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ISOLATION AND CHARACTERIZATION OF ANDROGEN-DEPENDENT NON-HISTONE CHROMOSOMAL PROTEIN FROM DORSOLATERAL PROSTATE OF RATS

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SUMMARY: Rat prostate contains a unique androgen-dependent non-histone protein (Matuo et al. (1)). The non-histone protein was isolated in homogeneous form by extraction of nuclei from the dorsolateral prostate with 0.35 M NaCl in the presence of 1 mM PMSF and chromatography on a CM-Sepharose column. The final fraction was greater than 98% pure as judged by electrophoreses in SDS- and acid/urea-gels. The purified protein had a molecular weight of approximately 20,000, and an isoelectric point of approximately 11.5. Its absorption peak was at 276 nm and A(1%, 276nm)=9.3. The protein is characterized by the absence of cysteine, histidine and tryptophan, and by the high content of methionine, tyrosine and phenylalanine.

We have recently described that a novel species of non-histone chromosomal protein having a molecular weight of approximately 20,000 (20K-NHP) was abundantly localized in the dorsolateral prostate of rats, and that the content of 20K-NHP was found to be decreased by castration and to be restored to normal by replacement of androgen (1). So far as we are aware, the existence of 20K-NHP whose content is dependent on the level of androgen has not been previously reported. The present paper deals with isolation and characterization of the androgen-dependent 20K-NHP from nuclei of the dorsolateral prostate of rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (16-20 weeks) were used. Preparation of nuclei(1), SDS-polyacrylamide gel electrophoresis (1), and disc gel isoelectric focusing (2) were carried out by the methods described previously. Polyacrylamide gel electrophoresis in the presence of 5.4% acetic acid and 6.25 M urea was carried out at 25°C by the method of Paniym and Chalkley (3). Total amino acids were measured using a Hitachi 835 automatic amino acid analyzer; the purified samples of 20K-NHP were lyophilized and hydrolyzed for 24 h at 110°C according to the methods of Moore and Stein (4) and Matsubara et al. (5). The

Abbreviations: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol.

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content of protein was determined by the method of Lowry et al.(6). Specific conductivity was measured at 25°C by a conductivity meter CDM 3 (Radiometer, Copenhagen).

RESULTS AND DISCUSSION

Extraction and purification of androgen-dependent 20K-NHP from nuclei of dorsolateral prostate of rats Hagiwara et al. (7) found that an alkaline protease capable of hydrolyzing H1 histone and non-histone proteins was extracted to a significantly low extent by NaCl lower than 0.7 M. In order to minimize the proteolytic degradation, the androgen-dependent 20K-NHP was extracted by 0.35 M NaCl in the presence of 1 mM PMSF as follows: Nuclear pellet prepared from the dorsolateral prostate, which had been centrifugally washed with 5 vol. (v/w) of 0.15 M NaCl containing 1 mM PMSF, was homogenized with 5 vol. (v/w) of 10 mM Tris-HCl (pH 7.5) containing 1 % Triton X-100, 0.25 M sucrose, 3 mM $CaCl_2$ and 1 mM PMSF, followed by centrifugation. The resulting precipitate was suspended in 3 vol. (v/w) of 5 mM Tris-HCl (pH 7.5) containing 0.35 M NaCl and 1 mM PMSF, and stirred for 30 min in an ice-bath, followed by centrifugation at 50,000 x g for 1 h. The supernatant thus obtained was collected. This extraction was repeated twice. When the resulting extracts were subjected to SDS-polyacrylamide gel electrophoresis, several species of non-histone protein including 21K-NHP, in addition to the 20K-NHP, were observed (Fig. 1 (A)). The content of the 20K-NHP in the residual precipitate was less than 5 % of the total content. As reported previously (1), the isoelectric point (pI) of the 20K-NHP was measured to be approximately 11.5 by two-dimensional gel electrophoresis. Consequently, the resulting extracts were applied to a column of CM-Sepharose CL-6B according to the method of Sanders (8). After washing the column with the running buffer, adsorbed proteins were eluted with NaCl gradient (Fig. 2). Polyacrylamide gel electrophoreses in SDS-gel (Fig. 1 (B)) and acid/urea-gel (data not shown) revealed that the purity of F-2 was found to be higher than 98%, and that the 21K-NHP coextracted from the nuclei was fractionated in F-1. The results for the purification are summarized in Table I. When CM-Sepharose chromatography was performed on a short column (1.5 x 5 cm), the recovery of proteins was increased

