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Registry No. Adenylate cyclase, 9012-42-4.

References

Bahl, O. P., März, L., & Kessler, M. J. (1978) *Biochem. Biophys. Res. Commun. 84*, 667–676.
Barondes, S. H. (1981) *Annu. Rev. Biochem. 50*, 207–231.
Chen, H.-C., Shimohigashi, Y., Dufau, M. L., & Catt, K. J. (1982) *J. Biol. Chem. 257*, 14446–14452.
Crestfield, A. M., Moore, S., & Stein, W. H. (1963) *J. Biol. Chem. 238*, 622–627.
Downs, F., Herp, A., Moschera, J., & Pigman, W. (1973) *Biochim. Biophys. Acta 328*, 182–192.
Edman, P., & Begg, G. (1967) *Eur. J. Biochem. 1*, 80–91.
Faiman, C., & Ryan, R. J. (1967) *J. Clin. Endocrinol. Metab. 27*, 444–447.
Fein, H. G., Rosen, S. W., & Weintraub, B. D. (1981) *Endocrinology (Baltimore) (Suppl.) 108* (Abstr. No. 330).
Greenwood, F. C., Hunter, W. M., & Glover, J. S. (1963) *Biochem. J. 89*, 114–118.
Ishizaka, T. (1982) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41*, 17–21.
Kalyan, N. K., & Bahl, O. P. (1981) *Biochem. Biophys. Res. Commun. 102*, 1246–1253.
Keutmann, H. T., & Williams, R. M. (1977) *J. Biol. Chem. 252*, 5393–5397.
Lee, C. Y., & Ryan, R. J. (1973) *Biochemistry 12*, 4609–4619.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Manjunath, P., & Sairam, M. R. (1982) *J. Biol. Chem. 257*, 7109–7115.
Manjunath, P., Sairam, M. R., & Schiller, P. W. (1982) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41*, 1160.
McIlroy, P. J., Richert, N. D., & Ryan, R. J. (1980) *Biochem. J. 188*, 423–430.
Morell, A. G., Gregoriadis, G., Scheinberg, I. M., Hickman, J., & Ashwell, G. (1971) *J. Biol. Chem. 246*, 1461–1467.
Morgan, F. J., Canfield, R. E., Vaitukatius, J. L., & Ross, G. T. (1973) *Methods Invest. Diagn. Endocrinol. 2B*, 733–742.
Mort, A. J., & Lamport, D. T. A. (1977) *Anal. Biochem. 82*, 289–309.
Niall, H. D. (1973) *Methods Enzymol. 27*, 942–1018.
Pernollet, J. C., Garnier, J., Pierce, J. G., & Salesse, R. (1976) *Biochim. Biophys. Acta 446*, 262–276.
Reinhold, V. N. (1972) *Methods Enzymol. 25*, 244–249.
Rodbard, D., Munson, P. J., & DeLean, A. (1978) *Radioimmunoassay Relat. Proced. Med., Proc. Int. Symp. 1*, 469–504.
Sairam, M. R. (1980) *Arch. Biochem. Biophys. 204*, 199–206.
Tarr, G. E., Beecher, J. F., Bell, M., & McKean, D. J. (1978) *Anal. Biochem. 84*, 622–627.
Thotakura, N. R., & Bahl, O. P. (1982) *Biochem. Biophys. Res. Commun. 108*, 399–405.
Zahlten, R. N., Rogoof, T. M., & Steer, C. J. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 2460–2468.

Isolation, Properties, and Androgen Regulation of a 20-Kilodalton Protein from Rat Ventral Prostate†

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ABSTRACT: An abundant 20-kilodalton protein has been isolated from the cytosol fraction of rat ventral prostate by ammonium sulfate precipitation, DNA–cellulose chromatography, and gel filtration. The purified 20K protein is a glycoprotein, containing 11% hexose by weight. It contains no fucose, hexosamine, or sialic acid. The 20K protein does not bind androgen. Binding of the 20K protein to DNA is nonspecific, showing affinity toward DNAs of various tissue origins, as well as poly(dA-dT), poly(rI-rC), and phosphocellulose. The 20K protein comprises about 9% of the total cytosolic proteins in rat ventral prostate. Examination of eight different rat organs, including prostate secretion, lateral and dorsal prostates, and rat ejaculate, for the presence of the 20K protein by double immunodiffusion analysis revealed that the protein is a rat ventral prostate specific secretory protein. Hybridization of prostatic poly(A) RNA with a cloned cDNA coding for the 20K protein indicated that the synthesis of the 20K protein is regulated by testosterone at the mRNA level.

