Prostate α-Protein

ISOLATION AND CHARACTERIZATION OF THE POLYPEPTIDE COMPONENTS AND CHEMICAL BONDING*

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α-Protein, a major glycoprotein in the cytosol fraction of rat ventral prostate, has a molecular weight of about 50,000 and can be dissociated, by sodium dodecyl sulfate, into two different subunits (A and B). α-Protein has three different polypeptide components with apparent molecular weights of 10,000 (I), 14,000 (II), and 15,000 (III). These components were purified to homogeneity and their amino acid compositions were determined. Subunit A is composed of Components I and III, whereas subunit B is composed of Components II and III. Carbohydrate was detectable only on Component III. Component III isolated from subunit A and Component III isolated from subunit B appear to be identical.

The purified α-protein contains 0.7–1 mol of cholesterol/mol of protein. If cholesterol was removed by acetone, about 1 mol of 5α-dihydrotestosterone or pregnenolone could bind to 1 mol of α-protein. In the presence of 2 mm ZnCl₂, α-protein can form dimers and tetramers.

In cell-free systems, α-protein can inhibit binding of the androgen-receptor complex to nuclear chromatin and also can promote the release of the complex already bound to chromatin. This effect is due to polypeptide Component I.

In 1971, we isolated a cytosol protein from the rat ventral prostate which could interfere with the association of the androgen-receptor complex with isolated cell nuclei (1). Since this protein can bind androgens and other steroids, we named it "α-protein" to distinguish it from the androgen receptor protein that was called "β-protein." α-Protein may be identical with a glycoprotein studied by others in recent years. This glycoprotein is a major secretory product of the rat ventral prostate and can promote the release of the complex already bound to chromatin. This effect is due to polypeptide Component I.

In this article, we will describe methods for isolation of the individual polypeptide chains that form the two subunits of α-protein. We will also show that the major ligand associated with the protein is cholesterol. One of the acidic polypeptide components of α-protein is also identified as the active unit that can inhibit binding of the androgen-receptor complex to nuclear chromatin.

EXPERIMENTAL PROCEDURES

ω-Protein was isolated and labeled with 1,2,3,5-

*Portions of this paper (including "Experimental Procedures," Figs. 2-4, and Tables I and II) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

†The abbreviations used are: 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; androstenedione, 4-androstene-3,17-dione; pregnenolone, 3β-hydroxy-5-pregnen-20-one; epitestosterone, 17α-hydroxy-11,17-dione; androstanediol, 3α- or 3β 17β-dihydroxyandrostenedione; androstenediol, 3α- or 3β 17β-dihydroxyandrost-4-en-3-one; TLC, thin layer chromatography; GC, gas chromatography; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
RESULTS

Isolation of a-Protein Components and Cholesterol Binding

a-Protein Components and Cholesterol Binding

Sephalogel chromatography—The protein fractions containing subunit A or B were dialyzed against medium ET, concentrated by Amberlite 110 protein, and loaded onto a Sephadex G-100 column (0.25 x 20 cm) eluted with medium ET containing 1.0 M NaCl and eluted at a flow rate of 0.4 ml/min.

Concluding remarks—The native fraction labeled 15 mg in 0.5 ml obtained in Sephalogel chromatography were dialyzed against 0.3 M NaCl and eluted in medium ET containing 0.1 M NaCl and 0.1 M NaCl by DEAE-Septagel chromatography. The subunits were eluted from a hydroxyapatite column (0.3 x 12 cm) eluted with medium ET containing 0.1 M NaCl by eluting with 0.2 M NaCl in 20% 2-mercaptoethanol.

Subunit A was collected as a linear gradient of 0 to 0.5 M NaCl in the above elution buffer. The protein fractions were dialyzed against distilled water and lyophilized.

Hydroxyapatite chromatography—in the presence of SDS and 0-mercaptoethanol—The protein fractions containing subunit A and B were dialyzed against 0.1 M sodium phosphate, pH 7.3, 0.1 M NaCl and 0.1 M NaCl by DEAE-Septagel chromatography. The subunits were then eluted from a hydroxyapatite column (0.4 x 13 cm) equilibrated with 20% 2-mercaptoethanol, 0.1 M NaCl, and then with 0.1 M NaCl in 0.1 M NaCl.

Subunits A and B were eluted from the column by a linear gradient of 0 to 0.5 M NaCl in the presence of 0.1 M NaCl in 0.1 M NaCl.

Subunits A and B were eluted from the column by a linear gradient of 0 to 0.5 M NaCl in 0.1 M NaCl.