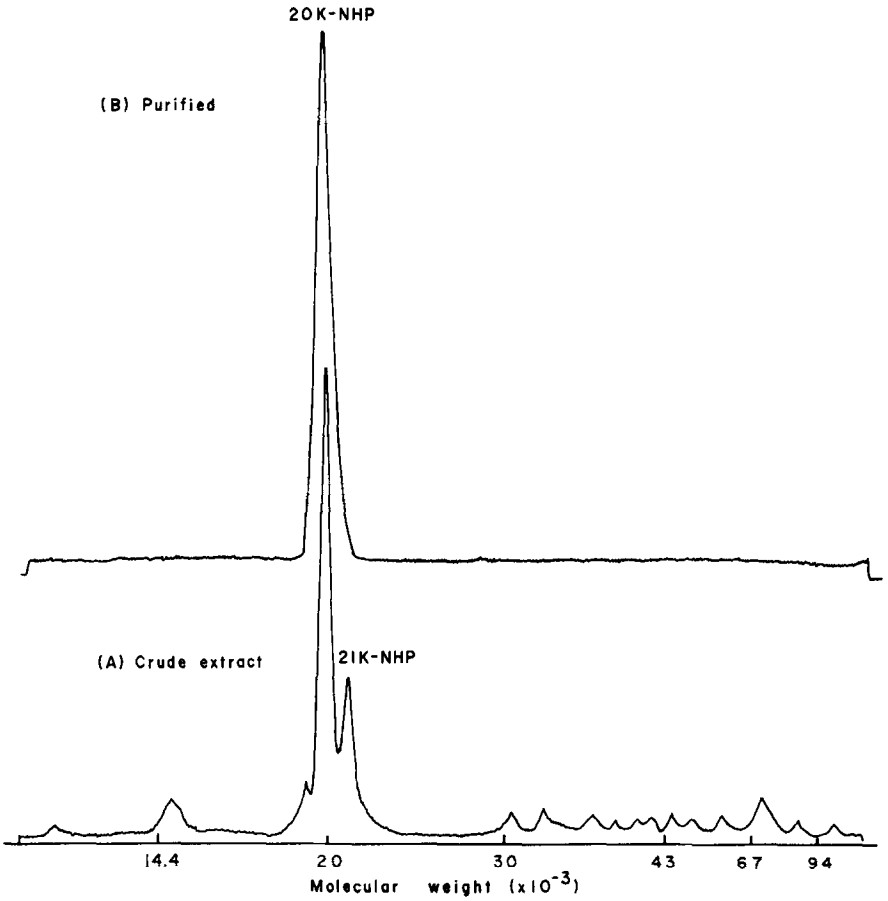


Fig. 1. SDS-polyacrylamide gel electrophoresis of the androgen-dependent 20K-NHP from nuclei of the dorsolateral prostate of rats. Samples containing 20 μg protein of the extract (A) and the purified 20K-NHP (B) were analyzed by SDS-polyacrylamide slab gel electrophoresis (1). 20K-NHP and 21K-NHP; non-histone protein having a molecular weight of approximately 20,000 and 21,000, respectively.

to 80%. However, the purity of the resulting 20K-NHP fraction was reduced to approximately 70% by the contamination of F-1. It is difficult to show the androgen dependency of F-1, because of low content and similar properties in pI and molecular weight to the 20K-NHP.