The rat prostate, a male accessory sex gland, produces several abundant proteins in response to testosterone stimulation. Parker et al. (1978) have reported the presence of three major androgen-regulated proteins in the postnuclear supernatant fraction of rat ventral prostate, identified electrophoretically as polypeptides of M_r values of 8K, 12K, and 20K. The 12K polypeptide actually contains two polypeptides of M_r 13K and 11K which, together with the 8K polypeptides, constitute the subunit components of PBP[1] (Heyns & De Moor, 1977; Heyns et all., 1978; Lea et al., 1977, 1979; Forsgren et al., 1979) or

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[1] Abbreviations: PBP, prostatic binding protein; $NaDodSO_4$, sodium dodecyl sulfate; TEM, 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 1 mM β-mercaptoethanol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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α-protein (Shyr & Liao, 1978). PBP has been purified (Heyns et al., 1978; Chen et al., 1982), extensively characterized (Heyns & De Moor, 1977; Peeters et al., 1981, 1982; Chen et al., 1982; Liao et al., 1982), and shown to be a steroid-binding prostate secretory protein, but the 20K protein has not been isolated so far. However, initial studies (Parker et al., 1978) of the 20K protein on polyacrylamide gel with Schiff's reagent and by electrophoresis of lectin-bound prostatic proteins indicate that the 20K protein is a glycopeptide. Two lines of evidence further indicate that this 20K protein is regulated by testosterone at the mRNA level. First, translation of total prostatic poly(A) RNA in vitro shows that the concentration of the mRNA coding for a 20K polypeptide decreases sharply after castration and is restored by androgen replacement (Parker & Scrace, 1979; Hiremath et al., 1981). Second, using a cloned cDNA for 20K protein in hybridizations with prostatic poly(A) RNA, Parker et al. (1980) have determined that the mRNA for a 20K polypeptide decreases by more than 30-fold after androgen withdrawal. Administration of testosterone for 4 h partially restores the level of the mRNA.

Since PBP and the 20K protein account for about 50% of the proteins synthesized in the rat ventral prostate (Parker et al., 1978) and are under androgenic control, there is a need to understand better the nature and androgen regulation of the 20K protein. In this paper, we report the purification and preliminary characterization of a cytosolic 20K protein from rat ventral prostate. The regulation of the mRNA coding for the 20K protein was also investigated by using a cloned cDNA.

Experimental Procedures

Animals and Materials. Male Sprague-Dawley rats of 300 g body weight were purchased from Harlan Sprague-Dawley, Madison, WI. The rats were castrated by the scrotal route under ether anesthesia. For androgen treatment, rats castrated for 3 days were each injected subcutaneously with 1.5 mg of testosterone in 0.15 mL of sesame oil containing 10% ethanol for time periods as indicated. Female New Zealand white rabbits of 5 lb body weight were bought from a local rabbitry.

Testosterone propionate and dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) were obtained from Sigma Chemical Co., St. Louis, MO. [1,2,4,5,6,7-^{3}H(N)]DHT (131 Ci/mmol) was from New England Nuclear, Boston, MA. Freund's complete and incomplete adjuvant were from Difco, Detroit, MI. Agarose and all electrophoresis reagents were from Bio-Rad, Richmond, CA. Sephadex G-100 was from Pharmacia, Piscataway, NJ, and Whatman CF11 cellulose powder from Whatman, Inc., Clifton, NJ. All other chemicals used were of the highest available purity.

Preparation of Prostate Cytosol Fraction. Rats were killed by decapitation. The ventral prostates were removed, collected on ice, minced, and homogenized in 3 volumes of 0.32 M sucrose, 0.05 M Tris-HCl, pH 7.4, 0.025 M KCl, and 5 mM $MgCl_2$. The homogenate was filtered through glass wool and centrifuged at 3000*g* for 10 min. The supernatant was successively centrifuged at 27000*g* for 20 min and at 130000*g* for 90 min to yield a supernatant which was the cytosol fraction. Cytosol fractions from other rat organs were prepared in the same way.

Polyacrylamide Gel Electrophoresis. Slab gels (14.5 × 14.5 cm) or cylindrical tube gels (9.5 × 0.5 cm) were prepared according to Laemmli (1970). Stacking and separating gels were 4% and 12.5% acrylamide, respectively. Protein samples were dissolved in sample buffer (2.3% $NaDodSO_4$,[1] 62.5 mM Tris-HCl, pH 6.8, 5% β-mercaptoethanol, and 10% glycerol) and dialyzed against the same buffer overnight. Bromphenol blue was added as the tracking dye, and the samples were boiled for 1 min before applying to the gel. Electrophoresis was run for 5–6 h at 100 V after which the gels were stained overnight in 0.25% Coomassie blue in 10% acetic acid and 45% methanol and destained in the acetic acid–methanol solution. For carbohydrate staining, gels were stained with periodic acid–Schiff reagent according to Zacharius et al. (1969).

