Molecular Cloning of Testicular 20α-Hydroxysteroid Dehydrogenase: Identity with Aldose Reductase†

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ABSTRACT: Complementary DNA (cDNA) clones encoding bovine testicular 20α-hydroxysteroid dehydrogenase (20α-HSD) have been isolated from a bovine testicular λgt11 library using polyclonal antibodies against 20α-HSD and DNA probe hybridization. Nucleotide sequencing of three independently isolated clones was used to establish a composite cDNA sequence that encodes the enzyme. It contains a coding sequence of 921 nucleotides, a stop codon, and a 264-nucleotide 3′-noncoding segment which allowed deduction of the amino acid sequence of the enzyme. A computer homology search of the 20α-HSD cDNA performed against the GenBank DNA sequence database revealed it to be identical with bovine lens aldose reductase (alditol:NADPH oxidoreductase; EC 1.1.1.21), and a literature search reveals the deduced amino acid sequence to be identical with that reported for the bovine enzyme. Sequences obtained from the N-terminus of purified testicular 20α-HSD and from random peptides obtained by treatment with endopeptidase Lys-C are all identical with regions of the deduced amino acid sequence of 20α-HSD and/or the published sequence of aldose reductase. Further, the enzyme purified to homogeneity by following activity with 17-hydroxyprogesterone as a substrate was shown to reduce glucose, glyceraldehyde, and benzaldehyde (all classic aldose reductase substrates). Finally, 17-hydroxyprogesterone inhibited the reduction of benzaldehyde and glyceraldehyde. Because aldose reductase has been implicated in the etiology of diabetic complications, acceptance of steroid substrates may offer new implications for therapy.

Shikita and co-workers reported the presence of a 20α-hydroxysteroid dehydrogenase (20α-HSD)[1] in rat and porcine testis (Shikita et al., 1967; Sato et al., 1972). The enzyme was shown to be a monomer (M_r 35 000) and to lack 17β-hydroxysteroid dehydrogenase (17β-HSD) activity toward estrone. The Nomenclature Committee of the International Union of Biochemistry recommended the name 20α-hydroxysteroid dehydrogenase for this enzyme and assigned it the number EC 1.1.1.149 (Nomenclature Committee, 1979). While engaged in studies designed to define the topography of the steroid binding site and the catalytic mechanisms of mammalian steroid-interconverting enzymes, we had crystallized human placental 17β-hydroxysteroid dehydrogenase (Chin et al., 1976) and identified the steroid domain and certain catalytic residues of this dimeric dehydrogenase (Chin & Warren, 1975; Murdock-Warren, 1982; Murdock et al., 1983, 1988) using affinity labeling techniques (site-directed, irreversible inactivation). Thinking that the monomeric testicular 20α-HSD might represent a second, distinct "model" steroid dehydrogenase, we purified it to homogeneity from bovine testis, characterized it as monomeric (M_r 38 000), and demonstrated that it used both NADPH and NADH as cofactor and lacked 17β-HSD activity (Pineda et al., 1985).

Because of the work of Strickler and co-workers (Strickler et al., 1981), the Nomenclature Committee, in 1984, deleted the entry 20α-hydroxysteroid dehydrogenase (EC 1.1.1.149) and included it with estradiol 17β-dehydrogenase (EC 1.1.1.62), which was thereafter also called 17β,20α-hydroxysteroid dehydrogenase (Nomenclature Committee, 1984). Subsequently, we determined the stereospecificity of hydrogen transfer by bovine testicular 20α-HSD and demonstrated that it transfers hydrogen from the 4-*pro*-*R* position of the cofactor nicotinamide ring (Pineda et al., 1989). In contrast, placental estradiol 17β-dehydrogenase transfers to (and from) the 4-*pro*-*S* position (Jarabak & Talalay, 1960; Warren et al., 1967).

As we began affinity-labeling studies of bovine testicular 20α-HSD, it became necessary to clone the cDNA that encodes the enzyme, sequence the cDNA, and deduce its amino acid sequence. This has been done, and we now report that the cDNA ecoding bovine 20α-HSD is not similar to that encoding estradiol 17β-dehydrogenase, but that it is identical with that encoding bovine lens aldose reductase (Petrash & Favello, 1989) and that the deduced amino acid sequence is identical with that reported for bovine aldose reductase (Schade et al., 1990).

