Cytoplasmic Androgen Binding Protein of Rat Liver: Molecular Characterization after Photoaffinity Labeling and Functional Correlation with the Age-Dependent Synthesis of α_{2u}-Globulin†

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ABSTRACT: The liver of the mature male rat contains a moderate affinity (K_d = 10^{-8} M), low-capacity, cytoplasmic androgen binding protein (CAB) whose appearance during puberty and disappearance during senescence correlate with the androgen-dependent synthesis of α_{2u}-globulin. Molecular properties of CAB were examined by photoaffinity labeling with tritiated methyltrienolone (R-1881), a synthetic androgen, and by its localization within the hepatocytes which are competent to produce α_{2u}-globulin. Photoaffinity labeling of the liver cytosol derived from postpubertal male rats, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and fluorography, showed a predominant androgen binding band corresponding to M_r 31 000. This 31-kilodalton (kDa) binding component was conspicuously absent in the liver of androgen-insensitive prepubertal and senescent male rats and in adult male rats treated with estradiol-17β. In addition, unlike the cytoplasmic extract, the nuclear lysate of the male rat hepatocytes did not contain the 31-kDa androgen binder. Disappearance of the 31-kDa androgen binding band from the cytosolic fraction of androgen-insensitive animals was associated with a concomitant appearance of a minor androgen binding component of apparent M_r 29 000. The livers of postpubertal male rats normally contain two subpopulations of hepatocytes, only one of which is highly active (competent) in α_{2u}-globulin synthesis. Separation of these two subpopulations through a fluorescence-activated cell sorter followed by whole cell labeling showed more than a 2-fold higher uptake of R-1881 by the competent cells. These results suggest that the 31-kDa androgen binder may play an important role in androgen action and that a conversion of the 31-kDa androgen binding component to a 29-kDa binder may be involved in the regulation of the hepatic androgen sensitivity.

Hormones are known to transmit their regulatory signals by interacting with specific binding proteins present in target cells. In the case of steroid hormones, the principal signal transducer is a class of DNA binding proteins characterized by their high binding affinity (K_d = 10^{-9} M) for the steroid ligand (Jensen & Jacobsen, 1962; Edelman & Gimognari, 1968; Gorski et al., 1968; Liao & Fang, 1969; Jensen & DeSombre, 1972; Munck & Leung, 1976; Wilson & French, 1976). These receptor proteins either are exclusively localized within the target cell nuclei or, upon steroid uptake, attain greater affinity for DNA and translocate into the nucleus. Sequence-specific binding of steroid receptors to the regulatory regions of target genes has also been established (Payvar et al., 1981; Compton et al., 1982; Mulvihill et al., 1982; Scheidereit et al., 1983). Such an interaction is believed to facilitate RNA polymerase action and increase specific mRNA synthesis (Mueller et al., 1958; O'Malley & Means, 1974). In addition to transcriptional regulation, hormonal control of processing, stability, transport, and translation of several mRNAs has been indicated (Palmiter & Carey, 1974; Brock et al., 1983; Moore et al., 1984; Vannice et al., 1984; Diamond et al., 1985; Fulton et al., 1985; Berger et al., 1986). Although the biochemical mechanism for such regulatory processes is largely unknown, it is reasonable to assume that these hormonally controlled steps in information transfer also require participation of specific cytoplasmic steroid binding proteins. Furthermore, a body of evidence indicates that the high-affinity steroid receptors may be localized exclusively in the nuclear compartment associated with the chromatin (Sheridan et al., 1979; King & Greene, 1984; Welshons et al., 1984). If this is proven to be a general phenomenon for all steroid targets, a separate class of cytoplasmic steroid binding proteins may be involved in concentrating steroid hormones from the extracellular envrionment. Examples of cytoplasmic steroid binding proteins which do not seem to translocate into the nucleus, such as the progesterone binding protein of the chicken oviduct, the estrogen binding protein of the *Xenopus* liver, and the androgen binding protein of the rat liver, have been described (Milin & Roy, 1973; Hayward & Shapiro, 1981; Taylor & Smith, 1982). Primarily on the basis of sucrose density gradient centrifugation, a considerable body of information has been obtained concerning the properties and hepatic level of the middle affinity (K_d = 10^{-8} M) cytoplasmic androgen binder of the rat liver (Milin & Roy, 1973; Roy et al., 1983; Kyakumoto et al., 1984). There is a strong correlation between the presence of the cytoplasmic androgen binding protein and the hepatic synthesis of α_{2u}-globulin, the androgen-dependent male urinary protein (Roy et al., 1983). Normally, α_{2u}-globulin is only synthesized in the liver of the male rat where it appears after puberty (~40 days) and disappears at senescence (>800 days). α_{2u}-globulin can also be induced in the ovariectomized female rat by androgen administration. It is of interest to note that the livers of both prepubertal and senescent rats are androgen insensitive, i.e., do not respond to androgen treatment. Earlier studies in our laboratory have shown that hepatic androgen insensitivity

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associated with both prepuberty and senescence is correlated with the disappearance of the cytoplasmic androgen binding peak (Roy et al., 1983).