Ouchterlony Double Diffusion. Antiserum was raised against the 20K protein in female rabbits by multiple intradermal injections with the purified protein in Freund's complete adjuvant. After 4 weeks, each rabbit was injected with the antigen emulsified in Freund's incomplete adjuvant. The injection was repeated twice in a 2-week period, and 1 month after the last injection, blood was collected by cardiac puncture. To concentrate the antibody, the antiserum was fractionated by ammonium sulfate precipitation according to Campbell et al. (1970).

Immunodiffusion gels were prepared by pipetting 2.5 mL of 1% agarose in 0.01 M Tris-HCl, pH 8.8, and 0.15 M NaCl onto glass microscope slides, and wells were cut by a metal cylinder 3 mm in diameter. Ten microliters of the γ-globulin fraction or protein solution was pipetted into the wells as indicated in the figure. The slides were incubated at room temperature overnight in a Petri dish containing wet filter paper, soaked in 0.15 M NaCl, and rinsed by two changes of distilled water for 15 min each time. The gels were covered with wet filter paper and air-dried. Slides were stained for 5–10 min in Coomassie Blue and destained in 10% acetic acid and 45% methanol for 15–30 min.

Samples were prepared for immunodiffusion analysis as follows. Prostatic secretion was collected according to the procedure of Levy & Fair (1973). Ejaculated semen was homogenized in 0.15 M NaCl and 0.01 M Tris-HCl, pH 8.8, and the homogenate was centrifuged to remove insoluble material. Cytosols from kidney, spleen, seminal vesicle, epididymis, coagulating gland, testes, ventral prostate, dorsal prostate, and lateral prostate of rats were prepared as described above. Total cytosol protein was precipitated by the addition of ammonium sulfate to 70% saturation. The precipitate was collected by centrifugation and dissolved in and dialyzed against 0.15 M NaCl in 0.01 M Tris-HCl, pH 8.8. All samples were adjusted to the same absorbance (0.78) at 280 nm.

Preparation of cDNA Clone. Total RNA was extracted from rat ventral prostate as described by Hastie et al. (1979). Poly(A) RNA was isolated from total RNA by chromatography on oligo(dT)–cellulose (Aviv & Leder, 1972). Single-stranded cDNA was synthesized from poly(A) RNA by AMV reverse transcriptase (a gift from the Division of Cancer Cause and Prevention, NCI) and made double stranded (ds) according to Rougeon et al. (1976). S1 nuclease treatment of ds cDNA, tailing of poly(dC) and poly(dG) of the ds cDNA and *Pst*1-treated plasmid pBR 322, respectively, and transformation into *E. coli* κ1776 were carried out according to Berger et al. (1981). Colony hybridization was performed as described by Grunstein & Hogness (1975), and plasmid DNA was isolated by the procedure of Clewell & Helinski (1970).

Selection and Translation of mRNA. Message was selected by hybridization of poly(A) RNA to cloned cDNA on, and elution from, nitrocellulose circles (Ricciardi et al., 1979) and translated in a cell-free system as described by Held et al. (1977). Immunoprecipitation of translation products was performed as described previously (Hiremath et al., 1981).

RNA–DNA Hybridization. RNA excess hybridization with purified [^{32}P]cDNA was done under the conditions as described by Hastie et al. (1979).

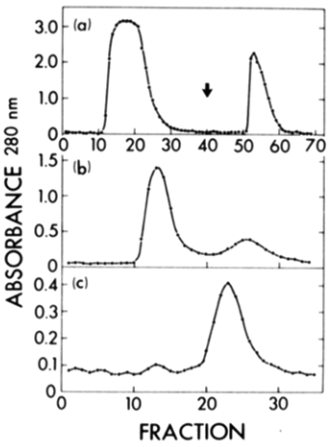

FIGURE 1: Purification of 20K protein. (a) Rat DNA–cellulose chromatography of the 40% saturated ammonium sulfate precipitated proteins from rat ventral prostate cytosol. The bound proteins were eluted with 1.0 M NaCl–TEM, and fractions of 1.5 mL were collected. Fractions 51–58 were pooled. (b) Gel filtration of the DNA–cellulose-bound proteins on Sephadex G-100. The pooled fraction from (a) was concentrated to 2 mL and chromatographed on a Sephadex G-100 column (100 × 1.2 cm) in 1.0 M NaCl–TEM. Fractions 23-29 were pooled. (c) Rechromatography of the second peak from (b) on Sephadex G-100. Conditions were the same as in (b). Fractions 20–25 were pooled, dialyzed against 0.01 M Tris-HCl, pH 8.8, and lyophilized.