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[1] Abbreviations: HSD, hydroxysteroid dehydrogenase; DNA, deoxynucleic acid; N-terminus, amino terminus; Lys-C, Sigma protease (type XXXIII-A); NADPH, β-nicotinamide adenine dinucleotide phosphate (reduced form); NADH, β-nicotinamide adenine dinucleotide (reduced form); RNase, ribonuclease; T_4 ligase, T_4 DNA ligase; kb, kilobase; IgG, immunoglobulin G; BT, bovine testis; bp, base pairs; SSC, sodium chloride–sodium citrate buffer at pH 7.0; SDS, sodium dodecyl sulfate; dATP, 2′-deoxyadenosine 5′-triphosphate; dGTP, 2′-deoxyguanaosine 5′-triphosphate; dCTP, 2′-deoxycytidine 5′-triphosphate; TCA, trichloroacetic acid; progesterone, 4-pregnen-3,20-dione; 17 hydroxyprogesterone (17-OHP), 17-hydroxy-4-pregnen-3,20-dione; isocortisol, 11β,17,20β-trihydroxy-3-keto-4-pregnen-21-al; isocorticosterone; 11β,20β-dihydroxy-3-keto-4-pregnen-21-al; PCR, polymerase chain reaction; mRNA, messenger ribonucleic acid; HnRNA, heterologous nuclear ribonucleic acid; C-terminal, carboxy terminal; IUB, International Union of Biochemistry.

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EXPERIMENTAL PROCEDURES

Materials. A bovine testicular cDNA library in the λgt11 expression vector was obtained from Clonetech (Palo Alto, CA). Restriction endonucleases, proteinase K, RNAse A, T_4 ligase, nucleotides, and the Protoblot immunoscreening system were from Promega (Madison, WI). The p-Bluescript II (KS^+ and SK^+) and VCS-M13 helper phage were from Stratagene (La Jolla, CA). The Geneclean kit was from Bio 101, Inc. (La Jolla, CA). The 1-kb DNA ladder standard was obtained from Bethesda Research Laboratories (Gaithersburg, MD). Amplitaq DNA polymerase was obtained from Perkin Elmer Cetus (Norwalk, CT). The pZ 523 Spin Column Plasmid purification system was from 5 Prime-3 Prime, Inc. (Boulder, CO). Nitrocellulose filters were obtained from Schleicher and Schuell (Passel, FRG). The [^{32}P] α-dCTP, >3000 Ci/mmol, was obtained from New England Nuclear (Boston, MA). Other reagents were the highest purity available from various suppliers. All solutions were made in deionized, glass-distilled water and were sterilized by autoclaving or filtration.

Antibody Screening of the λgt11 Library. Highly purified, homogeneous (Pineda et al., 1989) 20α-HSD was combined with Freunds adjuvant. Serial injection into rabbits resulted in a polyclonal antibody, which gave a positive Western blot with 20α-HSD at dilutions of 1:500 and 1:1000 but failed to do so with human placental estradiol 17β-dehydrogenase. Antiserum was diluted 1:10 with TBST buffer (10 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20) and absorbed for 30 min on an agar plate of λgt11 (bearing no insert) infected into *Escherichia coli* Y1090r$^-$. It was then taken to a 1:500 dilution with TBST buffer for use as the first antibody. Approximately 8×10^5 plaque forming units (pfu) of recombinant phage from an unamplified bovine testicular cDNA library were propogated in *E. coli* Y1090r$^-$ and screened on 20, 150-mm Petri plates with the anti-20α-HSD-antiserum. Expression was induced by overlaying plates with nitrocellulose filters which had been soaked in 10 mM IPTG (isopropyl β-D-thiogalactopyranoside) and patted dry. Filters were incubated in anti-20αHSD for 16 h, and then positive plaques were detected by the use of an alkaline phosphatase:goat-anti-rabbit IgG conjugate as second antibody (Protoblot system). Apparent positive plaques were further purified by rescreening at low plaque density and by selection of the persistently positive plaque.