The evidence for the presence of a classical androgen receptor in the rat liver has so far been controversial. Initially, the cytoplasmic androgen binding protein (CAB) in the liver of the male rat was thought to represent the same type of receptor as that found in reproductive tissues (Milin & Roy, 1973; Roy et al., 1974). However, contrary to the claims of Sato and his collaborators (Kyakumoto et al., 1984), we have so far been unable to obtain evidence for the presence of a nuclear androgen binding moiety in rat hepatocytes. Thus, in order to obtain further insight into the physicochemical nature of the hepatic androgen binding protein, we sought for a covalently linked radioligand. Such a labeled product would also allow monitoring the CAB under chaotropic and denaturing conditions which can facilitate its purification. Methyltrienolone (R-1881) is a synthetic androgen which offers two major advantages over natural androgens for the study of androgen binding proteins present in a crude tissue extract. Unlike natural androgens, R-1881 is not metabolized to inactive derivatives, and it can be irreversibly coupled to the binding protein upon ultraviolet irradiation (Bonne & Raynaud, 1975). We have therefore utilized [^{3}H]R-1881 to study the molecular properties of the CAB and to obtain further correlative evidence for its functional role in the androgen-dependent synthesis of α_{2u}-globulin.

Materials and Methods

Preparation of Rat Liver Cytosol. Experiments were performed on Sprague-Dawley rats obtained from Charles River Laboratory (Stoneridge, NY). Testicular feminized male (Tfm) rats were obtained from Dr. K. W. Chung of the University of Oklahoma. Animals were anesthetized by intraperitoneal injections of chloropent (0.25 mL/100 g; Fort Dodge Laboratories Inc., Fort Dodge, IA), and the liver was flushed with 0.15 M NaCl at 4 °C. The liver was then homogenized in a buffer containing 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 2 mM ethylenediaminetetraacetic acid (EDTA), 3 mM dithiothreitol (DTT), 20 mM sodium molybdate, 2 mM phenylmethanesulfonyl fluoride, 0.5 mM leupeptin, and 20% glycerol (cytosol buffer). Cytosol was prepared by centrifugation of the liver homogenate according to the procedure described earlier (Milin & Roy, 1973). Cytosol samples were divided into small aliquots (0.1 mL) and frozen at −70 °C until use. Protein concentrations were estimated according to Bradford (1976).

Binding of [^{3}H]R-1881 and Photoaffinity Labeling. For quantitative analysis of androgen binding, the cytosol samples were diluted in the cytosol buffer to a total protein concentration of 5 mg/mL; 20 μL of the diluted cytosol was incubated with 58 nM [^{3}H]R-1881 (New England Nuclear, Boston, MA) either in the absence or in the presence of 29 μM unlabeled R-1881 (unless mentioned otherwise) for 2 h at 0 °C in gelatin-coated glass tubes (Roy et al., 1974). After incubation, an equal volume of dextran-coated charcoal was added and incubated for 10 min at 0 °C (Korenman, 1969). Samples were centrifuged at 1500*g* for 10 min. Aliquots (100 μL) of the supernatant were mixed with 3 mL of Bray's solution and counted in a liquid scintillation spectrometer.

For cross-linking of [^{3}H]R-1881 to cytosolic binding proteins, diluted cytosol (5 mg/mL) protein) was incubated with [^{3}H]R-1881 at a final concentration of 58 nM either in the absence or in the presence of 29 μM unlabeled R-1881 for 2 h at 0 °C. After incubation, glass tubes (0.7 × 7.5 cm) containing the reaction mixture were placed in a larger glass tube (2.4 × 7.5 cm) which contained saturated copper sulfate solution. The copper sulfate layer served as a filter to screen light rays whose wavelengths are shorter than 300 nm. The reaction mixture was irradiated for 15 min at 4 °C with a 450-W mercury vapor lamp. The photolysis setup was assembled according to a published design (Katzenellenbogen et al., 1974). As determined by the radioactivity in the trichloroacetic acid (TCA)-insoluble proteins, the efficiency of photolabeling under the conditions described above varied from 10% to 15% cross-linking of the labeled steroid. After photolysis, samples were adjusted to 3% sodium dodecyl sulfate (SDS), 5% β-mercaptoethanol, and 10% sucrose and subjected to sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis according to the protocol of Laemmli (1970). Slab gels were treated with EN^{3}HANCE (New England Nuclear, Boston, MA), dried, and autoradiographed on Kodak XAR-5 X-ray films.