Other Procedures. DNA–cellulose was prepared according to the procedure of Alberts & Herrick (1971) or by coupling DNA to CNBr-activated Sepharose 4B according to Arnot-Jovin et al. (1975). Protein was determined by the procedure of Lowry et al. (1951). Hexose was determined by a procedure given in Nowotny (1969). Fucose and hexosamine were determined according to Winzler (1955). Sialic acid was determined by the procedure of Warren (1959). Dihydrotestosterone binding was measured by the charcoal absorption assay of Wilson & French (1976).

Results

Purification of the 20K Protein. The cytosol from 50 rat ventral prostates was made to 40% saturated ammonium sulfate by gradual addition of solid ammonium sulfate. The resulting precipitate was collected by centrifugation and washed twice with a solution of 40% saturated ammonium sulfate to remove any unprecipitated protein which may adhere to the precipitate. The washed pellet was dissolved in 0.05 M NaCl–TEM[1] and dialyzed against three changes of the same buffer. The dialyzed cytosol fraction was clarified by centrifugation and loaded on a rat DNA–cellulose column (9 × 2 cm) which had been previously equilibrated with 0.05 M NaCl–TEM. The column was washed with the same buffer until A_{280nm} of the wash was less than 0.02. The bound protein was eluted with 1.0 M NaCl–TEM (Figure 1a). The peak fractions eluted from DNA–cellulose were pooled and concentrated to about 2 mL in an Amicon ultrafiltration unit by using a PM-10 filter. The concentrated protein solution was loaded on a Sephadex G-100 column (100 × 1.2 cm) that had been equilibrated with 1.0 M NaCl–TEM. As seen in Figure 1b, the proteins appeared in two peaks. The second peak which contained the 20K protein was collected, concentrated by ultrafiltration, and rechromatographed on Sephadex G-100 (Figure 1c). The leading and central portions of the second peak were pooled, dialyzed against 0.01 M Tris-HCl, pH 8.8, and stored lyophilized as the purified 20K protein. Electrophoretic analysis of each fraction obtained during the course of fractionation is shown in Figure 2. From a scanning diagram of the electrophoretic pattern of the purified product,

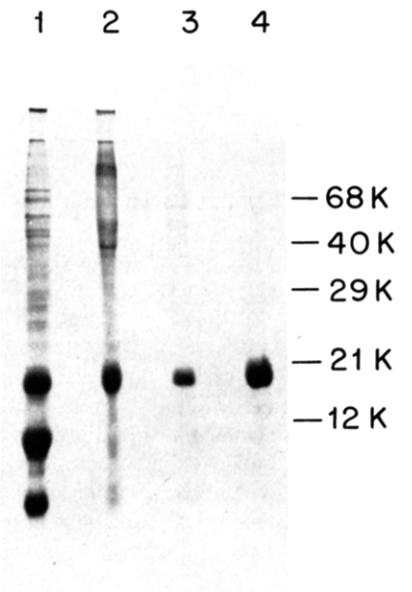

FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis of fractions obtained during purification of the 20K protein. Column 1, total cytosol from rat ventral prostate, 100 μg; column 2, 40% ammonium sulfate precipitate fraction of prostate cytosol; column 3, DNA–cellulose-bound protein, 20 μg; column 4, Sephadex G-100 fraction, 20 μg. Marker proteins used were bovine serum albumin (68K), alcohol dehydrogenase (40K), carbonic anhydrase (29K), soybean trypsin inhibitor (21K), and cytochrome *c* (12K).

Table I: Purification of 20K Protein from Rat Ventral Prostate

fraction	total protein (mg)	total 20K protein[a] (mg)	20K protein in fraction (%)
total cytosol	1157	102.4	9
40% $(NH_4)_2SO_4$ (ppt)	100.6	13.4	13
DNA–cellulose bound	10.6	4.9	46
Sephadex G-100 second peak	2.9	1.8	62
rechromatography of G-100 second peak	1.6	1.4	88

[a] The fractions were dialyzed against 90 mM Tris-borate buffer, pH 8.3, containing 0.053% calcium lactate and 0.065% sodium azide, and the 20K protein was quantitated by the radial immunodiffusion procedure as described under Experimental Procedures.

the protein is calculated to be 93% homogeneous. Table I summarized the purification steps and the yield of 20K protein determined by the immunodiffusion method.