DNA Probe Hybridization. Approximately 8×10^5 pfu of recombinant phage from the amplified bovine testicular library was screened by high-stringency hybridization using a fragment of the clone recovered from immunoscreening (BT 20α-1) as a probe. The probe fragment was prepared by simultaneous incubation of the BT 20α-1 cDNA with *Eco*RI and *Bgl*II restriction enzymes, by electrophoresis of the resultant DNA fragments on 1% agarose gels, and by recovery of the 250-bp *Eco*RI–*Bgl*II 5′ fragment with the Geneclean kit. This fragment was radiolabeled by the random priming procedure using Klenow DNA polymerase and [^{32}P] α-dCTP. The library was propogated in LE392 bacteria, and filter replicates were prepared (Zimmer et al., 1988). Hybridization was carried out by incubation of the filters with the probe fragment for 16 h at 65 °C in buffer containing 6 × SSc, 1x Denhartz, 0.05 M $NaHPO_4$ (pH 7.0), 1% SDS, and 100 μg/mL sheared salmon sperm DNA, and washing was for 3 h with 2x SSC and 0.1% SDS at 65 °C. Positive clones were identified by autoradiography and purified by rescreening at low plaque density until a homogeneous population of each positive plaque was obtained.

Amplification, Restriction Endonuclease Treatment, and Subcloning Procedures. Amplification of the inserts was accomplished by polymerase chain reaction of inserts in the λgt11 vector, after appropriate purification, utilizing a mixture of 0.2 mM each of dATP, dGTP, dCTP, and dTTP, specific λgt11 priming oligonucleotides, and Amplitaq DNA polymerase in a Ericomp Easy Cycler apparatus essentially as described by Friedman et al. (1988).

Incubation of inserts and vectors with restriction endonucleases was carried out using appropriately purified DNA and buffers from the Promega 4-Core system. Incubations were overnight at 37 °C in the optimum buffer using 2 units of enzyme/μg of DNA. Efficacy of the reaction was monitored by electrophoresis.

Subcloning usually involved incubation of the vector (after treatment with calf intestinal phosphatase and the Geneclean kit) and insert (after removal from electrophoresis gel by treatment with the Geneclean kit) overnight with T_4 ligase in ligation buffer. In some instances, restriction endonuclease-treated inserts were purified on NuSieve low melting agarose and identified by ethidium bromide staining, and the region of the agarose containing them was cut out, melted, and added to the ligation incubation. The ligation products were used to transform *E. coli* strain UT481 as described previously (Zimmer, et al., 1988). Preparative quantities of Phagemid-borne inserts were obtained by growth in Luria broth with ampicillin overnight, and subsequent purification and recovery of double-stranded plasmid DNA away from bacterial chromosomal DNA with the PZ 523 system.

cDNA Sequence Analysis. Colonies with recombinant phagemid were induced to synthesize single-stranded DNA by growth in Luria both containing ampicillin (75 μg/mL), with VCS-M13 helper phage and incubation at 37 °C for 12–16 h with vigorous shaking. Single-stranded DNA was purified from the media as described previously (Zimmer et al., 1988). It was resuspended in sterile water, and after quantification by absorption of UV light at 260 and 280 nm, its purity was monitored by electrophoresis. Sequence was obtained from single-stranded templates by the dideoxynucleotide chain termination method and automated analysis of the reaction mixture using fluorescent-labeled DNA primers with an applied Biosystems Model 370A DNA sequencing apparatus. Data from the multiple sequencing analyses were collated and analyzed using the University of Wisconsin Genetics Computer Programs (GCG) available through the Alabama Super Computer Network.

Generation and Sequencing of Peptides from 20α-Hydroxysteroid Dehydrogenase. The final enzyme purification to homogeneity was achieved by isolation on a Fast Flow Q column on the FPLC system (Pharmacia) as previously described (Pineda et al., 1989). The purification was monitored by following the oxidation of NADPH with 17-hydroxyprogesterone (17-OHP) as the substrate. The eluted enzyme (430 μg/mL of protein by Coomassie) was prepared for N-terminal analysis and limited proteolysis by endoproteinase Lys-C (protease type XXXIII-A, Sigma Chemical).

An aliquot of 450 μL (194 μg ≈ 5 nmol of enzyme) was precipitated with 50 μL of 100% TCA (10% final concentration), incubated on ice for 30 min, and centrifuged in a table-top microfuge for 8 min. The pellet was washed twice with 100 μL of cold acetone and air dried. The protein sample was submitted for N-terminal analysis in an Applied Biosystem Model 477 peptide sequencer.