Subunit A was eluted as a single peak of 0.3 to 0.5 M sodium phosphate, pH 7.3, containing 0.1 M SDS and 0.1 M 0-mercaptoethanol at room temperature.

Sephadex chromatography—The protein components eluted from hydroxyapatite chromatography were dialyzed against medium ET containing 0.1 M NaCl, equilibrated in 0.1 M SDS and 0.1 M NaCl. The column was then eluted with medium ET containing 0.1 M NaCl and 0.1 M NaCl. Fractions were eluted with a linear gradient of 0 to 0.5 M NaCl. Fractions containing Component I were pooled, lyophilized, restituted in 0.1 M SDS and 0.1 M NaCl.

The two subunits of a-protein could be separated by hydroxyapatite chromatography of the a-protein preparation. Subunits A and B were eluted from the column by 0.2 M and 0.3 M sodium phosphate buffer, respectively (Fig. 4, miniprint). Trace contaminants associated with these subunits could be eliminated by Sephacryl gel chromatography. Both subunits A and B contain carbohydrate and are retained by concanavalin A-Sepharose columns but are eluted from the column with 0.2 M a-methylmannoside.

Both subunits A and B yield two protein bands when analyzed by PAGE in the presence of SDS and 0-mercaptoethanol or dithiothreitol. One of the components, as judged by the migration pattern during electrophoresis under various conditions, appears to be common to the A and B subunits. We therefore designated the components in A as Components I and II, and those in B as Components I and II (Fig. 5).

Isolation of a-Protein Components and Cholesterol Binding

The two proteins of a-protein could be separated by Sephacryl gel chromatography of the a-protein preparation. Subunits A and B were eluted from the column by 0.2 and 0.3 M sodium phosphate buffer, respectively (Fig. 4, miniprint). Trace contaminants associated with these subunits could be eliminated by Sephacryl gel chromatography. Both subunits A and B contain carbohydrate and are retained by concanavalin A-Sepharose columns but are eluted from the column with 0.2 M a-methylmannoside.
$\alpha$-Protein Components and Cholesterol Binding

The molecular weights of $\alpha$-protein and subunits A and B, as determined by co-chromatography of these proteins labeled with $^{125}$I and standard proteins on a Sephadex G-150 (superfine) gel column, are 50,000, 24,000, and 26,000, respectively. In the presence of 5 mM EDTA, the fractionation pattern was not changed. In the presence of 2 mM ZnCl$_2$ (in 20 mM Tris-HCl buffer, pH 7.5), $\alpha$-protein appears to dimerize and tetramerize to give radioactive peaks with molecular weights of about 90,000 and 180,000.

Carbohydrate analysis showed that $\alpha$-protein has no detectable sialic acid. Of the three protein components, only Component III appears to contain carbohydrate detectable by the methods we employed (Table I, miniprint). The amino acid compositions of $\alpha$-protein and its subunits and components show that they are rich in aspartic acid and glutamic acid (Table II, miniprint), facts which are consistent with the low isoelectric points of the various components (Table I, miniprint).

**Biochemical Properties**—Fig. 8 shows the ability of $\alpha$-protein and its subunits and components to inhibit the retention of the radioactive androgen-receptor complex by isolated pros-

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Component by DEAE-Sephadex chromatography (Fig. 1). However, we found that the following procedure could provide a better yield of the components (60-80% from $\alpha$-protein) in a reproducible manner. In this alternative procedure, $\alpha$-protein is chromatographed on a DEAE-Sephadex column in the presence of urea and dithiothreitol. A protein component eluted from the column by 0.05 M NaCl (Fig. 6) is identified by PAGE as Component III. Components I and II are eluted together from the column by 0.15-0.25 M NaCl. These two components are separated by hydroxylapatite chromatography in the presence of SDS (Fig. 7). Minor proteins associated with these components could be removed by passing the protein preparations through a Sephadex G-100 column in the presence of SDS (results not shown).

