Structure and Tissue-Specific Expression of the Aldo–Keto Reductase Superfamily†

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ABSTRACT: We previously identified multiple proteins structurally related to 3α-hydroxysteroid dehydrogenase in rat liver, lung, kidney, and testis ((1991) *Arch. Biochem. Biophys. 291*, 258–262). We further used these monoclonal antibodies to screen several λ gt11 cDNA libraries derived from male rat liver, lung, and kidney. Five additional unique cDNA clones were isolated and sequenced; the proteins encoded by these cDNAs were found to exhibit 37–62% amino acid sequence homology to rat liver 3α-hydroxysteroid dehydrogenase. Because these encoded proteins belong to the aldo–keto reductase superfamily, we named these proteins RAKa to RAKf. RAK represents rat aldo–keto reductase, and RAKa is the previously described rat liver 3α-HSD. Northern blot analysis and reverse transcription–polymerase chain reactions were performed to examine their expression in various tissues. Only RAKe, which resembles human aldehyde reductase, was ubiquitously expressed in liver, kidney, lung, and other tissues, while the remaining mRNAs were found to have a more tissue- and sex-specific distribution. Genomic blot analysis showed complex, yet distinctive, restriction band patterns when different cDNAs were used as probes, suggesting that these cDNA clones are products of different genes and more related gene(s) may exist.

Aldo–keto reductases were so named because they catalyze the conversion of aldehydes and ketones to their corresponding alcohols. The primary sequences of several enzymes belonging to the aldo–keto reductase superfamily have been delineated recently by molecular cloning and sequencing of their cDNAs. These enzymes include human aldehyde reductase (Bohren et al., 1989), human and rat aldose reductase (Carper et al., 1987), human chlorodecone reductase (Winters et al., 1990), bovine prostaglandin F synthase (Watanabe et al., 1988), and European common frog eye ε-crystallin (Tomarev et al., 1984). Recently we and others have cloned the rat liver 3α-hydroxysteroid dehydrogenase (3α-HSD) cDNA and shown that 3α-HSD displays a high degree of amino acid sequence homology to the above aldo–keto reductases (Cheng et al., 1991; Pawlowski et al., 1991; Stolz et al., 1991).

Among these structurally related enzymes, 3α-HSD may be the most versatile enzyme due to its broad substrate specificity. Its substrates include androgens, progestins, glucocorticoids, bile acid precursors, prostaglandins, polycyclic aromatic hydrocarbons, and xenobiotics (Tomkins, 1956; Smithgall et al., 1988; Stolz et al., 1987; Worner and Oesch, 1984). Therefore, 3α-HSD may be involved in the detoxification of chemical carcinogens and xenobiotics, metabolism and transportation of bile acids, and metabolism of steroid hormones and prostaglandins. More recent studies show that some of the brain 3α-HSD products, such as allotetrahydropregnalonone and tetrahydrocortisone, are ligands of the brain $GABA_A$ receptor. These steroid hormone metabolites produce anxiolytic, analgesic, and anticonvulsive effects when administered to animals (Majewska et al., 1986). Other known aldo–keto reductases may be involved in various physiological and pathological pathways. For example, lung prostaglandin F synthase may regulate the metabolism and interconversion of various prostaglandins. Aldose reductase is involved in the function of the kidney and is implicated in the secondary pathogenic symptoms of diabetes (Moriyama et al., 1989). Human chlordecone reductase functions as a detoxification enzyme in liver (Winters et al., 1990).

We prepared several monoclonal antibodies against the enzyme. Interestingly, two of these monoclonal antibodies were capable of interacting with several antigenically related proteins in various tissues (Cheng, 1991). Several other groups also suggested that there are multiple proteins structurally related to 3α-HSD in rat liver (Hara et al., 1990). To investigate the structure and function of these related proteins, we screened several cDNA libraries derived from rat liver, kidney, and lung using monoclonal antibodies are probes. As reported here, our efforts have been rewarded by the isolation of multiple cDNAs encoding proteins which display various degrees of structural homology to rat liver 3α-HSD. This confirmed our previous hypothesis that the aldo–keto reductase superfamily contains a large number of structurally related proteins.