Properties of the 20K Protein. When the 20K protein was subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis and duplicates were stained separately with Coomassie blue and periodic acid–Schiff's reagent, both stained bands coincided (data not shown), indicating that the protein was a glycopeptide. Analysis of the 20K protein for carbohydrate showed that it contained only hexose. No sialic acid, fucose, or hexosamine was detectable. The amount of hexose present, based on two separate determinations, was 105 μg/mg of protein, representing 11% carbohydrate by weight or 10 mol of hexose/mol of protein.

Table II shows the amino acid composition of the 20K protein. Although the acidic amino acid residues appeared to predominate, their amide content was not determined.

From the amino acid composition, the partial specific volume of the protein was calculated to be 0.72 mL/g. The Stokes radius of the protein, determined by gel filtration on Sephadex G-75 (superfine) with references of ovalbumin (27.3

Table II: Amino Acid Composition of 20K Protein[a]

amino acid	mol %	amino acid	mol %
aspartic acid	12.73	tyrosine	7.34
glutamic acid	11.87	valine	4.75
lysine	9.49	arginine	4.96
glycine	4.21	proline	3.24
leucine	7.34	alanine	2.37
serine	5.39	half-cystine	2.81
threonine	5.82	methionine	2.37
phenylalanine	7.98	histidine	1.29
isoleucine	6.04		

[a] Hydrolysis of the 20K protein was carried out in constant boiling 6 N HCl in vacuo at 110 °C for 22 h. The amino acids were determined with a Beckman Model 120c amino acid analyzer.

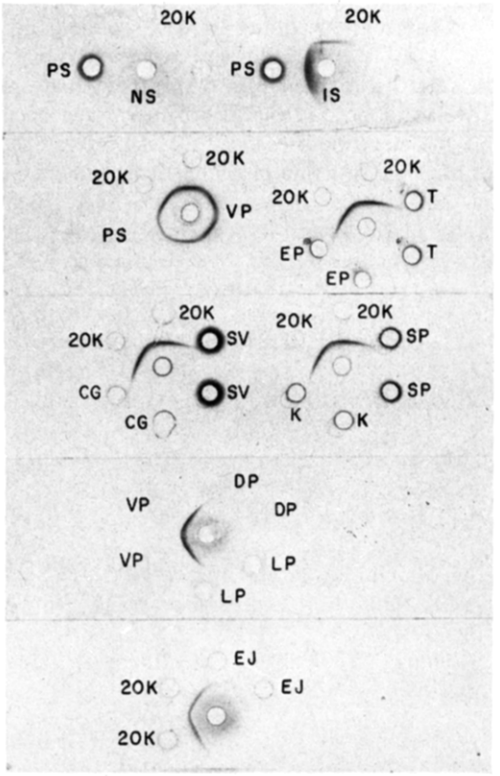

FIGURE 3: Double immunodiffusion analyses of cytosol fractions from various rat organs wiith anti-20K serum. Plates were prepared and analyzed as described under Experimental Procedures. 20K, purified 20K protein; PS, prostatic secretion; NS, nonimmune serum; IS, immune serum; VP, ventral prostate cytosol; EP, epididymis cytosol; T, testis cytosol; CG, coagulating gland cytosol; SV, seminal vesicle cytosol; K, kidney cytosol; SP, spleen cytosol; DP, dorsal prostate cytosol; LP, lateral prostate cytosol; EJ, saline extract of rat ejaculate.

Å), γ-globulin (52 Å), cytochrome *c* (17 Å), and alcohol dehydrogenase (46 Å), was estimated to be 18 Å. By use of these two parameters, the frictional coefficient was calculated to be 0.86, indicting a nearly spherical protein.

Unlike the PBP, which binds steroids nonspecifically, the 20K protein did not bind 5α-DHT. Direct incubation of the 20K protein with [^{3}H]DHT, followed by charcoal absorption of free steroid, did not result in [^{3}H]DHT binding to the protein. [^{3}H]DHT-labeled ammonium sulfate fraction of prostate cytosol, fractionated through the Sephadex G-100 gel filtration step, also yielded a nonradioactive 20K peak.

To determine whether the 20K protein was present in other male accessory sex glands or organs, cytosol fractions prepared from various organs, including lateral and dorsal prostates, prostate secretion, and rat ejaculate, were analyzed by Ouchterlony double diffusion gels. As shown in Figure 3, the

Table III: Binding of Prostate Cytosolic Protein to DNA[a]

DNA	unbound	bound	% bound
rat liver			
double stranded	13.9	1.9	12
single stranded	6.0	1.3	18
calf thymus			
double stranded	12.9	2.6	17
single stranded	10.2	3.3	24
chicken erythrocyte			
double stranded	12.8	1.8	12
single stranded	12.1	2.4	17
poly(I)-poly(C)	13.8	2.0	13
poly(dA)-poly(dT)	6.0	0.24	4
phosphocellulose	3.0	0.66	18