Limited proteolysis was carried out on a 900-μL (10 nmol) aliquot of the homogeneous enzyme which was precipitated

with 100 μL of 100% TCA, washed, and dried as above. The pellet was dissolved in 10 μL of 1 N NaOH and then 100 μL of 50 mM Tris buffer, (pH 8.0) was added, and the pH was readjusted to 8.0 with 1 N HCl. Endoproteinase Lys-C (25 μg) was added, and the mixture was incubated at 37 °C with 25-μL aliquots taken at times 0, 0.5, 1, 4 and 18 h. The aliquots were applied to a HPLC, and the peptides were separated on a C-18 reversed-phase column with an initial buffer of 0.06% trifluoroacetic acid (TFA) in H_2O and a limit buffer of 0.045% TFA in 80% acetonitrile. The peptides were eluted at a flow rate of 0.6 mL/min and were monitored at 214 nm.

Enzyme Activity Assays. The spectrophotometric assays were carried out in 3 mL of 0.1 M Na_xPO_4 buffer, pH 6.5, containing 100 μM NADPH and substrates (benzaldehyde, glyceraldehyde, glucose, and 17-OPH), separately or together, at various concentrations at room temperature. The reaction was initiated by the addition of 20 μg of 20α-HSD in 100 μL of buffer. Assays to determine K_m and k_{cat} for glyceraldehyde as substrate were carried out at pH 7.0.

To evaluate the kinetic mechanism of the inhibition of 17-hydroxyprogesterone reduction by glyceraldehyde, 0.32 nmol of enzyme was incubated in 0.1 M Na_xPO_4 buffer, pH 6.4, containing 500 μM NADPH with the steroid at various concentrations (7.5–56 μM) both with and without 20 μM glyceraldehyde, at room temperature for 30 min. Total volume of the final assay mixture was 1.0 mL. At the conclusion, steroids were extracted, and both substrate and product were separated and quantitated by HPLC.

RESULTS

Isolation and Nucleotide Sequencing of 20α-Hydroxysteroid Dehydrogenase cDNA Clones. The immunoscreening of ~8 × 10^5 bovine testicular cDNA clones with polyclonal antibodies directed against 20α-HSD yielded one positive clone which was designated as BT20α-1. *Eco*RI-digestion of the recombinant phage released a cDNA insert of 950 base pairs (bp). Additional 20α-hydroxysteroid dehydrogenase clones were obtained by screening 8 × 10^5 clones (amplified library) using a hybridization probe, from the BT 20α-1 clone. This probe, containing 250 bp, identified 10 additional clones which were persistent on rescreening and which after release with *Eco*RI yielded inserts of 0.5–1.8 kb that gave positive Southern blots with the probe. These were originally designated as W_1–W_{10}, respectively. They were studied (after PCR amplification) by digestion with *Bgl*II and *Xba*I (separately) to identify those extending upstream from the 5′ end of BT20α-1. Those that looked promising were further characterized by comparison of *Bgl*II digestion products with those of simultaneous *Bgl*II and *Nco*I treatment. From this analysis, two clones appeared to extend well upstream from the 5′ end of BT20α-1, and they were then redesignated BT20α-2 and -3, respectively (Figure 1).

For DNA sequencing analysis, various restriction fragments of the BT20α-cDNAs were subcloned into KS^+ and SK^+ vectors as necessary. Figure 1 shows the sequencing strategy utilized to obtain the composite sequence for the bovine testicular 20α-hydroxysteroid dehydrogenase. The composite cDNA sequence that encodes the bovine testicular 20α-hydroxysteroid dehydrogenase and the deduced putative amino acid sequence of the enzyme is shown in Figure 2.