MATERIALS AND METHODS

Materials. Restriction endonucleases and related reagents were purchased from International Biotechnologies. Sequenching enzyme and reagents were purchased from United States Biochemicals. Rat liver cDNA libraries were a gift of Dr. B.-J. Song of the National Institute of Alcohol Abuse and Alcoholism, and a rat lung cDNA library was a gift of Dr. F. Gonzalez of the National Cancer Institute. Antibodies and other reagents for immunoblotting were obtained from Bio-Rad, and other reagents were obtained from Sigma.

Library Screening. Rat cDNA libraries in the expression vector λ gt11 were screened using supernatants from two hybridomas (7D3 and 3G6) secreting monoclonal antibodies

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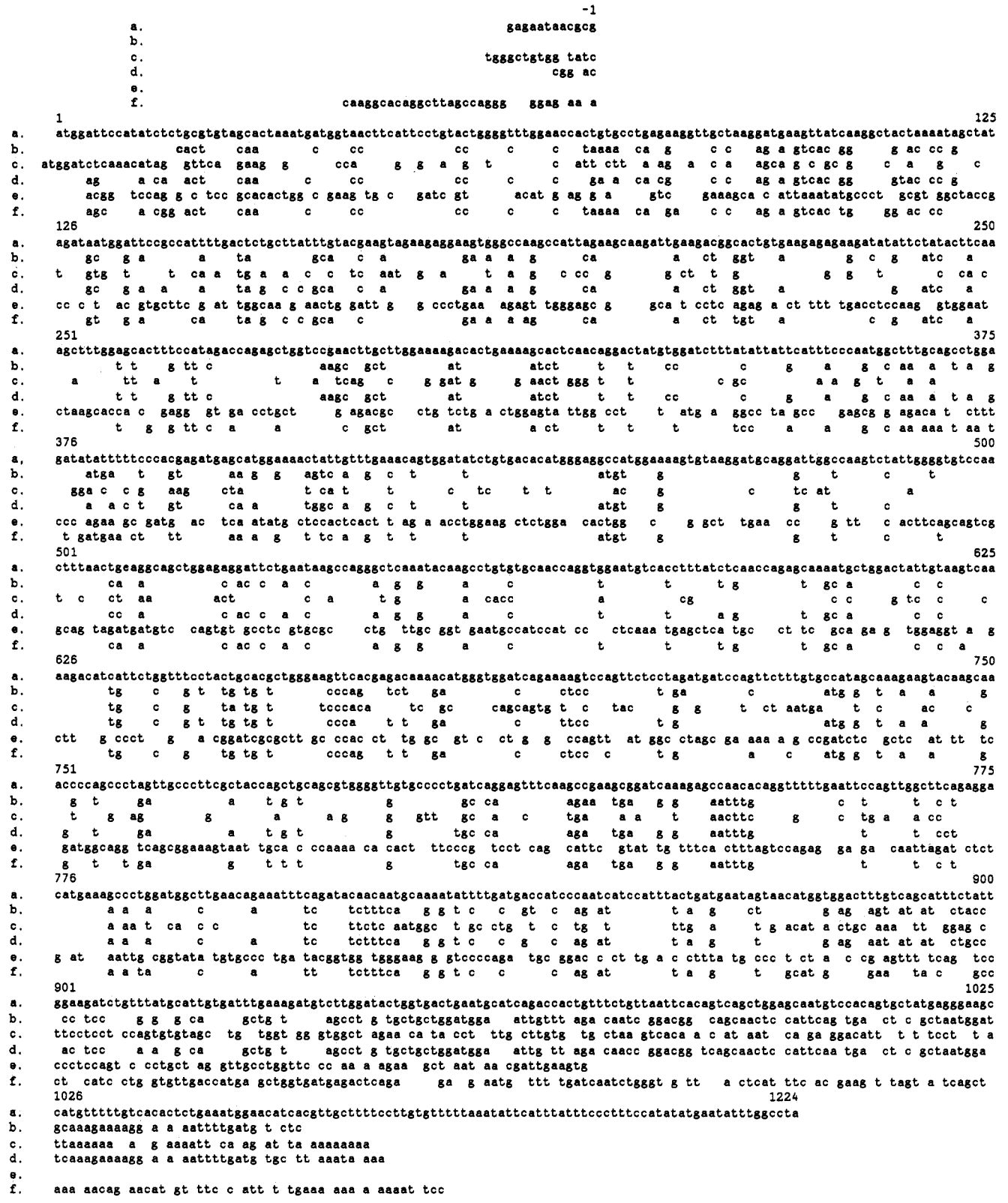

