α-DHT, 5α-dihydrotestosterone; PMSF, phenylmethylsulfonyl fluoride; BME, 2-mercaptoethanol.

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sucrose by adding 2.4 M sucrose containing 1 mM PMSF, 1 mM MgCl₂, and 3 mM BME, layered over a 3.0-M cushion of 2.2 M sucrose (containing 1 mM MgCl₂, 3 mM BME, and 1 mM PMSF), and centrifuged for 80 min at 78,000 × g in a Beckman SW-27 swinging bucket rotor. The nuclei were washed twice with 0.25 M sucrose, containing 1 mM MgCl₂ and 1 mM PMSF, by centrifugation for 5 min at 1,000 × g each time. These nuclei were used to prepare water-soluble chromatin as described previously (5). The ratio of protein to DNA in chromatin preparations from nuclei obtained from normal as well as BPH prostate was 1.5 to 2.5, regardless of the method of tissue procurement.

**Protein Phosphokinase Assays.** Protein phosphokinase activities were determined by measuring the rate of transfer of 32P from [γ-32P]ATP into 2 exogenous protein substrates, basic protein substrate LRH and the model acidic phosphoprotein DPV, as well as into endogenous chromatin proteins (5, 10, 17, 19). The reaction medium for assaying the protein kinase activity toward LRH as substrate contained 4 mM MgCl₂, 1 mM dithiothreitol, 30 mM Tris-HCI (pH 7.6 at 37°C), LRH (3 mg/ml), and 0.1 mM [γ-32P]ATP (specific radioactivity, 2000 dpm/nmol ATP) in a final reaction volume of 0.50 ml. The standard reaction medium for measuring protein kinase activity toward DPV consisted of 5 mM MgCl₂, 200 mM NaCl, 1 mM dithiothreitol, 2 mg DPV/ml, 3 mM [γ-32P]ATP (specific radioactivity, 3000 dpm/nmol), and 30 mM Tris-HCI (pH 7.4 at 37°C) in a final volume of 0.50 ml. The reactions were started by the addition of chromatin (equivalent to 1.5 to 5 µg of DNA) and were carried out for 30 min with DPV and for 20 min with LRH as substrates. The rate of phosphorylation of endogenous chromatin proteins was determined as described previously (10). The reaction mixture in this case contained 30 mM Tris-HCl (pH 7.6 at 37°C), 5 mM MgCl₂, 1 mM dithiothreitol, 24 mM NaCl, 0.1 mM [γ-32P]ATP (30,000 dpm/nmol), and chromatin (equivalent to 5 to 20 µg of DNA) in a final volume of 0.50 ml. The reaction was initiated by the addition of chromatin and was carried out for 1 min. The above protein kinase reactions were terminated by the addition of 0.50 ml of 30% (w/v) trichloroacetic acid containing 2 mM P⁰I and 5% Na₂P₂O₅. The precipitated protein was washed and prepared for the measurement of radioactivity as described earlier (5, 17). In all protein kinase assays using exogenous protein substrates, suitable controls were included in the experiments to take into account the incorporation of radioactivity into chromatin proteins as well as the nonspecific binding of radioactivity to added exogenous protein substrates. These control values were subtracted from the total to calculate the radioactivity incorporated into the appropriate experimental substrate. All assays were performed in triplicate, and variability within an assay was ±5%. The reaction times selected in the above assays were to ensure linear rates of incorporation of ³²P into various phosphoprotein substrates.

**Other Methods.** Phosvitin from hen egg yolk was prepared and partially dephosphorylated as described previously (7). Prostatic homogenates, nuclei, and chromatin were assayed for protein content by the method of Lowry et al. (29), and for DNA content according to the method of Burton (14), using bovine serum albumin and calf thymus DNA, respectively, as standards. The preparation of [γ-32P]ATP was carried out as described earlier (4). Gel electrophoretic separation and autoradiography of ³²P-labeled endogenous chromatin proteins of human normal prostate and BPH tissue were carried out according to the procedures described previously (17, 18).