Molecular weight, isoelectric point and absorption spectrum of purified 20K-NHP For the estimation of the molecular weight of 20K-NHP, the purified sample was subjected to molecular-sieve chromatography on a column of Ultrogel AcA 44 under non-denaturing conditions; 10 mM Tris-HCl (pH 7.5) containing 0.5 M NaCl. The proteins were not recovered from the column, and eluted with 0.5% SDS, by which a large amount of unknown substance having an absorption at 280

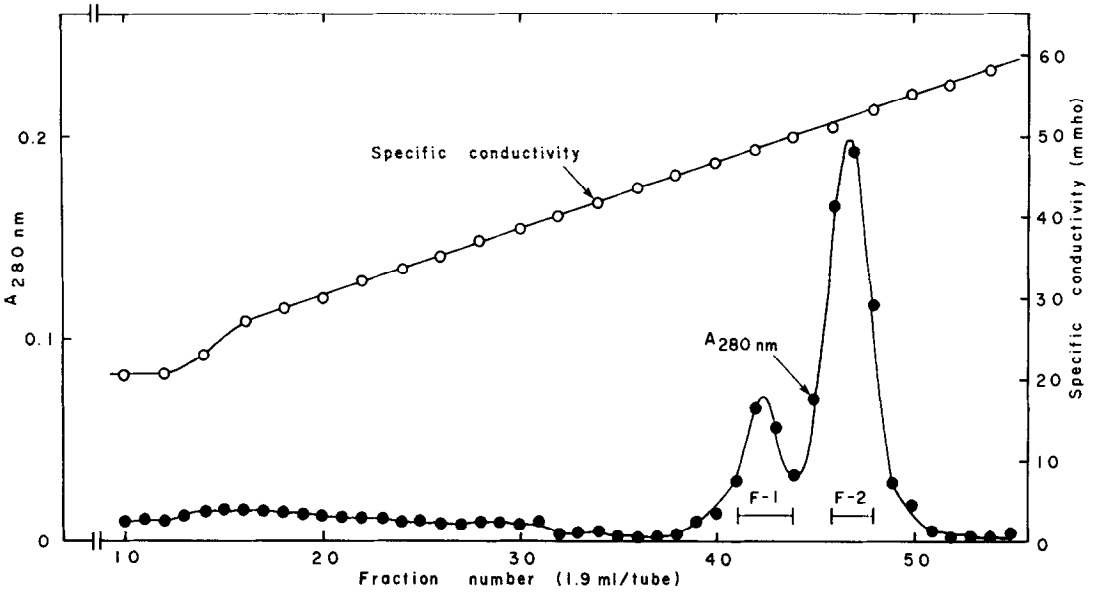


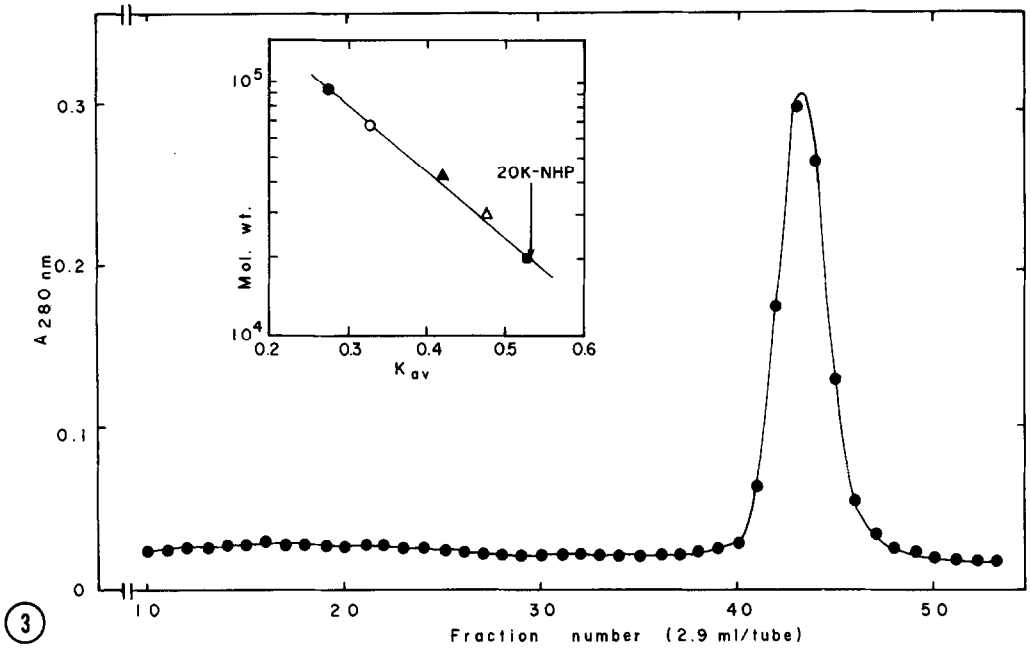
Fig. 2. Ion-exchange chromatography of 0.35 M NaCl-extract from nuclei of the dorsolateral prostate on a column of CM-Sepharose CL-6B. The 0.35 M NaCl-extract (34 ml containing 7.5 mg protein) was supplemented with 1/5 vol. (v/v) of 15 mM sodium borate buffer (pH 9.0), and adjusted to pH 9.0 by adding 1 M NaOH. Ionic strength of the solution was adjusted to the same specific conductivity (20 mmho/cm) as that for the running buffer (7.5 mM sodium borate (pH 9.0) containing 0.2 M NaCl), and applied to a column (0.7 x 29 cm) of CM-Sepharose CL-6B at a flow rate of 12 ml/h. After washing the column with 20 ml of the running buffer, adsorbed proteins were eluted with NaCl gradient. Fractions of (No. 41-44) and (No. 46-48) were combined as F-1 and F-2, respectively. F-2 was dialyzed against water, and subjected to gel electrophoreses and amino acid analysis.