Isolation of Hepatocytes and Cell Sorting after Fluorescent–Antibody Labeling. Hepatocytes were isolated from the liver after in situ perfusion with 0.04% collagenase (Roy et al., 1982; Seglen, 1976). Briefly, Hank's balanced salt solution (HBSS), without Ca^{2+} and Mg^{2+}, containing collagenase was perfused through the liver for 10 min. Subsequently, the liver was minced, and cells were disaggregated by repeated pipetting. Cells were washed twice in Dulbecco's-modified Eagle's medium containing 10% fetal bovine serum and cultured for 2 h in the same medium supplemented with insulin, inositol, and hydrocortisone, all at concentrations of 1 mg/100 mL. The hepatocytes were washed 3 times in HBSS and incubated with anti-α_{2u}-globulin antibody (unfractionated rabbit serum at a protein concentration of 1 mg/mL) for 45 min at 4 °C. The cells were washed 3 times with HBSS and incubated with 50 μg/mL fluorescein-labeled anti-rabbit goat immunoglobulin (IgG) (Hyclone Laboratories Inc., Logan, UT) for 45 min at 4 °C. Subsequently, the cells were washed extensively with HBSS and processed through an automatic fluorescence-activated cell sorter (FACS-III; Beckton–Dickinson, Mountain View, CA) interfaced to an Apple II computer (Motwani et al., 1984).

Specific Binding of [^{3}H]R-1881 by Isolated Hepatocytes. Isolated hepatocytes were incubated at 16 °C for 90 min in Dulbecco's phosphate-buffered saline (PBS) with [^{3}H]R-1881 either in the absence or in the presence of a 500-fold molar excess of unlabeled R-1881. Following the incubation, cells were layered over 0.5 mL of 10% sucrose (in PBS) and centrifuged for 1 min in an Eppendor centrifuge. The cell pellet was dissolved in Bray's solution, and the radioactivity was determined in a liquid scintillation spectrometer. The specific binding was determined from the radioactivity of the incubation mixture containing only labeled R-1881 minus the counts obtained with the 500-fold molar excess of unlabeled R-1881.

Isolation of Nuclei and Preparation of Nuclear Extract. Nuclei were prepared following the procedure described by Tata (1974). Briefly, the liver was chilled and homogenized at 4 °C with 5 volumes (w/v) of buffer A (10 mM Tris-HCl, pH 8.0, containing 2.5 mM magnesium acetate, 0.5 mM DTT, 0.25% Triton X-100, and 0.3 M sucrose). The homogenate was filtered through layers of glass wool and diluted with an equal volume of the same buffer containing different concentrations of sucrose (2.1 M) and Triton X-100 (0.1%) (buffer B). This suspension was layered over 15 mL of buffer B and centrifuged for 90 min at 70000*g* in a Beckman SW 25.1 rotor at 4 °C. The nuclear pellet was washed twice in

Table I: Specific Binding of [^{3}H]R-1881 to Adult (~100-Day-Old) Male Liver Cytosol and Competition by Nonandrogenic Steroids[a]

competing steroid	% displacement	competing steroid	% displacement
R-1881	100	progesterone	5.8
triamcinolone acetonide	6.2	estradiol-17β	31.1

[a] Binding assays were performed in the presence of 58 nM [^{3}H]R-1881 and a 500-fold molar excess of unlabeled competing steroids. In the absence of any unlabeled competing steroids, 52.0 fmol of R-1881 was specifically bound to 1 mg of cytosolic protein (100% binding).

Table II: Correlation between Age- and Sex-Specific Binding of [^{3}H]R-1881 to Liver Cytosol and Level of Hepatic α_{2u}-Globulin[a]

liver cytosol	specific R-1881 binding (fmol/mg of protein)	cytosolic α_{2u}-globulin (ng/mg of protein)
adult male (100 days)	52.98	240.00
adult female (100 days)	6.53	0.85
immature male (30 days)	5.14	0.41
old male (850 days)	7.99	2.66

[a] Values are means of three animals for each age group.

buffer C (10 mM Tris-HCl, pH 7.4, containing 25 mM KCl, 5 mM $MgCl_2$, and 0.35 M sucrose) by centrifugation at 500g. The nuclear suspension was lysed by sonication, and the protein concentration was determined according to Bradford (1976). The nuclear lysate and the liver cytosol of equal protein content (2.8 mg/mL) were used for [^{3}H]R-1881 binding and photoaffinity labeling.

Fractionation of the Slab Gel and Determination of Radioactivity. The appropriate lane in the dried slab gel was sliced into 2-mm segments, and each slice was incubated in 0.5 mL of hydrogen peroxide for 14 h at 37 °C in counting vials. The vials containing the gel slices were mixed with Bray's solution, and the radioactivity was determined in a liquid scintillation spectrometer.