FIGURE 1: Comparison of cDNA sequences for (a) 3α-HSD, (b) RAKb, (c) RAKc, (d) RAKd, (e) RAKe, and (f) RAKf. The nucleotide residues are numbered in the 5′ to 3′ direction. Only differences from the 3α-HSD are shown.

against 3α-HSD (Young and Davis, 1983). Antibodies bound to the fusion proteins adsorbed to nitrocellulose filters were detected by sequential incubation with goat anti-mouse IgG-peroxidase and 4-chloronaphthol plus hydrogen peroxide.

Positive plaques were replaced at lower titers and rescreened with monoclonal antibodies until all plaques reacted positively. Bacteriophage DNA was isolated by the plate lysate method (Synder et al., 1987).

Subcloning and Sequence Analysis. Bacteriophage DNA was digested with *Eco*RI, subjected to electrophoresis in a low-melting agarose gel, and isolated by binding to glass beads. DNA fragments were subcloned into the *Eco*RI site of pUC19, and chain termination sequencing was performed on denatured supercoiled plasmid DNA using T7 DNA polymerase (Chen and Seeburg, 1985). Alternatively, cDNA inserts were amplified directly using two λ gt11 primers by the polymerase

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     1                                                                  65
a    MDSISLRVALNDGNFIPVLGFGTTVPEKVAKDEVIKATKIAIDNGFRHFDSAYLYEVEEEVGQAI
b         HC K  H H   A      YK    P SKSLE AHL   A Y  I T  A QI   I
c    MDLKH RS K   P LM        FASKEIP SKAAE   V   V    I A  F QN       L
d     MS KHHC K    H   A      SI NE P SKSLE VHL   A YH I T SA QI   I
e       MTASS L HT QKM LI L   WKS PGQVKAAI--- Y LSV Y  I C SV GN T I E L
f     MS KLHC K  N H   A      YK KE P SKSLE AHL   V Y  I T SA Q    I
g      DPKYQ  E    H M        YA PE PRNRAVEV  L   EA   I      NN  Q  L
h     M PK Q  K    H          RA  E P S ALE   F  EV    V   H  QN  Q
i       MAASC L HT QKM L  L   WKS PGQVKAAV--- Y LSV Y  I C AI GN P I E L
j        MAS LL  NCAKM I  L   WKSPPGQVT AV--- V   V Y  I C HV QN N   V

     66                                                                 129
a    RSKIEDG-TVKREDIFYTSKLWSTFHRPELVRTCLEKTLKSTQQDYVDLYIIHFPMALQPGDIFF
b    Q   KA -V     M I T   C CF     KPA   S  NL L  A    M Y VPMKS  ND
c     D MA  -      L   T I I  L      Q   RS  KLGL     C   I I MK  EELL
d    Q   KA -V     M I T   C CF     KPA   S  NL L  A    M Y VPMKS  KYL
e    KESVGA KA P  EL V     N K H  D EPAVR   ADL LE L   LM W Y FER  NP
f    Q   KAC-V     M I T   CSCF T M  PA    S  NL L     FL  Y VPIKSSVSES
g        A  -S             C  FQ QM QPA  SS  KL L      LL      K  ETPL
h        AE -              CNSLQ     PA   S QNL L         S VS K  NK V
i    KEDVPG KA P  EL V     N K H  D EPA R   ADL LE L   LM W Y FER  NP
j    QE LREQ-V    EL IV    C Y EKG  KGACQ   SDLKL  L   L  W TGFK  KE