**RESULTS**

**Recovery of Nuclei from Human Prostate**

Although difficulty in preparing nuclei from BPH tissue has been encountered in the past (31), such preparations were successfully obtained more recently (12). We have carried out a detailed analysis of the recovery of nuclei from BPH tissue obtained using different surgical procedures. The homogenization of the BPH tissue as described in "Materials and Methods" was facilitated by the use of a Tissuemizer. The recovery of DNA in the filtered homogenate was 60 to 70% of that present in the unfiltered homogenate. Presumably, the loss of this DNA is due to the removal of stromal elements present in the homogenate. Recovery of DNA in the final nuclear preparation was 50 to 85% of that in the filtered homogenate for tissue obtained at open prostatectomy, 40 to 50% for tissue obtained at autopsy, and 18 to 35% for tissue obtained from transurethral resection. The marked reduction in the recovery of nuclei from BPH tissue obtained from a transurethral resection reflects the tissue damage inflicted by this technique. The final recovery of DNA in purified chromatin as compared with the DNA in the filtered homogenate was 36 to 65% for open prostatectomy tissue, 12 to 40% for BPH tissue obtained at autopsy, and 4 to 12% for BPH tissue obtained from transurethral resection. By contrast, the recovery of chromatin from normal prostate was better than 70% of the tissue DNA. This was somewhat higher than that reported previously (31). In all cases, the ratio of protein to DNA in the chromatin preparations was relatively constant.

**General Characteristics of Human Prostatic Chromatin-associated Protein Kinase Reaction**

**Effect of Varied ATP Concentration on Phosphorylation of Different Substrates.** The data presented in the following (Charts 1 and 2; Table 1) are based on experiments with normal prostate or BPH tissue, as indicated in the legends to the charts. Because of limitations of tissue availability (especially the normal prostate tissue), select representative experiments were carried out to establish that the various kinetic parameters established were similar in the 2 types of tissue (i.e., normal and BPH). Chromatin preparations from human normal prostate and BPH tissue were able to catalyze phosphorylation of exogenous substrates, such as DPV and LRH, as well as the endogenous chromatin-associated proteins. The kinetic values of activation of the BPH chromatin-associated protein kinases by exogenous ATP and phosphate acceptor proteins as substrates were examined. An apparent Kₘ of 0.06 mM for ATP was found for the phosphovitin kinase (Chart 1A) and 0.01 and 0.07 mM ATP for kinase activated toward LRH (Chart 1C). These data suggest the presence of more than one protein kinase activity in human prostatic chromatin, as was found originally for rat ventral prostate chromatin-associated protein kinases (Table 1). In addition to the striking similarities of the kinetic parameters for ATP as a substrate for human and rat prostate chromatin protein kinases, other assay conditions such as the pH optima and effects of NaCl, MgCl₂, and dithiothreitol were also much alike (Table 1). The kinetic values of various substrates versus activity were also examined (Chart 1, B and D) and are summarized in Table 1. Two apparent Kₘ values for DPV (0.15 and 0.90 mg/ml) and 2 for LRH (0.15 and 0.90 mg/ml) as substrate were found. Under the experimental conditions described in "Materials and Methods," rates of incorporation of ³²P into DPV, LRH, and endogenous chromatin-associated proteins were linear for a period of 20, 60, and 1 min, respectively (data not shown). All these reactions were stimulated by sulfhydryl-protecting agents such as dithiothreitol; the maximum protective effect was afforded by dithiothreitol at 1 to 3 mM.

**pH Optima.** Measurement of protein kinase activity with LRH as substrate showed a shoulder of activity in the pH range of 7.2 to 7.6.
Effects of Monovalent and Divalent Cations. The protein kinase activities of chromatin were dependent on divalent cations for activity and were each maximally active in the presence of 4 mM MgCl₂ (Chart 2B). The incorporation of ³²P into endogenous chromatin proteins was maximal at about 5 mM MgCl₂. The rates of reaction of the histone and phosvitin kinases of human prostatic chromatin were also stimulated by increased ionic strength of the reaction medium; the kinase activity toward LRH was optimal at 60 mM NaCl and was strongly inhibited at NaCl concentrations greater than 100 mM, whereas the phosvitin kinase activity was maximal at NaCl concentrations of 200 to 400 mM (Chart 2C). The phosphorylation of endogenous chromatin-associated proteins did not demonstrate a requirement for the presence of monovalent salts. This was not due to the presence of salt in the chromatin preparations.