[a] The 40% saturated ammonium sulfate fraction of rat prostate cytosol was used. Various DNAs and synthetic polynucleotides were coupled to CNBr-activated Sepharose 4B as described under Experimental Procedures. The amount of nucleic acid coupled to the Sepharose was approximately 50 μg/mg dry weight of Sepharose, except for poly(dA)-poly(dT) which was 4 μg/mg dry weight of Sepharose. Columns containing about 3–3.5 mL packed volume of the coupled Sepharose [equivalent to 35–40 mg of nucleic acid; 1;2 mg for poly(dA)-poly(dT)] were used for the binding experiments. Samples containing 18 mg of protein, except that for chromatography on poly(dA)-poly(dT) which contained 9 mg of protein, were loaded on and eluted from the columns at 2 °C as described for the DNA–cellulose chromatography.

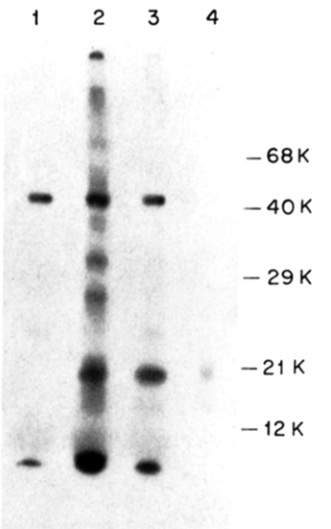

FIGURE 4: Translation in vitro of message selected by hybridization with cloned cDNA and immunoprecipitation of translational product of selected mRNA. (1) Minus mRNA; (2) total prostate poly(A) RNA; (3) message selected by cloned cDNA; (4) immunoprecipitated translated product of cDNA selected mRNA.

20K protein is exclusively localized in the rat ventral prostate and is a prostatic secretory protein.

Since DNA binding was used in the purification procedure for isolation of the 20K protein, the specificity of the protein binding to DNA was examined and quantitated by chromatography on Sepharose 4B coupled to DNA from various sources. The presence of the 20K protein in the DNA-bound fractions was verified by NaDodSO$_4$–polyacrylamide gel electrophoretic analysis. As shown in Table III, the protein binds to all the DNAs tested as well as poly(dA-dT), poly(rI-rC), and phosphocellulose. DNA binding of the 20K protein is therefore nonspecific.

Androgen Regulation of the 20K Protein. Cloned cDNA was prepared as described under Experimental Procedures. Message selection, translation, and immunoprecipitation of the cDNA clone, shown in Figure 4, indicated that the isolated

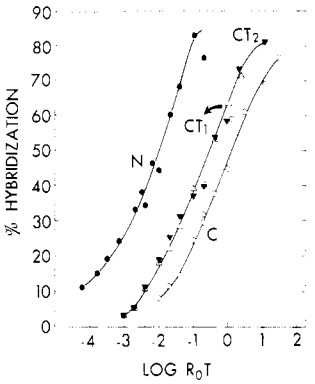

FIGURE 5: RNA excess hybridization of cloned cDNA. Prostate poly(A) RNA, prepared from normal (N) and castrated (C) rats and castrated rats injected with testosterone for 1 (CT_1) and 2 h (CT_2), were each hybridized with the cloned [^{32}P]cDNA as described under Experimental Procedures.

cDNA clone codes for the 20K protein.

To determine the effect of testosterone on the 20K protein, total prostate poly(A) RNA was prepared from castrated rats injected with testosterone for 1 (CT_1) and 2 (CT_2) hours as well as from normal and castrated rats. The poly(A) RNAs were hybridized with the cloned cDNA labeled with ^{32}P by nick translation. Figure 5 shows the results of the hybridization kinetics. As can be seen, 3-day castration drastically decreased the concentration of 20K mRNA by 2 orders of magnitude. The level of the 20K mRNA responded to androgen treatment by increasing 3-fold within 1 h following testosterone injection. Thus, the 20K protein is regulated by androgens at the mRNA level. From the $R_0t_{1/2}$ values of the hybridization data and assuming 15 pg of RNA per prostate cell, the number of copies was calculated to be 43×10^3 per normal prostate cell.