     130                                                                194
a    PRDEHGKLLFETVDICDTWEAMEKCKDAGLAKSIGVSNFNCRQLERILNKPGLKYKPVCNQVECH
b     V  K  S LD   F     ML        V         HKG   L
c     K AN  FI D    R     L        S         LK   L           T
d     V DN  W LD   F     ML        V         HKQ   LI
e     KNAD TVKYDSTHYKE  K L ALVAK  V AL L   SS  IDDVLSVASV--R AVL
f     V  K    -D   F     ML        V         HK    L          PV
g     K  N  VI D   LSA   V                       M
h     K  S   I DS  L H    L        T         HK   K
i     KNAD TICYDSTHYKE  KEL ALVAK  VQAL L    S  IDD  SVASV--R AV
j     L  S NVVPSDTN L   A   ELV E  V A  I    HL V M           AV  I

      195                                                               259
a    LYLNQSKMLDYCKSKDIILVSYCTLGSSRDKTWVDQKSPVLLDDPVLCAIAKKYKQTPALVALRY
b          L         V  A GA  TQ Y E    N     N     DV   N R    I
c    P     L EF      V  A SA   H  SS  SSD  Y  E    MT   HN   GQ
d      M   L         V  A GA  TQ Y E    N           DV   N RS   I
e    P  A N LIAH QARGLEVTA SP    - RA RHPDE    EE  CL L EEHGRS  QIL  W
f          L         V  A GA  TQ YEE    N        I  DV   N RS   I
g    P     L  F      V  AHSA  TQ H L   PN     E      L   H R    I
h    P     L EF   HE V  A AA  AQLLSE  NSNN    E          H
i    P  A NELIAH QARGLEVTA -P    - RA RDPDE    EE  VL L E  GRS  QIL  W
j    P  T E LIQ  Q  G VVTA SP   P- RP AKPED S  E  RIK   A HNK T Q LI F

     260                                                               332
a    QLQRGVVPLIRSFKPKRIKEPTQVFEFQLASEDMKALDGLNRNFRYNNAKYFDDHPNHPFTDE
b    LV        AQ   ENEMR NL   D   SP    T     K    LS EFLAG  EY  SE Y
c           V AK  NE   I NF   D E TP    TI S       SQMAFAL   DY  LE Y
d    LV  E     AQ   ENEMR NL   D   HP    T     K    LS EFLAG  GC  SE Y
e     V  K ICIPK IT S  LQNI   D TFSP E  Q  A  K W  IVPMITV GKTV RDAGHPLYPFNDPY
f    LF        AQ   ENEMR NL       SP    T     K F
g           V AK YNEQ  R NI        T     V       Y  VVMDFLM   DY  S  Y
h     V      V AK  NK     NM   D E TP     I      I  YDFQKGIG  EY  SE Y
i     V  K ICIPK IT S  LQNIK  D TFSP E  Q NA  K W  IVPMLTV GKRV RDAGHPLYPFNDPY
j    PM  NL VIPK VT E  A NFK  D E S Q  TT LSY   W VCALLSCTS KDY  HE F
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FIGURE 2: Comparison of deduced amino acid sequences for (a) 3α-HSD, (b) RAKb, (c) RAKc, (d) RAKd, (e) RAKe, (f) RAKf, (g) human chlordecone reductase (h) bovine prostaglandin F synthase, (i) human aldehyde reductase, and (j) human aldose reductase. Only differences from the 3α-HSD sequence are displayed. Boldface one-letter codes denote the beginning and the ending of each sequence. The dashed lines represent gaps necessary for optimal alignment of different sequences. The amino acid residues are numbered in the amino terminus to carboxyl terminus direction.

chain reaction (PCR). Amplified DNA was sequenced in the presence of DMSO.

Northern Blot Hybridization Analysis and RT–PCR Analysis. Total RNA was extracted from tissues by homogenization in 4 M guanidine thiocyanate followed by ultracentrifugation (Chirgwin et al., 1979). Forty-microgram aliquots of each RNA sample were subjected to electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde and blotted to nylon membranes (Lehrach et al., 1977). Blots were hybridized in buffer containing 50% formamide, 6× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.5), 5× Denhardt's solution, and 0.5% SDS for 16 h at 42 °C and then washed at 65 °C in solution containing 0.2× SSC and 0.1% SDS.