**Physical and Chemical Properties**—The PAGE patterns of various purified protein fractions are shown in Fig. 5. In nondenaturing gels (gel 1), purified $\alpha$-protein gives three bands in the order of subunit A, $\alpha$-protein, and subunit B from the anode (bottom end of the gel). In the presence of SDS but without $\beta$-mercaptoethanol, $\alpha$-protein migrates as two bands (gel 2) having mobilities identical with that of purified subunits A and B (not shown). In the presence of SDS and $\beta$-mercaptoethanol, purified $\alpha$-protein exhibits three bands (gel 3) that migrate to the same position as Components I (gel 6), II (gel 7), and III (gel 8). Subunits A (gel 4) and B (gel 5), respectively, show two bands that correspond to Components I and III (for A) and Components II and III (for B). From SDS-PAGE, the apparent molecular weights of the component proteins are: 10,000 (I), 14,000 (II), and 15,000 (III). As shown in the accompanying paper (34), the molecular weight of Component I, as determined by the amino acid sequence, is 10,191.
a-Protein Components and Cholesterol Binding

![Graph](http://www.jbc.org/)

**Fig. 6.** Separation of Component III from the core unit of a-protein. a-Protein (102 mg protein in 21 ml) was made to 6 M urea and 10 mM dithiothreitol and chromatographed on a DEAE-Sephadex column (1.6 × 15 cm). Proteins were eluted by a linear gradient (500 ml) of 0-0.5 M NaCl, containing 6 M urea, 2 mM dithiothreitol, 1 mM EDTA, and 20 mM Tris-HCl, pH 7.5. Fractions of 5 ml were collected. The first absorbance peak (fractions 7-10) was due to excess dithiothreitol in the protein sample. The second sharp peak (fractions 23-29) was due to Component II, whereas the third peak (fractions 50-60) contained core unit having Components I and II.

**Fig. 7.** Separation of Components I and II. The protein fraction (146 mg protein) containing Components I and II (isolated by DEAE-Sephadex chromatography shown in Fig. 5) was chromatographed on a hydroxylapatite column (2.6 × 12 cm). Proteins were eluted by a linear gradient (300 ml) of 0.01-0.5 M sodium phosphate containing 0.1% SDS and 0.1% β-mercaptoethanol. Fractions of 2.1 ml were collected. The first absorbance peak (fractions 10-59) was due to excess β-mercaptoethanol in the protein sample. The second and third peaks were due to Components I and II, respectively.

tate cell nuclei. Subunit A was active, but subunit B was not. The inhibitory activity of subunit A appears to be due to Component I, since Component I but not Component III is inhibitory. In fact, on a molar basis, Component I is about 5 times more active than subunit A.

α-Protein freshly isolated from the prostate could bind less than 0.2 mol of radioactive androgen per mol of protein. If the purified protein was delipidated by acetone, the steroid-binding capability of the protein was significantly increased. At steroid concentrations higher than 5 μM, about 1 mol of 5α-dihydrotestosterone could bind to 1 mol of protein. The association constants (Kₐ) for delipidated α-protein toward various steroids are: androsterone, 1.15 × 10⁶ M⁻¹; pregnenolone, 1.1 × 10⁶ M⁻¹; 5α-dihydrotestosterone, 0.87 × 10⁶ M⁻¹; cholesterol, 0.64 × 10⁶ M⁻¹; testosterone, 0.58 × 10⁶ M⁻¹; and epitestosterone, 0.53 × 10⁶ M⁻¹. As described before for the crude preparation, the purified α-protein does not bind corticosteron. On a molar basis, subunit B appears to bind steroid nearly as well as α-protein, but steroid binding by subunit A is feeble. Of the three components, only Component II shows a significant steroid binding activity, but a detailed study is difficult because of the insolubility of this component in aqueous solution without SDS.

When α-protein was extracted with acetone or chloroform and the organic solvent extract was analyzed by thin layer chromatography, we found a major iodine-stainable spot that co-migrated with authentic cholesterol. On the thin layer chromatogram, we also found two minor iodine-stainable spots (less than 10%), but these minor components were not androstenedione, androstanediol, 5α-dihydrotestosterone, testosterone, androstanediol, or androstanediol, This is in line with the fact that the lipid extract was negative in the Zimmernan test (35). Analysis of the organic solvent extract of α-protein by gas chromatography-mass spectrometer also showed that there was only one major compound and that this major compound has a mass spectrum identical with that

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**Table I**

| Protein | Kₐ × 10⁻⁶ | Carbohydrate content | m & B |
|---------|------------|---------------------|------|
| A       | 5.0        | 0.3                 | 30   |
| B       | 2.4        | 0.8                 | 40   |
| C       | 2.8        | 0.9                 | 60   |
| D       | 1.0        | 0.6                 | 100  |
| E       | 1.4        | 0.8                 | 200  |