Discussion

The presence of a major 20-kilodalton glycopeptide in rat ventral prostate regulated by testosterone was first reported by Parker et al. (1978). A subsequent study by Parker et al. (1980) further showed that an abundant prostatic mRNA coding for 20K polypeptide responded to androgen withdrawal and replacement. Despite these manifestations, the 20K protein had not been isolated. In the present work, we have purified the prostatic 20K glycoprotein to 93% homogeneity and shown that it is a secretory protein unique to the ventral prostate of rat. One difficulty encountered during purification of the 20K protein was its tendency to form aggregates, both by self-association and with other proteins in the cytosol. The use of high pH (8.8) improved its solubility by 60% (data not shown). Liao (1976) has observed the formation of aggregates between androgen receptor and other cytosolic proteins. He also noted that high pH (9.0) reduced the extent of aggregation. Such aggregation most likely involves the 20K protein which is present in the same ammonium sulfate fraction as the androgen receptor.

The 20K protein and PBP represent the major proteins in rat ventral prostate, constituting about 50% of the cytosolic proteins in this organ. Comparison of these two proteins reveals some similarities as well as differences. First, PBP is an oligomeric protein, whereas the 20K protein is a single polypeptide. Second, both are glycosylated secretory proteins. Although the compositions of their carbohydrate moieties are unknown, binding of the 20K protein to specific lectins (Parker et al., 1978) suggests that the carbohydrate moiety of the 20K protein contains glucose and mannose. The present study rules out the presence of fucose, hexosamine, and sialic acid. Third, PBP binds androgens nonspecifically (Heyns & De Moor, 1977), whereas the 20K protein is not androgen binding. In addition, PBP also binds estramustine (Forsgren et al., 1979), 7′,12-dimethylbenzanthracene, and benzo[*a*]pyrene (McKeehan & Fast, 1981) with high affinity. The binding properties of the 20K protein toward these compounds and other steroids are a subject for future study. Fourth, unlike PBP, the 20K protein binds DNA, but the DNA binding is nonspecific. Similar binding efficiency of the 20K protein to DNAs from different tissue origins and to synthetic polynucleotides and phosphocellulose indicates that phosphocellulose or any polyphosphate can be used for facile fractionation of the 20K protein. The nonspecific DNA binding also rules out any significance of the interaction of the 20K protein with DNA.

In order to determine whether the 20K protein is involved in sperm interaction, a preliminary experiment in which prostate secretion was incubated with epididymal sperms, followed by electrophoretic analysis of sperm-adsorbed proteins, failed to detect the presence of 20K protein adsorbed to the sperms. The function of the 20K protein remains to be investigated.

Registry No. Poly(I)-poly(C), 24939-03-5; poly(dA)-poly(dT), 24939-09-1; phosphocellulose, 9015-14-9; testosterone, 58-22-0.

References

Alberts, B. M., & Herrick, G. (1971) *Methods Enzymol. 21*, 198–217.

Arnot-Jovin, D. J., Jovin, T. M., Bahr, W., Frischauf, A. M., & Marquardt, M. (1975) *Eur. J. Biochem. 54*, 411–418.

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Berger, F. G., Gross, K. W., & Watson, G. (1981) *J. Biol. Chem. 256*, 7006–7013.

Campbell, D. H., Garvey, J. S., Cremer, N. E., & Sussdore, D. H. (1970) *Methods in Immunology*, pp 189–191, W. A. Benjamin, New York.

Chen, C., Schilling, K., Hiipakka, R. A., Huang, I.-Y., & Liao, S. (1982) *J. Biol. Chem. 257*, 116–121.

Clewell, D. B., & Helinski, D. R. (1970) *Biochemistry 9*, 4428–4440.

Forsgren, B., Björk, P., Carlström, K., Gustafsson, J.-Å., Pousette, Å., & Högberg, B. (1979) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5969–5973.

Grunstein, M., & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3961–3965.

Hastie, N. D., Held, W. A., & Toole, J. J. (1979) *Cell (Cambridge, Mass.) 17*, 449–457.

Held, W. A., West, K., & Gallagher, J. F. (1977) *J. Biol. Biochem. 252*, 8489–8597.

Heyns, W., & De Moor, P. (1977) *Eur. J. Biochem. 78*, 221–230.

Heyns, W., Peeters, B., Mous, J., Rombauts, W., & De Moor, P. (1978) *Eur. J. Biochem. 89*, 181–186.

Hiremath, S. T., Mpanias, O. D., & Wang, T. Y. (1981) *Exp. Cell Res. 134*, 193–200.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lea, O. A., Petrusz, P., & French, F. S. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 780.

Lea, O. A., Petrusz, P., & French, F. S. (1979) *J. Biol. Chem. 254*, 6196–6202.

Levy, B. J., & Fair, W. R. (1973) *Invest. Virol. 11*, 173–177.

Liao, S. (1976) *Mod. Pharmacol.-Toxicol. 8*, 176–181.