Reverse transcription–polymerase chain reaction (RT–PCR) was performed using gene-specific oligonucleotide primers (Saiki et al., 1988). The following oligonucleotide primers were synthesized for the PCR: RAKb, 5′-CGCCATATTGATACTGCTTAT-3′ and 5′-GGAAAATCATTATCCCCTGAC-3′; RAKd, 5′-CACCATATTGATACGGCCTCT-3′ and 5′-GGAAGATATTTATCCCCTGAC-3′; RAKf, 5′-CGCCACATTGATACGGCCTCT-3′ and 5′-GGAGATTCACTAACACTTGAC-3′. Synthesis of the first strand of the cDNA was achieved using reverse transcriptase

Table 1: Sequence Similarity between Different Aldo–Keto Reductases: Percentage of Amino Acid Sequence Identity

	RAKa	RAKb	RAKc	RAKd	RAKe	RAKf	HAD	HAL	BPF	HCD
RAKa[a]	100	62	61	62	37	61	38	42	64	66
RAKb		100	64	94	41	91	41	47	68	68
RAKc			100	56	40	61	40	51	71	70
RAKd				100	39	91	40	45	67	67
RAKe					100	38	92	51	40	43
RAKf						100	38	45	66	67
HAD[b]							100	50	40	41
HAL[c]								100	48	47
BPF[d]									100	73
HDC[e]										100

[a] RAKa: rat liver 3α-hydroxysteroid dehydrogenase. [b] HAD: human aldehyde reductase. [c] HAL: human aldose reductase. [d] BPF: bovine prostaglandin F synthase. [e] HCD: human chlordecone reductase.

and random primers provided in the RT–PCR kit (Perkin-Elmer). Thirty-five cycles of PCR amplification were routinely performed under the following conditions: 1 min at 94 °C, 1 min at 60°C, and 2 min at 72 °C. Amplified DNA was resolved in a 2% agarose gel containing 5 μg/mL ethidium bromide and visualized under UV light.

Southern Blot Hybridization Analysis. Genomic DNA was prepared from rat liver by treatment with sodium dodecyl sulfate (SDS) and proteinase K followed by several chloroform extractions (Wyman and White, 1980). DNA (10 μg) was digested with restriction endonucleases, fractionated in a 0.7% agarose gel, and blotted to a nylon membrane (Southern, 1975). Blots were hybridized with ^{32}P-labeled cDNA in buffer containing 50% formamide, 6× SSC, 5× Denhardt's solution, 0.5% SDS, and 1 mg/mL salmon sperm DNA for 16 h at 42 °C, and then they were washed at 65 °C in solution containing 0.2× SSC and 0.1% SDS. The washed blots were exposed to Kodax X-AR5 film for 16 h prior to development.

RESULTS

Isolation of Multiple cDNAs. We have previously shown that two monoclonal antibodies (3G6 and 7D3) against rat 3α-HSD recognized multiple structurally related proteins in various tissues by Western blot analysis (Cheng, 1991). These two monoclonal antibodies were further used to screen several λ gt11 cDNA expression libraries derived from rat liver, lung, kidney, and brain. Approximately 100 cDNA clones reacting with monoclonal antibodies were identified, their inserts amplified by PCR, and the resulting DNAs differentiated on the basis of the existence of internal *Eco*RI and/or *Hin*dIII restriction sites and their ability to cross-hybridize with the 3α-HSD cDNA probe under high stringent hybridization conditions. Subsequently, both the 5′ and 3′ partial sequences of the PCR products were determined in order to delineate the identity of each cDNA clone. At least five groups of cDNAs were identified; the longest clones in each group were completely sequenced. Four of the new clones contained full-length coding sequences as determined by analogy with other aldo–keto reductases, and one lacked only a short segment of the 5′-coding sequence. While the full-length cDNAs of rat liver 3α-HSD (RAKa) displayed large variations in size (1.3–2.3 kb), the other full-length cDNAs ranged from 1.3 to 1.4 kb (Figure 1). Only one positive clone isolated from a brain expression library showed sequence homology to the other cDNA clones. Sequence analysis of this brain clone indicated that the DNA sequence is identical to that of the liver 3α-hydroxysteroid dehydrogenase. Therefore, both the brain and the liver seem to express the identical 3α-HSD isozyme.