Our composite sequence contains 1188 nucleotides, of which 924 constitute an open reading frame specifying a 307 amino acid polypeptide, and a TGA stop codon. Additionally, it contains 264 nucleotides and 3′ nontranslated sequences from

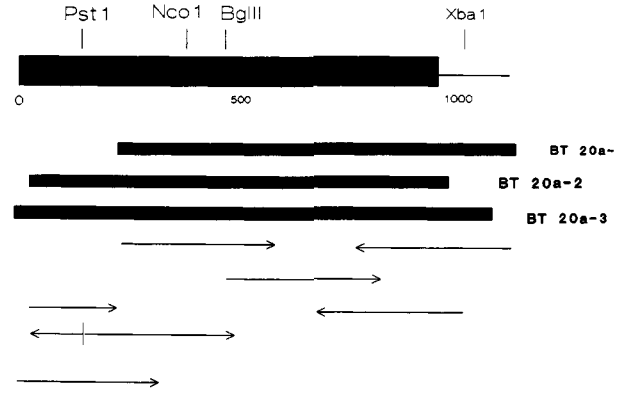

FIGURE 1: Structure and sequencing strategies for bovine testicular 20α-hydroxysteroid dehydrogenase cDNA clones. Restriction endonucleases sites shown. Each clone was sequenced according to the strategies shown.

the 20α-HSD mRNA molecule. There was no apparent polyadenylation tract associated with the 3′ end of clone BT20α-1 (most 3′ clone, Figure 1), suggesting that our sequence does not represent a complete copy of the noncoding region of the 20α-HSD mRNA. Although BT20α-2 extended some 200 bp upstream from the 5′ end of BT20α-3, this region of BT20α-2 contained stop codons and sequences clearly dissimilar from BT20α-3. We believe that this arose from an incompletely spliced HnRNA and that the additional sequences represented intervening sequences of the bovine 20αHSD gene.

Peptides Obtained from 20α-Hydroxysteroid Dehydrogenase. Figure 3 shows the sequences obtained by analysis of peptides derived from the purified 20αHSD enzyme. Comparison with Figure 2 allows assignment of amino acid residue number in the peptides. It can be seen that in all four cases a peptide has been obtained from the enzyme that is identical with a region of the amino acid sequence as deduced from the composite cDNA sequence that encodes the enzyme or (in the case of N-terminal analysis) the published enzyme sequence. These obervations provide convincing evidence that the deduced enzyme sequence is indeed, correct.

Nucleotide and Amino Acid Sequence Comparisons. A computer homology search of the 20α-HSD cDNA (Figure 2) performed against the GenBank DNA sequence database revealed that the nucleotide sequence was identical with that of the cDNA that encodes bovine lens aldose reductase [alditol: $NAD(P)^+$ 1-oxidoreductase, EC 1.1.1.21] as reported by Petrash and Favello (Petrash & Favello, 1989). Bovine aldose reductase is also a monomeric enzyme of similar molecular weight which uses both NADPH and NADH as cosubstrate. The amino acid sequence of 20α-hydroxysteroid dehydrogenase deduced from our cDNA is identical with the 307 C-terminal amino acid sequence of bovine lens aldose reductase (Schade et al., 1990).

Substrate Specificity and Interaction. The identify of 20α-hydroxysteroid dehydrogenase cDNA, its deduced amino acid sequence, and the peptides actually isolated from the enzyme with the cDNA that encodes for and the published amino acid sequence of bovine aldose reductase led us to examine substrate specificity. The data, shown in Table I, clearly indicate that the enzyme purified from bovine testis using 17-hydroxyprogesterone as a substrate also reduces glucose, benzaldhyde, and glyceraldehyde (classic aldose reductase substrates) with high efficiency and that 17-hydroxyprogesterone inhibits reduction of both glyceraldehyde and acetaldehyde. Further, K_m and k_{cat} values determined for glyceraldehyde as a substrate for 20α-HSD (18.8 μM and 0.717 s^{-1}) are in agreement with