Radioimmunoassay of α_{2u}-Globulin. α_{2u}-Globulin contents in rat liver cytosol were determined by double antibody radioimmunoassay as described earlier (Roy, 1977).

Results

Binding of R-1881 to the cytosolic proteins of the adult male rat liver displays a high degree of steroid specificity (Table I). Among the three other classes of steroid hormones used for this experiment (i.e., glucocorticoid, progesterone, and estrogen), only the estrogenic steroid (estradiol-17β) showed significant (30%) displacement of the labeled R-1881 when incubated at a molar ratio of 1:500 (R-1881 to estradiol-17β). In addition to steroid specificity, specific binding of R-1881 showed age- and sex-dependent variations (Table II). The livers of immature and senescent male rats and females of all ages do not synthesize appreciable amounts of α_{2u}-globulin. These animals contain relatively low (10–15% of the adult male) levels of the R-1881 binding activity in their livers (Table II).

Earlier studies have indicated cellular heterogeneity with respect to the hepatic synthesis of α_{2u}-globulin (Antalky et al., 1982; Motwani et al., 1984). In the male rat, a small population of hepatocytes becomes competent to synthesize α_{2u}-globulin during puberty (about 40 days of age). Subsequently, the number of these competent cells increases rapidly (Sarkar et al., 1986). The competent and noncompetent hepatocytes can be separated and isolated through fluorescence-activated

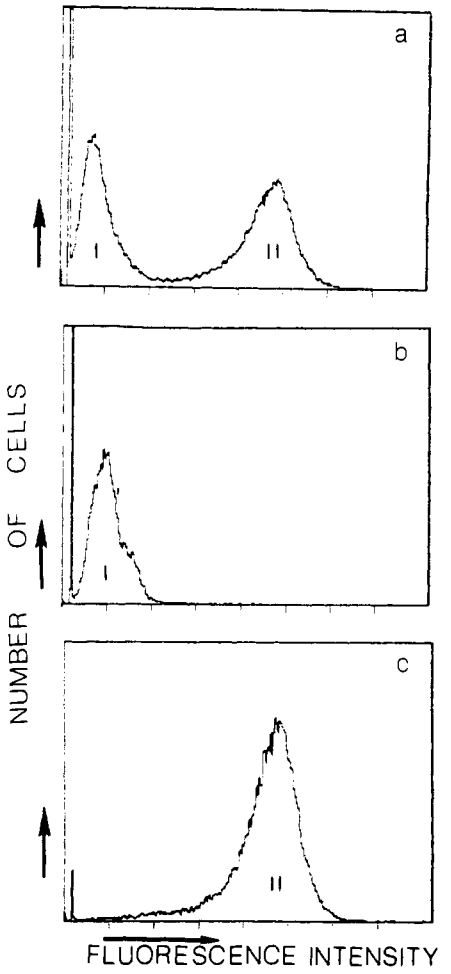

FIGURE 1: FACS pattern of hepatocytes isolated from a 45-day-old male rat. (a) Total hepatocyte population showing two distinct subpopulations (peaks I and II); (b and c) rerun of the cells isolated from peaks I and II. Both the relative fluorescence intensity and the number of cells (arbitrary scale) are expressed in linear increments. Control cells incubated only with the second antibody showed a fluorescent pattern which resembled the peak I cells.

cell sorting (FACS) (Motwani et al., 1984). We have examined the extent of [^{3}H]R-1881 uptake by the two populations of hepatocytes obtained from the liver of the 45-day-old (postpubertal) male rat. This particular age was chosen because at this stage the two cell populations are almost equally distributed. Figure 1 shows the FACS pattern of the hepatocytes derived from a 45-day-old male rat liver. Two cell populations can be distinctly separated by FACS, and upon reprocessing, they maintain their distinctive fluorescence intensities. Figure 2 summarizes the results of specific cellular uptake of [^{3}H]R-1881 by the unfractionated and fractionated hepatocytes. The subpopulation of hepatocytes which showed only weak immunoreactivity to anti-α_{2u}-globulin displayed about 40% specific uptake of [^{3}H]R-1881 as compared to the competent cell populations. The binding activity of the total unfractionated cell population can be accounted for by the sum of the two fractionated cell populations. Under identical binding conditions, the binding activity of the unfractionated hepatocytes from the liver of female rats was found to be comparable to the noncompetent fraction of hepatocytes isolated from the postpubertal male rat.