Figure 2 shows the alignment of the amino acid sequences of the encoded proteins and several other sequences belonging to the aldo–keto reductase gene family. Although the cDNA clones identified by our monoclonal antibodies encode proteins exhibiting moderate degrees of homology (37–62%) to rat liver 3α-HSD, it is unclear at this time whether or not they are 3α-HSD isozymes. We therefore tentatively named these enzymes RAKa to RAKf, where RAK reprsents rat aldo–keto reductases and RAKa represents previously characterized 3α-HSD. Each clone was assigned a small letter in alphabetical order according to its chronological identification.

Comparison of the aldo–keto reductases identified in this study and other known related proteins revealed that RAKe highly resembles human aldehyde reductase (Table 1). The high degree of amino acid sequence homology (92%) suggests that RAKe is the rat homolog of aldehyde reductase. A moderate homology (71%) was found between RAKc and bovine prostaglandin F synthase, as well as between rat 3α-HSD (RAKa) and human chlordecone reductase (66%). Interestingly, RAKb, RAKd, and RAKf are highly similar; their sequence exhibit more than 90% homology to each other. The protein encoded by RAKf is somewhat unique, however, in that it is 19 amino acids shorter than the others at the COOH terminus. It is not known whether such a deletion affects the catalytic function of the protein.

Expression of mRNA in Various Tissues. The sizes of the transcripts and the tissue-specific expression of the various rat aldo–keto reductases were first studied by Northern blot analysis. As shown in Figure 3, the size of the 3α-HSD transcript is approximately 2.3 kb, while those of the others range from 1.4 to 1.6 kb. This difference is largely due to a longer 3′-noncoding sequence in the 3α-HSD (RAKa) cDNA (data not shown).

Expression of liver 3α-HSD (RAKa) mRNA was most abundant in livers of female and male rats (Figure 3A) and was only moderate in intestine (data not shown). Although many other tissues such as testis, brain, and lung also display some 3α-HSD activities, none of these tissues express any detectable level of 3α-HSD transcript under our Northern blot hybridization conditions. By contrast, expression of RAKc was only found in lung, while RAKe was found to be ubiquitously expressed in liver, lung, kidney, and other tissues we examined such as brain, eye, intestine, skeletal muscle, prostate, and testis (data not shown).

On the basis of the Northern blot analysis, RAKb, RAKd, and RAKe are expressed in livers of both male and female rats as well as in kidneys of male rats. Since these three cDNA clones are highly homologous to each other, it was expected that their hybridization patterns are also similar. To obtain a more definite answer of their tissue distribution, we used gene-specific oligonucleotides as PCR primers in reverse transcription–polymerase chain reaction (RT–PCR) to analyze the presence of the mRNA. These oligonucleotide primers were chosen from the most diverse sequence regions

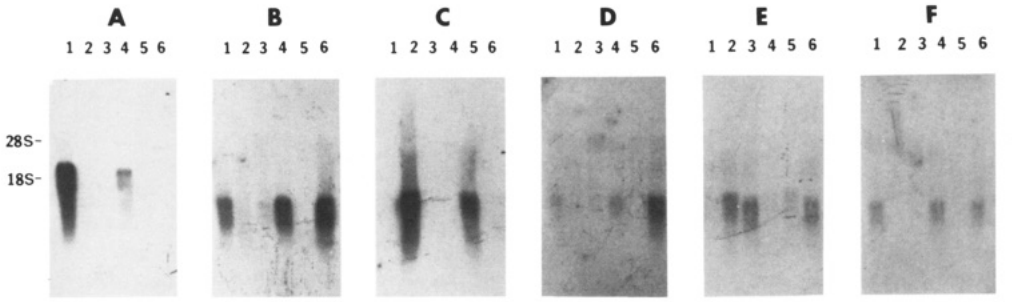

FIGURE 3: Blot hybridization of rat RNA with cDNA probes. Forty-microgram aliquots of total RNA from rat liver (1, female; 4, male), lung (2, female; 5, male) and kidney (3, female; 6, male) were subjected to Northern blotting as described. Blots were hybridized with the following radiolabeled cDNA probes: A, RAKa; B, RAKb; C, RAKc; D, RAKd; E, RAKe; and F, RAKf. Positions of 28S and 18S rRNA on the blots are indicated on the left of the autoradiogram.