nm was eluted from the column. Accordingly, the protein was applied to a column of Sepharose CL-6B in the presence of 10 mM Tris-HCl (pH 8.0) containing 0.5% SDS and 1 mM DTT. The protein was eluted in the fractions centered at 20,000 daltons (Fig. 3). Hayashi et al. (9) demonstrated that, in

Table I. Summary for purification of the androgen-dependent 20K-NHP from nuclei of the dorsolateral prostate of rats.

Fraction	Amount of protein (mg)	Yield (%)
(Tissue)	(30 g wet weight)[a]	
Nuclei[b]	157.4	100
0.35 M NaCl-extract	48.8	31.0
CM-Sepharose:		
Flow-through fraction	5.5	3.5
Adsorbed fraction; F-1[c]	2.2	1.4
F-2[d]	5.8	3.7

a) Obtained from 100 rats. b) Washed with the medium containing 1% Triton X-100 (amount of DNA: 25.8 mg). c) From fraction number 41 to 44 in Fig. 2. d) From fraction number 46 to 48 in Fig. 2. Recovery of total proteins in CM-Sepharose chromatography was approximately 50%.



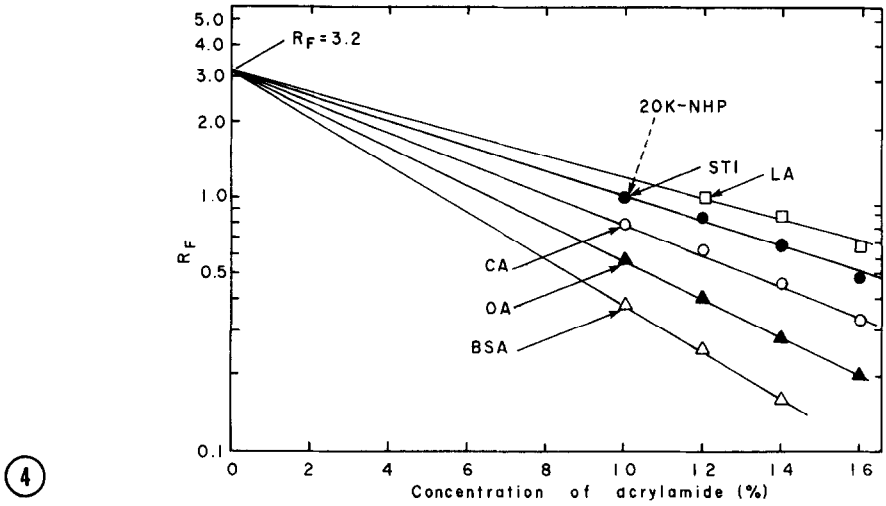


Fig. 3. Molecular-sieve chromatography of the androgen-dependent 20K-NHP on a column of Sepharose CL-6B in the presence of SDS and DTT. The 20K-NHP fraction (22.8 ml containing 3.34 mg protein) prepared by CM-Sepharose CL-6B chromatography was supplemented with 2.0 ml of conc.HCl and 149 ml of acetone, and allowed to stand overnight in an ice-bath, followed by centrifugation at 25,000 x g for 30 min. The resulting precipitate containing 2.9 mg protein was dissolved in 3 ml of 10 mM Tris-HCl (pH 8.0) containing 0.5% SDS and 1 mM DTT and applied to the column (1.5 x 92 cm). Marker proteins used were phosphorylase b (●) (94,000), bovine serum albumin (○) (67,000), ovalbumin (▲)(43,000), carbonic anhydrase (△)(30,000) and soybean trypsin inhibitor (■)(20,000). The values of Kav with the marker proteins and the 20K-NHP were calculated from Kav=(Ve-Vo)/(Vt-Vo), where Vo is the elution volume (68.64 ml) of Blue dextran and Vt is the elution volume (174.24 ml) of DNP-alanine.

Fig. 4. Ferguson plot of marker proteins and the androgen-dependent 20K-NHP in SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (10) in glass tubes (0.5 x 13 cm) having various concentrations of acrylamide. Ferguson plot was obtained by the method of Hayashi et al. (9). BSA (△); bovine serum albumin (67,000): OA (▲);ovalbumin (43,000): CA (○); carbonic anhydrase (30,000): STI and 20K-NHP (●); soybean trypsin inhibitor (20,000): LA (□); α-lactalbumin (14,400).

Table II. Amino acid composition of the androgen-dependent 20K-NHP isolated from nuclei of dorsolateral prostate of rats.