(Continued from Table I)

| Protein | Kₐ × 10⁻⁶ | Carbohydrate content | m & B |
|---------|------------|---------------------|------|
| F       | 1.1        | 0.7                 | 300  |
| G       | 2.3        | 0.9                 | 400  |
| H       | 1.2        | 0.8                 | 600  |
| I       | 1.5        | 0.9                 | 1000 |

(Continued from Table I)

**Table II**

| Amino acid | α | B | I | M² | M³ |
|------------|---|---|---|----|----|
| Lys        | 8.3 | 6.3 | 8.1 | 8.2 | 6.2 |
| His        | 5.5 | 5.4 | 6.3 | 6.5 | 6.6 |
| Arg        | 3.9 | 3.9 | 4.8 | 4.8 | 4.8 |
| Asp        | 2.8 | 2.8 | 3.8 | 3.8 | 3.8 |
| Ala        | 1.9 | 1.9 | 2.9 | 2.9 | 2.9 |
| Ser        | 1.8 | 1.8 | 2.8 | 2.8 | 2.8 |
| Gly        | 1.7 | 1.7 | 2.7 | 2.7 | 2.7 |
| Val        | 1.6 | 1.6 | 2.6 | 2.6 | 2.6 |
| Pro        | 1.5 | 1.5 | 2.5 | 2.5 | 2.5 |
| Thr        | 1.4 | 1.4 | 2.4 | 2.4 | 2.4 |
| Ser        | 1.3 | 1.3 | 2.3 | 2.3 | 2.3 |
| Cys        | 1.2 | 1.2 | 2.2 | 2.2 | 2.2 |

(Continued from Table II)

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*α-Component isolated from subunit A.

*β-Component isolated from subunit I.

*Component isolated from subunit A.

*Component isolated from subunit I.

*Component isolated from subunit A.

*Component isolated from subunit I.

*Component isolated from subunit A.

*Component isolated from subunit I.
protein fractions tested were α-protein Components I and II. The results were compared with the amount of the radioactive preparations that had been dialyzed extensively against medium ET. A nuclear retention assay was performed in the presence of various amounts of α-protein preparations that had been dialyzed extensively against medium ET. The results were compared with the amount of the radioactive-receptor complex retained in the absence of α-protein preparations and plotted as per cent inhibition of retention on the ordinate. The protein fractions tested were α-protein (AB), subunits A and B, and Components I and III at the concentrations shown on the abcissa.

The radioactive preparations that had been dialyzed extensively against medium ET. was not due to the exposure of the protein to much higher temperatures or the formation of a protein-protein complex by nuclei. The results were compared with the amount of the radioactive-receptor complex retained in the absence of α-protein preparations and plotted as per cent inhibition of retention on the ordinate. The protein fractions tested were α-protein (AB), subunits A and B, and Components I and III at the concentrations shown on the abcissa. 

α-Protein Components and Cholesterol Binding

![Graph](http://www.jbc.org/)

**FIG. 8.** The effect of α-protein subunits and components on the retention of the radioactive 5α-dihydrotestosterone-receptor complex by prostate cell nuclei. The nuclear retention assay was performed in the presence of various amounts of α-protein preparations that had been dialyzed extensively against medium ET. The results were compared with the amount of the radioactive-receptor complex retained in the absence of α-protein preparations and plotted as per cent inhibition of retention on the ordinate. The protein fractions tested were α-protein (AB), subunits A and B, and Components I and III at the concentrations shown on the abcissa.

of authentic cholesterol. With two colorimetric methods, we found that our α-protein preparations generally contains about 0.7–1.0 mol of cholesterol/mol of protein. The cholesterol-bound α-protein sediments as a discrete 3.5 S component.

To assure that exclusive binding of cholesterol by α-protein was not due to the exposure of the protein to much higher concentrations of cholesterol than of other steroids during the isolation, we added tritiated cholesterol, pregnenolone, or 5α-dihydrotestosterone to the minced prostate before homogenization. By comparing the total cholesterol and radioactive steroids bound to isolated α-protein, we could conclude that at least 80% of cholesterol binding occurs before prostate cells are disrupted by homogenization. In a previous article (10), we noted that α-protein can bind radioactive spermine at pH 8.7 but not at pH 7.5. At pH 8.7, α-protein or a mixture of subunits A and B, but not subunits A or B alone, can bind spermine weakly (Ka < 106 M⁻¹). Besides spermine, purified α-protein also binds cadaverine, spermidine, and putrescine without notable specificity. This is in contrast with the androgen-sensitive spermine-binding protein that is highly specific toward spermine and does not bind other polyamines well (33). Spermine binding by α-protein is not affected by 1 μM 5α-dihydrotestosterone.