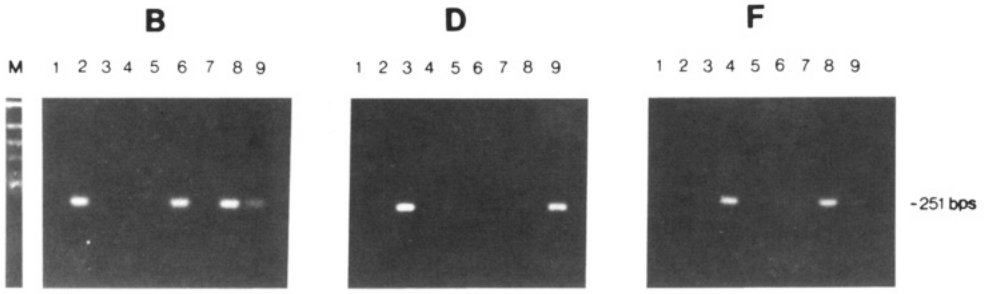

FIGURE 4: Polymerase chain reaction (PCR) and reverse transcription–polymerase chain reaction (RT–PCR) analysis of the expression of RAKb, RAKd, and RAKf in rat liver and kidney. Oligonucleotide PCR primers used in panels B, D, and F were specific for genes of RAKb RAKd, and RAKf, respectively. PCRs were performed using the following cDNAs as templates: 1, control; 2, RAKb; 3, RAKd; and 4, RAKf. RT–PCRs were performed using the total RNA isolated from the following tissues as template: 5, control; 6, female rat liver; 7, female rat kidney; 8, male rat liver; 9, male rat kidney. DNA size standards are shown on the left side of the figure.

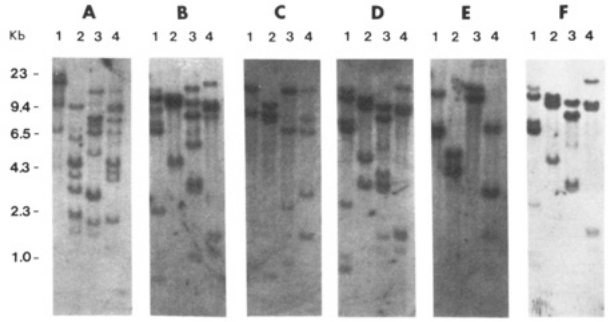

FIGURE 5: Genomic blot hybridization analysis of rat DNA with cDNA probes. Ten-microgram aliquots of DNA were digested with restriction enzymes (1, *Bam*H1; 2, *Bgl*II; 3, *Pst*I; 4, *Xba*I) and subjected to Southern blotting hybridization. Blots were hybridized with the following radiolabeled cDNA probes: A, RAKa; B, RAKb; C, RAKc; D, RAKd; E, RAKe; and F, RAKf. DNA size standards are indicated on the left of the autoradiogram.

which could amplify only their specific target cDNA (Figure 4). As shown in Figure 4, RAKb is expressed in the livers of both female and male rats as well as in the kidneys of male rats, whereas RAKd is expressed exclusively in the kidneys of male rats, and RAKf in the liver of male rats.

Genomic Blot Analysis. Rat genomic DNA was digested separately with four different restriction enzymes, *Bam*H1, *Bgl*II, *Pst*I, and *Xba*I. After agarose gel electrophoresis and Southern blotting, the blots were hybridized with ^{32}P-labeled cDNA probes and washed under high stringency conditions. As shown in Figure 5A, the band patterns for liver 3α-HSD were extremely complex; digestion of the genomic DNA with each of the four restriction enzymes resulted in at least four large genomic fragments hybridizing to the cDNA probe. The complexity of the band patterns strongly suggests the existence of multiple related genes. Similarly, the other restriction band patterns of the genes of RAKb, RAKd, and RAKf were equally complex (Figure 5). Upon close examination, it was found that the band patterns of RAKb, RAKd, and RAKf genes were very similar but not identical. Considering the high degrees of homology between these three cDNA clones, it is not surprising to find that many genomic DNA fragments hybridize to all three cDNA probes, whereas the distinct bands presumably correspond to more diverse regions in different cDNAs. The band patterns of RAKc and RAKe appeared to be less complex but still displayed multiple bands (Figure 5C,E).