Comparison of Chromatin-associated Protein Kinase Activities of Prostatic Tissue from Different Sources

Since benign hyperplastic prostate is the most readily available pathological human prostate tissue removed by different procedures, we compared protein kinase activities in chromatin prepared from such tissue samples obtained at open prostatectomy, transurethral resection, and autopsy (Table 2). The specific activities of both the histone and phosvitin kinases, as well as the percentage of stimulation of phosvitin phosphorylation by 1 mM spermine (see later), were not altered significantly in chromatin preparations from prostate tissue from each of these sources. Thus, although the recovery of nuclei and chromatin from the transurethral resection specimens was low, the protein kinase activities were similar to those in nuclei obtained from noncauterized tissue. However, regardless of the method of tissue procurement, the rate of ³²P incorporation into chromatin proteins was higher in BPH than in normal prostate (Table 3). The average endogenous protein kinase activity for normal prostatic chromatin was 6.7 nmol ³²P/mg of DNA/h (range, 4.2 to 13.5), whereas that for chromatin from BPH tissue was 14.7 nmol ³²P/mg of DNA/h (range, 16.0 to 26.9). These data also indicate that cadaver prostate can be used effectively in the study of chro-

Table 1

Comparison of enzymic properties of chromatin-associated protein phosphokinases from human benign hyperplastic prostate and rat ventral prostate

<table>
<thead>
<tr>
<th>Protein kinase activity towards substrates</th>
<th>pH optima</th>
<th>MgCl₂ (mM), optimal</th>
<th>NaCl (mM), optimal</th>
<th>ATP (mM), optimal</th>
<th>Protein substrate (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human BPH chromatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPV</td>
<td>7.2–7.5; 8.2</td>
<td>4</td>
<td>260</td>
<td>0.06</td>
<td>0.15, 0.9</td>
</tr>
<tr>
<td>LRH</td>
<td>7.8–8.0</td>
<td>4</td>
<td>60</td>
<td>0.01, 0.07</td>
<td>0.15, 0.9</td>
</tr>
<tr>
<td>Endogenous proteins²</td>
<td>7.45</td>
<td>4</td>
<td>0</td>
<td>0.01</td>
<td>ND²</td>
</tr>
<tr>
<td>Rat ventral prostatic chromatin*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPV</td>
<td>7.0–7.4; 7.9</td>
<td>5</td>
<td>280</td>
<td>0.04, 0.41</td>
<td>0.12, 0.9</td>
</tr>
<tr>
<td>LRH</td>
<td>8.0–8.2</td>
<td>6</td>
<td>80</td>
<td>0.01</td>
<td>0.9</td>
</tr>
<tr>
<td>Endogenous proteins</td>
<td>7.45</td>
<td>4</td>
<td>0</td>
<td>0.01</td>
<td>ND</td>
</tr>
</tbody>
</table>

* DPV as substrate (2 mg/ml).
² LRH as substrate (5 mg/ml).
² Phosphorylation of endogenous chromatin-associated proteins (equivalent to 5 to 20 µg of DNA per ml) catalyzed by the intrinsic protein kinase activity.
² ND, not determined.
* Data from Refs. 2, 5, 8, and 38.
of spermine in the reaction).

Mean ± SD. Polyamines such as spermine and spermidine have also been shown to stimulate the phosphorylation of certain endogenous chromosomal proteins of rat ventral prostate and liver (2, 10). Spermine (at a concentration of 1 mM) stimulated the phosphorylation of chromatin proteins (mediated via the endogenous protein kinase activity) from human normal prostate as well as BPH by 62 and 75%, respectively (Table 3). Thus, there was no significant difference in the effect of spermine on the 2 types of preparations. The presence of spermine not only enhanced the initial rate but also the extent of phosphorylation of chromosomal proteins, suggesting that additional phosphates were incorporated into new sites in the protein substrates (data not given).

Gel Electrophoresis and Autoradiography of ³²P-labeled Chromatin Proteins in the Human Normal and BPH Prostatic Tissue

The results presented in Table 3 were further confirmed by gel electrophoretic and autoradiographic analyses of ³²P-labeled chromatin proteins from normal and BPH prostatic tissues. The gel electrophoretic profile of the chromatin-associated proteins did not indicate any apparent change in the staining of the various proteins present in the BPH chromatin as compared with those in the normal. The autoradiographic visualization of ³²P incorporation clearly indicated that the rate of phosphorylation of the chromatin-associated proteins in the BPH tissue was considerably higher than that for the normal prostatic chromatin (result not shown). The extent of ³²P incorporation in the chromatin preparations from normal and BPH tissues was also compared. The results shown in Fig. 1 indicated that the extent of ³²P incorporation in the proteins of BPH chromatin was significantly higher than that of the normal prostate. The presence of 1 mM spermine stimulated the phosphorylation of the normal as well as the BPH chromatin proteins. These results demonstrate that, in the BPH chromatin, there is an increase not only of the initial rate but also of the extent of phosphorylation of the endogenous proteins catalyzed by the intrinsic protein kinase activity.