The electrophoretic distribution of the photoaffinity-labeled androgen binding proteins within the cytosol derived from prepubertal, adult, and senescent male and adult female rats is shown in Figure 3A. Among these four types of animals, only the liver of the adult male is sensitive to androgen and

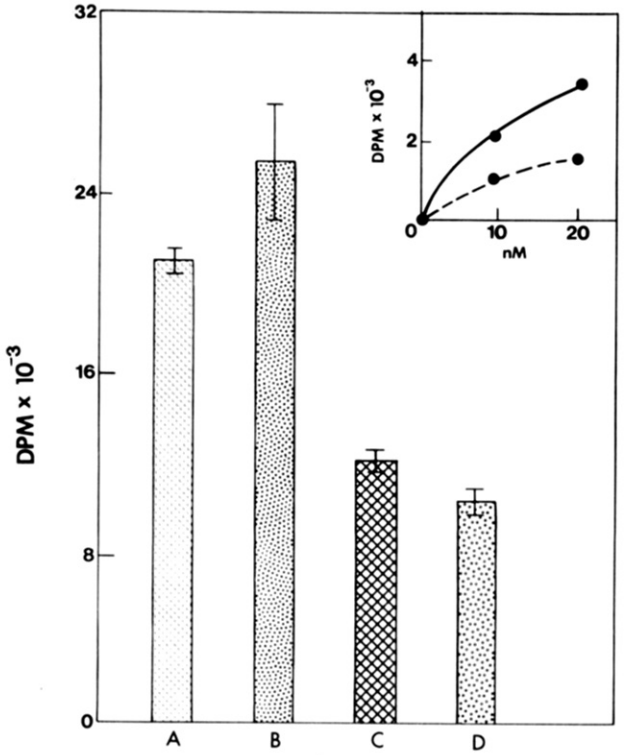

FIGURE 2: Whole cell binding of [^{3}H]R-1881. Bar graphs from left to right show (A) specific uptake by unfractionated hepatocytes from 45-day-old male rat (filled with angular signs), (B) uptake by the cell collected from the FACS peak II (filled with solid dots), (C) uptake by cells collected from the FACS peak I (filled with cross-hatchings), and (D) uptake by unfractionated hepatocytes from female rat (filled with open circles). In all four experiments, equal numbers of hepatocytes (5×10^4) were incubated with 18 nM [^{3}H]R-1881. Nonspecific binding (in the presence of a 500-fold excess unlabeled R-1881) was subtracted from the total radioactivity bound by the hepatocytes. Each bar graph represents an average of five separate binding assays ± SD. The inset shows the dose–concentration dependence for R-1881 binding by FACS-fractionated peak I (●---●) and peak II (●—●) hepatocytes. For these experiments, 5×10^3 cells were used for each point, and the nonspecific binding (in the presence of a 500-fold molar excess of R-1881) was subtracted from the total uptake of [^{3}H]R-1881.

produces α_{2u}-globulin. In all of these animals, several proteins were found to cross-link with [^{3}H]R-1881 upon ultraviolet irradiation. However, only in the case of the adult male rat which synthesizes α_{2u}-globulin under androgenic influence can a dominant R-1881 binding band of M_r 31 000 be seen. Furthermore, androgen-insensitive testicular feminized male (Tfm) rats which do not synthesize α_{2u}-globulin under androgenic stimulation also lack the 31-kilodalton (kDa) androgen binder (lane 10).

The relative distribution of radioactivity in the various androgen binding components was determined by gel fractionation. Results show that in the adult male rat the 31-kDa androgen binding component accounts for 30–50% of the total covalently bound radioactivity. In the case of non-α_{2u}-globulin-producing and androgen-insensitive animals such as females and prepubertal, senescent, and testicular feminized males, the 31-kDa binding component is almost totally replaced by a 29-kDa protein which accounts for about 5–10% of the total androgen binding activity (data not shown). Unlike unlabeled R-1881, a 500-fold molar excess of dexamethasone and progesterone was totally ineffective in reducing the binding of labeled R-1881 from the 31-kDa band. However, unlabeled estradiol-17β partially inhibited the binding of [^{3}H]R-1881 (Figure 1b). It is of interest to note that unlabeled estradiol-17β is also able to inhibit the binding of labeled R-1881 by two closely spaced bands around the 24-kDa range and the