Amino acid	mole %[a)]	Number of residue[b)]
Asp + Asn	13.0	23
Thr	6.2	11
Ser	3.0	5
Glu + Gln	12.0	21
Pro	1.7	3
Gly	6.7	12
Ala	4.8	8
Cys[c)]	n.d.[d)]	0
Val	7.0	12
Met	4.5	8
Ile	6.1	11
Leu	5.9	10
Tyr	5.8	10
Phe	4.6	8
Lys	8.7	15
His	n.d.	0
Trp[e)]	n.d.	0
Arg	10.0	18
(Total numbers of residue)		(175)
(Molecular weight calculated from amino acid compositions)		(20,630)

Fractions (No. 46-48) from CM-Sepharose CL-6B chromatography were dialyzed against water. An aliquot (336 μg protein) of the resulting turbid solution was lyophilized and subjected to amino acid analysis.
a) Average of duplicate. b) Calculated on the basis of molecular weight=20,000, which was determined by SDS-electrophoresis.
c) Determined by the method of Moore and Stein (4). d) Not detected.
e) Determined by the method of Matsubara et al (5).

SDS-polyacrylamide gel electrophoresis, the slope in the Ferguson plot is linearly related to the molecular weight of a protein, and that basic proteins such as histones show abnormal behavior. The purified 20K-NHP was subjected to SDS-polyacrylamide gel electrophoresis at various concentrations of acrylamide, and the log R_F vs. acrylamide concentration (T, %) were plotted (Fig. 4). A linear relationship between log R_F and T (%) was confirmed with all the marker proteins and with the purified 20K-NHP. These results indicate that the 20K-NHP shows similar behavior in SDS-gel electrophoresis to the marker proteins, and that the observed molecular weight (20,000) of the 20K-NHP measured by SDS-gel electrophoresis is reliable. By disc gel isoelectric focusing, the isoelectric point of the purified 20K-NHP was measured to be

approximately 11.5. Absorption spectrum was measured with the purified 20K-NHP in the presence of 0.02% SDS; it had absorption peak at 276 nm and trough at 250 nm. The value of $A_{276\ nm}^{(1\%)}$ was 9.3.

Amino acid composition of purified 20K-NHP The amino acid compositions of the purified 20K-NHP are shown in Table II. The data revealed that the 20K-NHP was characterized by the absence of cysteine, histidine and tryptophan. The 20K-NHP was found to be abundant in methionine, tyrosine and phenylalanine. These are distinctly different from those of various kinds of histones including H1 histones, its variants (11-15) and high mobility group non-histone proteins (8, 16, 17), indicating that the 20K-NHP was not caused by the modification of histones, but a novel species of non-histone protein.

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