Liao, S., Chen, C., & Huang, I.-Y. (1982) *J. Biol. Chem. 257*, 122–125.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
McKeehan, W. L., & Fast, D. (1981) *Cell Biol. Int. Rep. 5*, 2.
Nowotny, A. (1969) in *Basic Exercises in Immunochemistry*, pp 102–104, Springer-Verlag, New York.
Parker, M. G., & Scrace, G. T. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1580–1584.
Parker, M. G., Scrace, G. T., & Mainwaring, W. I. P. (1978) *Biochem. J. 170*, 115–121.
Parker, M. G., White, R., & Williams, J. G. (1980) *J. Biol. Chem. 255*, 6996–7001.
Peeters, B., Rombauts, W., Mous, J., & Heyns, W. (1981) *Eur. J. Biochem. 115*, 118–121.
Peeters, B., Heyns, W., Mous, J., & Rombauts, W. (1982) *Eur. J. Biochem. 123*, 55–62.
Ricciardi, R. P., Miller, J. S., & Roberts, B. E. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4927–4931.
Rougeon, F., & Mach, B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3418–3422.
Shyr, C.-I., & Liao, S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5969–5973.
Warren, L. (1959) *J. Biol. Chem. 234*, 1971–1976.
Wilson, E. M., & French, F. S. (1976) *J. Biol. Chem. 251*, 5620–5629.
Winzler, R. J. (1955) *Methods Biochem. Anal. 2*, 292–296.
Zacharius, R. M., Zell, T. E., Morrison, J. H., & Woodlock, J. J. (1969) *Anal. Biochem. 30*, 148–152.

Human Serum Procarboxypeptidase A†

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ABSTRACT: Zymogen activation is an important biochemical control process and has important physiological and pathological implications. We have simultaneously measured both procarboxypeptidase A, the enzyme precursor, and carboxypeptidase A, its active product, in serum by using an affinity resin and the synthetic peptide substrate *N*-(2-furanacryloyl)-L-phenylalanyl-L-phenylalanine. Serum procarboxypeptidase A is activated by trypsin, chymotrypsin, plasmin, subtilisin, or urokinase but not by thrombin or enteropeptidase. The molecular weight of the precursor is approximately 5000–10 000 greater than that of the active product. Both enzyme and precursor increase in serum in the course of pancreatic inflammation, but the degree of activation can vary up to 2000-fold, independent of the amount of precursor present. The existence of this pancreatic proteolytic precursor in serum opens new avenues for the investigation of zymogen activation and its regulation.

Conversion of inactive enzyme precursors to their physiologically active forms by limited proteolysis is a major biological control mechanism (Neurath & Walsh, 1976). Several of the digestive enzymes are stored in the pancreas as precursors or zymogens that are activated in the duodenum by the limited proteolytic removal of a peptide from the proenzyme (Maroux et al., 1971). The precursors in the pancreas are actually synthesized as pre-proenzymes that contain an amino-terminal peptide which is removed prior to release of the proenzyme from the acinar cell (Blobel & Dobberstein, 1975). Both limited proteolytic processes are irreversible under physiological conditions; as a result, the enzymatic activity measured is a reflection of the concentration of the proenzyme. Most important, generation of activity by an activating proteolytic enzyme is strong evidence for the existence of the precursor.

It has long been known that the presence of amylase in serum serves as a diagnostic guide for the detection of various disorders of the pancreas. The utility of serum amylase is limited, however, since the pancreas is not the sole tissue of origin for this enzyme, but the search for other pancreatic enzymes in serum that have greater diagnostic significance has been largely futile. The presence of protease inhibitors in serum and the lack of tissue specificity for most of the pancreatic proteases have contributed to this problem. Our efforts in this regard have focused on the procarboxypeptidase–carboxypeptidase A system and are based on the properties and characteristics of zymogens and their constituent enzymes outlined above.

We have synthesized a new substrate and developed a new method for measuring low concentrations of carboxypeptidase A, thereby overcoming technical problems in detecting the activity of this enzyme in serum (Peterson et al., 1982). Yet, despite the sensitivity of the assay and the detection of high activities in some patients with pancreatitis, carboxypeptidase A activity was not detectable in the serum of normal individuals. We have found, however, that their sera do contain the precursor, procarboxypeptidase A, which can be detected following proteolytic activation.

This report details the evidence for the existence of procarboxypeptidase A in human serum and describes conditions for its activation. The demonstration of such a precursor and the capacity to measure it in serum constitute a new approach for the investigation of zymogen activation, in general, and for the evaluation and diagnosis of human pancreatic disease. Furthermore, simultaneous measurement of both the active and inactive forms of this enzyme allows assessment of its state of activation as well as concentration. The ability to determine the degree of activation of such biological molecules introduces

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