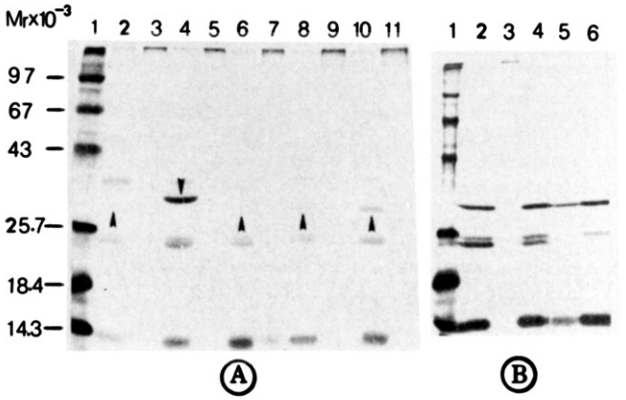

FIGURE 3: (A) Electrophoretic distribution of proteins labeled with [^{3}H]R-1881 in hepatic cytosols derived from animals of different ages and sexes. Lane 1 displays labeled molecular weight markers as identified by their respective molecular weight × 10^{-3}. The rest of the figure represents autoradiographic patterns of cytosols photoaffinity labeled with [^{3}H]R-1881. All even-numbered lanes are patterns of samples without any unlabeled R-1881, and the odd-numbered lanes show patterns with a 500-fold excess of unlabeled R-1881 included during photolysis. 70 μg of total protein was applied to each lane. Lanes 2 and 3, 30-day-old male; lanes 4 and 5, 100-day-old male; lanes 6 and 7, 850-day-old male; lanes 8 and 9, 100 day-old female; lanes 10 and 11, androgen-insensitive testicular feminized male (Tfm) rats which do not synthesize α_{2u}-globulin. The downward arrow marks the 31-kDa binding protein, and the upward arrows mark the 29-kDa binder. Time of exposure of the X-ray film = 11 days. (B) Competition of [^{3}H]R-1881 binding to the 31-kDa band with other classes of steroid hormones. The cytosol samples were all from 100-day-old male rat liver. Lane 1, labeled molecular weight markers; lane 2, cytosol photoaffinity labeled with [^{3}H]R-1881 alone; lanes 3, 4, 5, and 6, [^{3}H]R-1881 in the presence of a 500-fold molar excess of unlabeled R-1881, dexamethasone, estradiol-17β, and progesterone, respectively. Time of exposure of the X-ray film = 11 days.

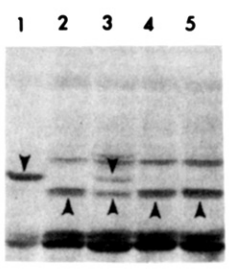

FIGURE 4: Effects of castration and estrogen treatment on the 31- and 29-kDa androgen binding proteins. Cytoplasmic extracts containing 70 μg of total protein photoaffinity labeled with [^{3}H]R-1881 were applied to each lane. The figure shows the autoradiogram of the dried slab gel. Samples in various lanes are from (1) 125-day-old normal male, (2) 125-day-old male treated for 10 days with estradiol-17β [50 μg (100 g)$^{-1}$ day^{-1}], (3) 125-day-old male castrated 25 days prior to killing, (4) 125-day-old male castrated 25 days prior to killing and injected daily with estradiol for last 10 days [50 μg (100 g)$^{-1}$ day^{-1}], and (5) 125-day-old normal female. The 31-kDa bands are marked with downward arrows, and the 29-kDa bands are marked with upward arrows. Time of exposure of the X-ray film = 14 days.

unlabeled progesterone inhibits the lower molecular weight band of this doublet as well.

Similar to the androgen-insensitive state observed in the prepubertal and senescent male rats and Tfm rats, hepatic androgen insensitivity can also be produced in the adult male rat by estrogen treatment (Roy et al., 1975). As shown in Figure 4, such estrogen-mediated androgen insensitivity is also associated with the replacement of the 31-kDa band with the 29-kDa androgen binding moiety. Castration of the adult male leads to an in-between situation where both 31- and 29-kDa binding components are observed.

The 31-kDa androgen binding component cannot be found within the nuclear extracts of either male or female liver

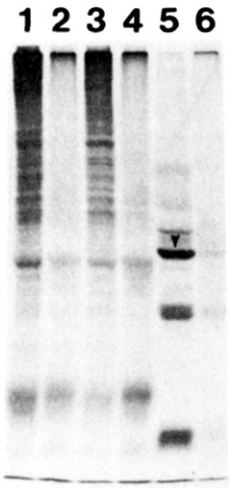

FIGURE 5: Autoradiogram of the electrophoretically separated [^{3}H]R-1881-labeled proteins present in the nuclear and cytoplasmic extracts of the hepatocytes derived from 100-day-old rats. 70 μg of total protein was applied to each lane. The even-numbered lanes represent cytosol photolyzed in the presence of a 500-fold excess of unlabeled R-1881. Lanes 1 and 2, nuclear extracts from female; lanes 3 and 4, nuclear extracts from male; lanes 5 and 6, cytoplasmic extracts from male. The 31-kDa major cytoplasmic androgen binding component is marked with a downward arrow. Time of exposure of the X-ray film = 14 days.

following photoaffinity labeling with [^{3}H]R-1881. Exclusive cytoplasmic localization of the male-specific 31-kDa androgen binding protein is substantiated by the results presented in Figure 5. Although the autoradiogram of the nuclear proteins displays many labeled bands, none corresponds to the 31-kDa cytosolic binding component. No major sex difference in the photoaffinity labeling of the nuclear proteins was observed, either.