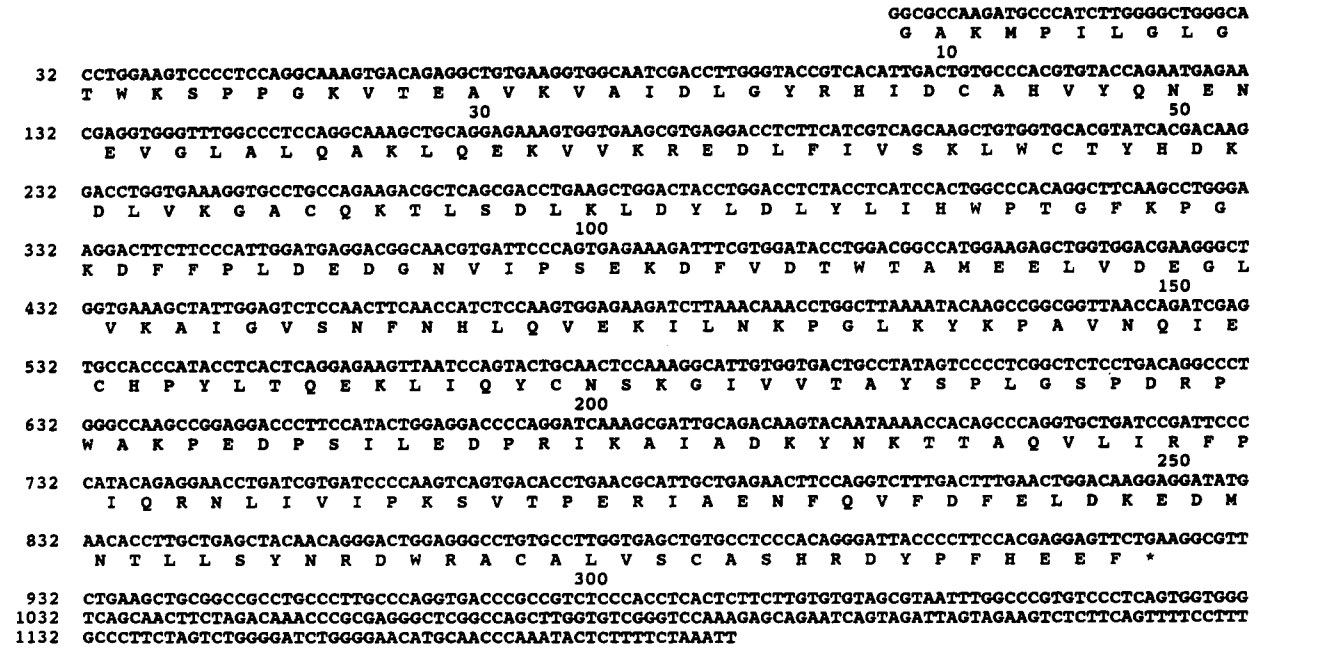

FIGURE 2: Nucleotide sequence and deduced amino acid sequence obtained from bovine testicular 20α-hydroxysteroid dehydrogenase cDNA clones. *Eco*RI linkers located at either end are not shown.

-Ile^{4}-Val-Leu-Tyr-

-Val^{27}-Thr-Glu-Ala-Val-Lys-

-Asp^{86}-Leu-Val-Lys-

-Ala^{235}-Ile-Ala-Asp-Lys-Tyr-Asn-Lys-

FIGURE 3: Peptides obtained from homogeneous testicular 20α-hydroxysteroid dehydrogenase by methods described in text and their identification as to regions of the amino acid sequence deduced from bovine testicular 20α-hydroxy dehydrogenase cDNA and the published sequence of aldose reductase (Schade et al. 1990).

Table I: Reductase Activity of Bovine Testicular 20α-HSD with Various Substrates

	nmol of NADP$^+$ generated/min[a]	
substrate (mM)	no steroid	+17-OHP (125 μM)
benzaldehyde (0.1)	17.8	
benzaldehyde (0.05)	15.4	13.0
glyceraldehyde (5)	20.2	16.4
glucose (5)	8.0	
17-OHP (0.25)	9.0	

[a] Assay conducted at room temperature, pH 6.5, as described in the text. Reaction initiated by addition of 20 μg of 20α-HSD. Activity calculated by decrease of absorbance at 340 nm. Progesterone (20 μM) and 17α,20α-dihydroxy-4-pregnen-3-one (60 μM) did not inhibit reduction of 0.05 mM glyceraldehyde.

values reported for aldose reductase from bovine lens (Del Corso et al., 1989) and human testis (Tanimoto et al., 1991). K_m and k_{cat} values determined for 17-hydroxyprogesterone as a substrate are 71.7 μM and 0.049 s^{-1}, respectively. Finally, a Lineweaver–Burk plot indicated that glyceraldehyde appeared to be a noncompetitive inhibitor of 17-hydroxyprogesterone (data not shown).