DISCUSSION

In this report we demonstrated the existence of multiple proteins structurally related to rat liver 3α-HSD. Alignment of the amino acid sequences of proteins encoded by our cDNAs suggested that they clearly belong to the aldo–keto superfamily. Enzymes known to belong to this aldo–keto reductase superfamily include human chlordecone reductase, aldehyde reductase, aldose reductase, bovine prostagladin F synthase, rat 3α-hydroxysteroid dehydrogenase, and frog eye ϵ-crystallin. Except RAKe, which displays a high degree of sequence homology to human aldehyde reductase, the other cDNAs isolated in this study showed only moderate amino acid sequence homology (40–71%) to those previously identified and sequenced. Rather interestingly, RAKb, RAKd, and RAKf display very high degrees of homology (>90%) to each other and, therefore, may belong to the same subclass. However, the expression patterns of their transcripts showed distinct tissue and sex specificities. Although the above three cDNAs encode proteins which display approximately 65% homology to rat liver 3α-HSD, our preliminary studies on the expression, purification, and characterization of various rat aldo–keto reductases in bacteria indicated that these proteins do not have 3α-HSD activity (data not shown). RAKc, like bovine prostaglandin F synthase, is expressed in lung; however, the moderate homology (71%) between these two sequences suggests that they may not be counterpart enzymes. Since

our previous study (Cheng, 1991) suggested the existence of immunologically distinct proteins in many other tissues such as testis and brain, additional enzymes may exist in a tissue-specific manner. The likelihood of the existence of more related genes is also supported by the genomic blotting using different cDNA as probes. Given their complexity, especially that of liver 3α-HSD, it appears that additional genes have yet to be identified.

As previously reported by us, there appears to be no significant homology between aldo–keto reductases and other alcohol dehydrogenases such as 3β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase, and 15-hydroxyprostaglandin dehydrogenase (Baker, 1990). In the latter superfamily a distinct nucleotide cofactor binding site has been identified near the amino-terminal region of the proteins. Among various aldo–keto reductase, approximately 20% of the amino acid residues are invariantly conserved. Since these residues are distributed evenly in the sequences, it is impossible to predict which region(s) may be involved in the binding of either the nucleotide cofactor or substrates. Having expressed and purified a large quantity of 3α-HSD (RAKa) in *Escherichia coli*, we obtained crystals from the purified protein. We thus anticipate that a more definite structural analysis by X-ray crystallography may create a general framework for all the aldo–keto reductases.

The fact that the expression of most of the genes identified is restricted to selective tissues tempts us to speculate that each tissue may have its own set of aldo–keto reductases for maintaining specific function(s). From what we already know, 3α-HSD in liver is a general metabolic enzyme required for maintaining the homeostatic balance of steroid hormones. 3α-HSD in the brain apparently is responsible for generating the tetrahydro metabolites of progesterone and deoxycortisone that modulate GABA receptors under stress conditions. Aldose reductase in eye lens and in nerves is implicated in pathological symptoms related to diabetes. Prostaglandin dehydrogenase in the lung may be involved in asthma by regulating the catabolism and interconversion of prostaglandins and may also function in the detoxification of chemical carcinogens. 3α-HSD and other enzymes in the intestine may be utilized for the transportation of bile acids and xenobiotics. In kidney, these enzymes may regulate the excretion of solutes by modulating the metabolism of corticosteroids; therefore, they could be involved in the regulation of sodium homeostasis and possibly the pathogenesis of hypertension. These questions regarding the functional roles of each of the related enzymes cannot be answered until we fully understand their substrate specificity. To this end we successfully expressed rat liver 3α-HSD and several other proteins in bacteria. Currently, we are evaluating the functional role(s) of these proteins by characterizing their substrate specificities.

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