Discussion

Photoaffinity labeling has proved to be a powerful tool for molecular characterization of hormone binding proteins (Katzenellenbogen et al., 1974; Taylor et al., 1980; Birnbaumer et al., 1983; Chang et al., 1983; Liang et al., 1985). Since the liver is the major site of steroid metabolism, a nonmetabolizing synthetic androgen such as R-1881 provides a significant additional advantage for the identification of the hepatic androgen binding protein. Results presented in this paper identify several major protein components in the hepatic cytosol that can specifically bind [^{3}H]R-1881. Among these proteins, the one with M_r 31 000 is the predominant binding component in the hepatic cytosol of the adult male rat and is almost abesent in the liver of animals such as prepubertal and senescent males and females of all ages which do not synthesize α_{2u}-globulin. It should be noted not only that prepubertal and senescent male rats are incapable of synthesizing α_{2u}-globulin but also that they fail to respond to androgen administration (Roy et al., 1983).

Competition analysis with other hormonal steroids reveals that the 31-kDa androgen binder possesses high steroid specificity. In addition to the androgen, the estrogenic steroid estradiol-17β also shows weak binding activity with this protein. This finding is consistent with previous observations based on sucrose density gradient analysis of the CAB (Roy et al., 1974). Furthermore, a comparative analysis of the photoaffinity-labeled nuclear and cytoplasmic extracts provides definitive evidence for the cytoplasmic localization of this male-specific hepatic androgen binding protein. The mammalian androgen receptor is known to have a molecular weight of about 90 000 and is predominantly localized in the nuclear compartment (Chang et al., 1983). Thus, the hepatic androgen binding protein may belong to a different class of cytoplasmic steroid hormone binding proteins distinct from the steroid hormone receptors.

Since the hepatic synthesis of α_{2u}-globulin can be totally inhibited by estrogen treatment and the estrogen-inhibited rats become temporarily (for about 4 weeks) androgen insensitive (Roy et al., 1975), we have examined the effect of estrogen on the relative hepatic levels of 31- and 29-kDa bands. The observation that estrogen treatment leads to an almost complete replacement of the 31-kDa band with the 29-kDa band is consistent with our contention that the 31-kDa band may be involved in conferring androgen sensitivity to the liver. Furthermore, about 50% replacement of the 31-kDa band to the 29-kDa binding moiety after castration is also supportive of such a hypothesis.

The nature of the other binding components which are observed in the autoradiogram and which do not show any age or sex specificity has not been critically examined in this study. It is possible that some of these may represent various steroid metabolizing enzymes. Despite the fact that R-1881 does not undergo hepatic metabolism, some of the steroid-modifying enzymes may be specifically able to accept this steroid in their active site to allow enough residency time for cross-linking during photoactivation (Liang et al., 1985). Although the sum of the counts in the androgen binding bands other than the 31-kDa component (which do not show sex or age specificity) constitutes at least 50% of the total radioactivity, sucrose density gradient analysis failed to demonstrate any androgen binding peak in the liver of prepubertal and senescent male and female rats (Roy et al., 1974). This may be due to the fact that these binding components are of sufficiently low affinity and may undergo dissociation during the lengthy centrifugation step.

Immature and old male and female rats which are virtually inactive in α_{2u}-globulin synthesis show 10–15% cytoplasmic androgen binding activities as compared to the adult male. This residual binding activity may be due to the 29-kDa binding component. It is, therefore, possible that the 29-kDa binder represents a biologically inactive species. Since the 31-kDa binder seems to be replaced by the 29-kDa binder during the transition from the androgen-sensitive to the androgen-insensitive state, the possibility of interconversion of these two binding components is highly intriguing. Presently, we do not have any evidence for a precursor–product relationship between 31- and 29-kDa binding components. If future studies prove this to be the case, it will provide a novel mechanism for the age-dependent regulation of sex hormone responsiveness in the target tissue.

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References

Antalky, T., Lynch, K. R., Nakhasi, H. L., & Feigelson, P. (1982) *Am. J. Anat. 165*, 211–224.

Berger, F. G., Loose, D., Meisner, H., & Watson, G. (1986) *Biochemistry 25*, 1170–1175.

Birnbaumer, M., Schrader, W. T., & O'Malley, B. W. (1983) *J. Biol. Chem. 225*, 1637–1644.