DISCUSSION

The present work was undertaken to determine the properties of human prostatic nuclear protein kinase reactions and to identify any underlying differences in the normal and BPH tissues with regard to these reactions. We have demonstrated that, despite low yield, nuclei from BPH tissue can be obtained and utilized for such studies. This is further substantiated by the observation that the ratio of protein to DNA in chromatin preparations from normal prostate as compared with that from BPH tissue (obtained from different sources) was fairly constant. In

matin protein kinase activities. This is important, since autopsy material is generally the source of "normal" prostate tissue.

Effects of Spermine. Naturally occurring aliphatic polyamines (spermine and spermidine) have been shown to stimulate protein kinase activity of rat ventral prostate chromatin toward the acidic phosphoprotein substrate DPV, but not towards the basic protein substrate LRH (10). Spermine, present in the reaction at 1 mM,
nuclear protein kinases had suggested an androgen sensitivity of the kinase activity towards phosphorlase and nonhistone proteins (19). However, by comparison, the androgen sensitivity of the latter reaction was significantly greater, which further supports the possibility of the presence of distinct protein kinases active towards nonhistone proteins and towards DPV. However, despite such indications, a protein kinase which is specific only for nonhistone protein substrates has not been isolated. Kinases active towards phosphorlase or casein (generally called N1 and N2) have been purified from rat prostate tissue* but demonstrate significant activity towards nonhistone proteins also. Since phosphorylation of the chromatin-associated proteins catalyzed by the endogenous protein kinase activity occurs primarily in the nonhistone proteins, it may be concluded that the increase in phosphorylation of nonhistone proteins in the BPH tissue reflects either a change in the kinase activity specific for these substrates or a qualitative or a quantitative change in the content of the nonhistone proteins present in this chromatin. A possible reason for these alterations is perhaps the relative contribution of the epithelial versus the stromal cells, owing to the nature of the pathological tissue change. It is also conceivable that the nonhistone proteins from BPH tissue have a higher number of phosphorylatable sites, as is indicated by a greater extent of $^{32}$P incorporation in those proteins. The results on the characterization of $^{32}$P-labeled phosphoproteins of human normal and BPH prostatic chromatin by one-dimensional gel electrophoresis and autoradiography do not give a clear indication of the presence of distinct new proteins in BPH chromatin. Thus, the increased phosphorylation of chromatin proteins in BPH tissue may be due to an elevation in the activity of an existing protein kinase or the presence of a novel protein kinase in the BPH tissue compared with the normal tissue. All these possibilities will need to be explored further.

It may be argued that the differences in the ages of normal and BPH subjects (minimally 26 years) may play a role in the differences in chromatin phosphorylation of equal or greater importance than the presence or absence of the BPH pathology. For obvious reasons, such a possibility is difficult to explore. However, it should be noted that cyclic AMP-independent protein kinase activity of prostatic cytosol from aging rats was found to decrease rather than increase (20). Preliminary results of similar experiments on nuclear-associated protein kinase reactions have also not shown any increase in the activity of these reactions as a result of aging.5 In view of the androgen sensitivity of rat prostatic nuclear enzymes of this type (19), it is noteworthy that the increased rate of phosphorylation of BPH chromatin-associated nonhistone proteins appears to correlate with the presence of elevated 5a-DHT (see, e.g., Refs. 13 and 21) and the presence of elevated levels of nuclear salt-resistant androgen receptors (12) in the BPH tissue. These receptors appear to be associated with the nuclear matrix. We have demonstrated recently that rat ventral prostate nuclear matrix protein phosphorylation is highly sensitive to the androgenic status of the animal and that the rate of its phosphorylation is controlled by the level or activity of the protein kinases present in the nucleus (18). Although the presence of elevated 5a-DHT is well known in BPH (21), it is not fully established if elevated 5a-DHT is responsible for the appearance of BPH or rather is a consequence of it. Likewise, it is difficult to

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determine at present if an elevation of the nuclear nonhistone protein kinase reaction in BPH tissue occurs in conjunction with the development of BPH, owing to elevated levels of 5α-DHT.

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REFERENCES


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