Immunological comparison of the various components of α-protein by double diffusion on agar plates using antibodies to subunit A revealed that Components I and II are antigenically similar (fused precipitin arcs). An immunological analysis of Component III isolated from subunits A and B revealed that these components are also antigenically related. Comparison of Components I and II with Component III revealed antigenic dissimilarity (crossed precipitin arcs).

**DISCUSSION**

Since the presence of α-protein in the rat ventral prostate was first described by us (1, 22), several investigators have described major secretory proteins in this organ. These proteins were called prostatein, by Lea et al. (4), prostatic binding protein by Heyns and De Moor (2), and estramustine binding protein by Forsgren et al. (3). A similar steroid binding protein was also described by Ichii (36). Although there are distinct differences in the estimated sizes of these proteins and their subunit components and in the steroid-binding affinities, some or all of these proteins may be identical with α-protein. A definite identification, however, cannot be made, since the protein components for these proteins have not been isolated in large amounts in pure forms for chemical analysis and for comparison with the α-protein components.

How the two subunits A and B interact with each other is not clear. During the establishment of the purification procedure, we found that Component II could associate with Component I through various chromatographic processes if SDS was not present; the Components I and II, therefore, may form a core unit but are individually linked to carbohydrate-containing Component III by disulfide linkages (Fig. 5).

Heyns et al. (9) also isolated the two subunits of prostatic binding protein and showed, by PAGE, that they contain dissimilar polypeptide components. Our present data, based on the isolation of the individual polypeptide components and their amino acid compositions, also clearly show that Component I in subunit A and Component II in subunit B are clearly different. These observations are in accord with the conclusion of Lea et al. (4) that "prostaticin" contains two identical subunits (4). Judging from electrophoretic mobility, the two subunits of α-protein contain a common polypeptide (Component III). The same conclusion was also obtained for "prostatic binding protein" (9). To provide additional support that Component III in subunits A and B is identical, we have isolated the component from the individual subunits. The electrophoretic mobility and the amino acid composition of the two preparations are essentially identical. They also have the same NH₂-terminal sequence (Ser-Gly-Ser-Gly⁻¹) and are antigenically related.

The biological role of α-protein is not clear. It is the major protein in the prostate secretory fluid and in the cytosol fraction of the rat ventral prostate. Using antibody raised in rabbits with α-protein and subunit A, we have found that only the dorsal and lateral prostate contain significant amounts of immunologically cross-reactive protein. However, the α-protein level in these prostate lobes was less than 5% of that in the ventral lobe which we used in this study. No traces of immunologically cross-reactive proteins are detectable in the cytosol preparations of rat seminal vesicle, liver, kidney, testis, brain, and spleen, as well as human prostate. α-Protein, therefore, is a species- and organ-specific protein that may be useful as a marker protein for rat ventral prostate.

Since α-protein binds steroids with an affinity several orders of magnitude lower than the androgen receptor in the same organ (1, 12), it is unlikely that α-protein can compete well with the receptor for binding active androgens. α-Protein may, however, compete well with other steroid metabolizing enzymes and retard metabolic transformation of steroids. α-Protein may also play a role in steroid accumulation in cells and extracellular spaces of the prostate. Steroids that are not recognized by the steroid receptor in the prostate may, with the help of α-protein, be retained or transported. The fact that α-protein, freshly isolated and not treated with acetone, contains no other steroid but cholesterol in nearly stoichiometric amounts is interesting, since the synthesis of cholesterol (37) and the secretory protein (38) in the rat ventral prostate are both enhanced by androgens. Whether α-protein plays a role in the synthesis, accumulation, and/or secretion of cholesterol in the prostate is an intriguing question.

Although α-protein was first identified in this laboratory as a steroid-binding protein that can prevent retention of the androgen-receptor complex by nuclei in vitro, the steroid-
binding property is not responsible for the inhibitory effect (11). Our present study clearly shows that the inhibitory activity is due mainly to Component 1 that does not bind steroid but is the interaction of androgen-receptor complex with nuclear chromatin in the intact cells, as we have envisioned previously (10, 11), the active unit may be Component 1 itself or a smaller but more potent oligopeptide derived from the component. Since the secretory component may be present in the endoplasmic reticulum adjacent to the nuclear membrane, the inhibitory unit may have to enter the nuclei through nongenous areas of the envelope or by nuclear blebbing that has been indicated to play a role in nucleocyttoplasmic exchanges (39–41).

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