Additional Amino Acid Sequence Comparisons. A computer homology search of the amino acid sequence of 20α-HSD as deduced from our cDNA composite performed against the GenBank translated sequence database reveals it to be 100% identical with bovine aldose reductase, 87.5% identical with human aldose reductase, 87.2% identical with rabbit aldose reductase, 85.5% identical with rat aldose reductase, and 69.3% identical with mouse aldose reductase. Thus, across a wide range of mammalian species identity varies from 69.3 to 87.5%. As to be expected, lower but significant levels of identity are noted on comparison with amino acid sequences of related enzymes of the superfamily of NAD(P$^+$)-dependent aldo-keto reductases: human alcohol dehydrogenase, 50.4%; bovine prostaglandin-D2 11 reductase, 50.5%; human chlordecone reductase, 48.3%; and 48.3%, and rat 3α-hydroxysteroid dehydrogenase 47.1%. Finally there was 49% identity with frog lens ρ crystallin.

DISCUSSION

The findings of this work provide incontrovertible evidence that the 20α-HSD activity which we purified from bull testis using 17-hydroxyprogesterone as a substrate is not due to a distinct 20α-HSD or estradiol 17β-dehydrogenase but is attributable to aldose reductase. This identification is based upon the following: (a) The cDNA clones that encode 20α-HSD are 100% identical in sequence with cDNA clones encoding bovine lens aldose reductase. (Indeed, they even provide the identity of 31 additional, previously unreported nucleotides at the 5′ end.) (b) The cDNA clones that encode 20α-HSD allow the deduction of the sequence of 307 amino acid residues which prove to be 100% identical with the 307 C-terminal amino acids of aldose reductase. (c) Four peptides obtained from 20α-HSD are identical with regions in aldose reductase (one peptide consists of amino acids 3–7). (d) 20α-HSD accepts benzaldehyde, glyceraldehyde, and glucose as substrates (all classic aldose reductase substrates). Futher, previous work has indicated that both are monomers (M_r 35 000–38 000), both transfer hydrogen from the 4-*pro-R* position of NADPH, and each uses NADPH or NADH as cofactor (Feldman et al., 1977; Pineda et al., 1989).

It is of interest that the attempt at N-terminal analysis, using large amounts of 20α-HSD, failed to reveal the first three amino acid residues but did reveal residues 4–7. Schade and co-workers (Schade et al., 1990) have shown that the N-terminal alanine of aldose reductase is acetylated (and hence, blocked). The most likely explanation is that, in the

large quantity of enzyme used, a small proportion was unblocked or deacetylated during purification, and it was this fraction that was sequenced. The first three residues could not be identified because of the "noise" commonly seen at the initiation of sequence, but residues were identified later in cycles 4–7 as the "noise" subsided.

Wiest and co-workers (Wiest, 1959; Wiest & Wilcox, 1961) reported the presence of 20α-HSD in the rat ovary in 1959. This enzyme accepted progesterone and 17-hydroxyprogesterone as substrate, mediated hydrogen transfer from the 4-*pro-R* side of NADPH (Kersey & Wilcox, 1970) and lacked estradiol 17β-dehydrogenase activity. Nevertheless, the recommended name and number assigned by the IUB was based on the report of a testicular 20α-HSD (Shikita et al., 1967) and the report of two 20α-HSD activities in adrenal, one acting on 21-desoxysteroids and one acting on 21-hydroxysteroids (Krehbiel & Darrach, 1968). Although the IUB later (Nomenclature Committee, 1984) deleted 20α-hydroxysteroid dehydrogenase (EC 1.1.1.149) and included it with estradiol 17β-dehydrogenase (EC 1.1.1.62), numerous observations hinted that in the case of the testicular enzyme this is not the case. It is monomeric, favors 17-hydroxyprogesterone over progesterone, and transfers from the 4-*pro-R* side of the nicotinamide of NADPH (as does ovarian 20α-HSD). Estradiol 17β-dehydrogenase is dimeric and transfers from the 4-*pro-S* side.

The observations reported in this paper indicate that 20α-HSD activity in bovine testis and quite likely those in the testis of rat, pig, and other species is due to aldose reductase, which now has the IUB recommended name aldehyde reductase (EC 1.1.1.21), the IUB systematic name alditol: $NAD(P)^+$ 1-oxidoreductase, and is also called polyol dehydrogenase and ALR_2. This is not to say that estradiol 17β-dehydrogenase will not catalyze 20α-oxidoreduction of progesterone. Indeed, the work of both Strickler (Strickler et al., 1991) and Purdy (Purdy et al., 1964) clearly demonstrates that the enzyme performs that catalysis. What is indicated, however, is that there are other enzyme(s) capable of effecting 20α-oxidoreduction of steroids.