Bonne, C., & Raynaud, J. P. (1975) *Steroids 26*, 227–232.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Brock, M. L., & Shapiro, D. J. (1983) *Cell (Cambridge, Mass.) 34*, 207–214.
Chang, C. H., Rowley, D. R., & Tindall, D. J. (1983) *Biochemistry 22*, 6170–6175.
Compton, J. G., Schrader, W. T., & O'Malley, B. W. (1982) *Biochem. Biophys. Res. Commun. 105*, 96–104.
Diamond, D. J., & Goodman, H. M. (1985) *J. Mol. Biol. 181*, 41–62.
Edelman, I. S., & Fimognari, G. M. (1968) *Recent. Prog. Horm. Res. 24*, 1–33.
Fulton, R., Birnie, G. D., & Knowler, J. T. (1985) *Nucleic Acids Res. 13*, 6467–6482.
Gorski, J., Toft, D., Shyamala, G., Smith, D., & Notides, A. (1968) *Recent Prog. Horm. Res. 24*, 45–80.
Hayward, M. A., & Shapiro, D. J. (1981) *Dev. Biol. 88*, 333–340.
Jensen, E. V., & DeSombre, E. R. (1972) *Annu. Rev. Biochem. 41*, 203–230.
Jensen, E. V., & Jacobsen, H. I. (1962) *Recent Prog. Horm. Res. 18*, 387–414.
Katzenellenbogen, J. A., Johnson, H. J., Carlson, K. E., & Meyers, H. N. (1974) *Biochemistry 13*, 2986–2994.
King, W. J., & Greene, G. L. (1984) *Nature (London) 307*, 745–747.
Korenman, S. G. (1969) *Steroids 13*, 163–177.
Kyakumoto, S., Sato, N., Nemoto, T., Ohara-Nemoto, Y., & Ota, M. (1984) *Biochim. Biophys. Acta 800*, 214–219.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Liang, T., Cheung, A. H., Reynolds, G. F., & Rasmusson, G. H. (1985) *J. Biol. Chem. 260*, 4890–4895.
Liao, S., & Fang, S. (1969) *Vitam. Horm. (N.Y.) 27*, 17–90.
Milin, B., & Roy, A. K. (1973) *Nature (London), New Biol. 242*, 248–250.
Moore, J. T., Norvitch, M. D., Weiben, E. D., & Veneziale, C. M. (1984) *J. Biol. Chem. 259*, 14750–14756.
Motwani, N. M., Caron, D., Demyan, W. F., Chatterjee, B., Hunter, S., Poulik, M. D., & Roy, A. K. (1984) *J. Biol. Chem. 259*, 3653–3657.
Mueller, G. C., Herranen, A. M., & Jervell, K. F. (1958) *Recent Prog. Horm. Res. 14*, 95–139.
Mulvihill, E. R., LePennec, J. P., & Chambon, P. (1982) *Cell (Cambridge, Mass.) 28*, 621–632.
Munck, A., & Leung, K. (1976) in *Receptors and Mechanism of Action of Steroid Hormones* (Pasqualini, J. R., Ed.) pp 311–316, Marcel Dekker, New York.
O'Malley, B. W., & Means, A. R. (1974) *Science (Washington, D.C.) 183*, 610–615.
Palmiter, R. D., & Carey, N. H. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2357–2361.
Payvar, F., Wrange, O., Carlstedt-Duke, J., Okret, S., Gustafsson, J. A., & Yamamoto, K. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6628–6632.
Roy, A. K. (1977) *Eur. J. Biochem. 73*, 537–543.
Roy, A. K., Milin, B. S., & McMinn, D. M. (1974) *Biochim. Biophys. Acta 354*, 213–232.
Roy, A. K., McMinn, D. M., & Biswas, N. M. (1975) *Endocrinology (Philadelphia) 97*, 1505–1508.
Roy, A. K., Chatterjee, B., Nath, T. S., & Motwani, N. M. (1982) *J. Biol. Chem. 257*, 7834–7838.
Roy, A. K., Chatterjee, B., Demyan, W. F., Milin, B. S., Motwani, N. M., Nath, T. S., & Schiop, M. J. (1983) *Recent Prog. Horm. Res. 39*, 425–461.
Sarkar, F. H., Mancini, M. A., Nag, A. C., & Roy, A. K. (1986) *J. Endocrinol. 111*, 205–208.
Scheidereit, C., Sabine, G., Westphal, H. M., & Beato, M. (1983) *Nature (London) 304*, 749–752.
Seglen, P. O. (1976) *Methods Cell Biol. 13*, 30–78.
Sheridan, P. J., Buchanan, J. M., Anselmo, V. C., & Martin, P. M. (1979) *Nature (London) 282*, 579–582.
Taylor, R. N., & Smith, R. G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1742–1746.
Taylor, C. A., Smith, H. E., & Danzo, B. J. (1980) *J. Biol. Chem. 255*, 7769–7773.
Vannice, J. L., Taylor, J. M., & Ringold, G. M. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4241–4245.
Welshons, W. V., Lieberman, M. E., & Gorski, J. (1984) *Nature (London) 307*, 747–749.
Wilson, E. M., & French, F. S. (1976) *J. Biol. Chem. 251*, 5620–5629.