There is a previous report that human brain aldose reductase will reduce steroid C-21-aldehydes (Wermuth et al., 1982). Isocortisol and isocorticosterone (20-hydroxy-21-aldehydes), which are intermediates in the indirect, or "long-loop", pathway of glucocorticoid metabolic degradation, are excellent substrates. The products are the 20,21-dihydroxy compounds which are conjugated and excreted (Lippman & Monder, 1978). The observations in this current paper constitute the first report that a biologically active steroid (progesterone) and a major precursor (17-hydroxyprogesterone) of the biologically active androgens, estrogens, and glucocorticoids are substrates for aldose reductase. The enzyme will reduce the 20-carbonyl group and reduce or eliminate actual or potential biologic activity. It has been demonstrated by Shikita (Sato et al., 1972) and in our laboratory (Pineda et al., 1985) that testicular 20-hydroxysteroid dehydrogenases (now identified as aldose reductases) favor 17-hydroxyprogesterone as a substrate over progesterone. These observations raise the question as to how many other enzymes, previously characterized in various species as "20α-dehydrogenases", particularly those that favor substrates with 11β-, 17-, or 21-hydroxylated substituents (polyols) are, in reality, aldose reductase.

There is also the question as to how many other enzymes that are currently thought to be exclusive steroid dehydrogenases are in reality members of the $NAD(P)^+$-dependent aldo-keto reductase superfamily. Pennington and co-workers (Pawlowski, 1991) and Stolz et al. (1991) have cloned rat hepatic 3α-hydroxysteroid dehydrogenase and have shown it to have 75% amino acid sequence homology with bovine lung prostaglandin F synthetase and 50% homology with human aldose reductase. Further, this enzyme, which catalyzes the stereospecific degradative reduction of several biologically active steroids (and indeed terminates their biological activity), accepts a wide variety of substrates and actually possesses prostaglandin F synthetase activity (Penning et al., 1987). Finally Dunbar and co-workers (unpublished observations, presented at the meeting of the Society for Gynecologic Investigation, San Antonio, TX, 1992) sequenced the cDNA that encodes rabbit ovarian 20α-HSD and reported that it resembles human liver chlordecone reductase and frog lens ρ crystallin. While these steroid dehydrogenase activities are certainly not due to aldose reductase (sequence data for rat and rabbit aldose reductases were available when all these authors did their search), they are almost certainly due to members of the $NAD(P)^+$-dependent aldo-keto reductase family. When all this is sorted out, it may be that hepatic 3α-hydroxysteroid dehydrogenase and prostaglandin F synthetase are identical (75% homology across species allows that speculation) as are rabbit ovarian 20α-HSD and chlordecane reductase. Nevertheless, currently available information now suggests that degradation of steroids by reduction of their aldehyde and ketone groups may be primarily due not to specific steroid dehydrogenases but result from the broad substrate specificity of enzymes of the $NAD(P)^+$-dependent aldo-ketase family.

Our analyses of the mechanism of inhibition of 17-hydroxyprogesterone reduction by glyceraldehyde (since both are substrates) had to be carried out by evaluation of steroid substrate and product concentrations. The results suggest that glyceraldhyde is a noncompetitive inhibitor. Thus, we cannot produce strong evidence that both substrates utilize the same active site at this time. Nevertheless, the recent demonstration that the active site pocket of aldose reductase is of an extremely hydrophobic nature (Wilson et al., 1992) signals, to us, the need for further investigation of this question.

Aldose reductase reduces glucose to sorbitol. It appears to be responsible (via the sorbitol or polyol pathway) for certain of the complications (cataract, retinopathy, and neuropathy) seen in diabetes mellitus (Kinoshita & Nishimura, 1988). While there is debate as to whether sorbitol itself, or metabolic events resulting from excess sorbitol levels associated with hyperglycemia, is the culprit (Pugliese et al., 1991), these complications can be prevented or delayed by the administration of aldose reductase inhibitors (Kador, 1988). While there is a rich literature on inhibitors, many have side effects. The current work raises the possibility that a steroid which lacks biological activity might be useful in this role.

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