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Identification of a Principal mRNA Species for Human 3α-Hydroxysteroid Dehydrogenase Isoform (AKR1C3) That Exhibits High Prostaglandin D_2 11-Ketoreductase Activity[1]

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Human 3α-hydroxysteroid dehydrogenase exists in four isoforms, which belong to the aldo-keto reductase (AKR) superfamily and are named AKR1C1-AKR1C4. The properties of AKR1C3 have not been fully characterized compared to the other isoforms. In addition, a cDNA that shows more than 99% homology with AKR1C3 cDNA has been cloned from human myeloblasts. We have here expressed and purified a recombinant enzyme (designated as DBDH) from this cDNA. DBDH oxidized xenobiotic alicyclic alcohols and 3α- or 17β-hydroxy-5β-androstanes, and catalyzed the reversible conversion between prostaglandin D_2 and 9α,11β-prostaglandin F_2 more efficiently than that of 3α- or 17β-hydroxysteroids: the respective K_m values were 0.6 and 6.8 μM, and k_{cat}/K_m values were about 1,000 $min^{-1} \cdot mM^{-1}$. Anti-inflammatory drugs highly inhibited the enzyme. The recombinant AKR1C3 prepared by site-directed mutagenesis of DBDH also showed the same properties as the wild-type DBDH. Analyses of expression of mRNAs for DBDH and AKR1C3 by reverse transcription-PCR indicated that only one mRNA species for DBDH is expressed in 33 human specimens of liver, kidney, lung, brain, heart, spleen, adrenal gland, small intestine, placenta, prostate, and testis. These results suggest that AKR1C3 acts as prostaglandin D_2 11-ketoreductase, and that its principal gene in the human has a coding region represented by DBDH cDNA.

Key words: aldo-keto reductase family, dihydrodiol dehydrogenase, 3α-hydroxysteroid dehydrogenase, polymorphism, prostaglandin D_2 11-ketoreductase.

3α-Hydroxysteroid dehydrogenase (3αHSD, EC 1.1.1.213) is involved in the biosynthesis and inactivation of steroid molecules and in the regulation of steroid hormone action in endocrine and steroid target tissues (*1*). In the liver, 3αHSD inactivates circulating steroid hormones and plays a role in the bile acid synthesis (*1, 2*). In addition, hepatic 3αHSD has been thought to act as prostaglandin (PG) oxidoreductase, carbonyl reductase, dihydrodiol dehydrogenase, and bile acid-binding protein because of its broad specificity for PGs, drug ketones, and *trans*-dihydrodiols of aromatic hydrocarbons (*3-6*), and its ability to bind bile acids (*7*).

In human liver, 3αHSD with dihydrodiol dehydrogenase activity exists in multiple forms (*8, 9*), and four types of cDNAs for the enzyme have been cloned (*10-13*). The 3αHSD isoforms reveal 83-98% sequence identities, belong to the aldo-keto reductase (AKR) superfamily, and have been systematically named AKR1C1-AKR1C4 (*14*). AKR1C1, 1C2, and 1C4 have been shown to be identical to human liver dihydrodiol dehydrogenases, DD1, DD2, and DD4, respectively (*8, 15, 16*). AKR1C3 has been designated as 3αHSD type 2 because dehydrogenase activity for some androstanes and chenodeoxycholic acid was detected with its crude recombinant enzyme preparation (*13*). However, the enzyme has not been purified and further characterized, and its protein has not been identified in human tissues. In addition, there are three nucleotide differences in the coding regions of AKR1C3 gene (*13*) and its cDNA (*10*), causing one amino acid substitution at position 75. Furthermore, a cDNA (accession No. D17793) that shows two amino acid differences at positions 75 and 175 from the AKR1C3 gene and its cDNA has been cloned from human immature myeloid cell line KG-1 (*17*). Although this cDNA has been thought to code for *trans*-1,2-dihydrobenzene-1,2-diol dehydrogenase (DBDH), its recombinant protein has not been characterized.

AKR1C1, AKR1C2, and AKR1C4 have been well characterized, and, despite their high sequence identity, their specificities for steroids and PGs and inhibitor sensitivity are remarkably different (*8, 15, 16*). In this study, we first characterized substrate specificity and inhibitor sensitivity

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Abbreviations: DBDH, *trans*-1,2-dihydrobenzene-1,2-diol dehydrogenase; HSD, hydroxysteroid dehydrogenase; PG, prostaglandin; AKR, aldo-keto reductase; RT, reverse transcription.

of the homogeneous recombinant DBDH and two AKR1C3 proteins, K75M/M175I and K75E/M175I, prepared by site-directed mutagenesis of DBDH cDNA, to elucidate functional differences among the recombinant AKR1C3-type enzymes and the other 3αHSD isoforms. Second, we examined the expression of mRNAs for DBDH in 33 specimens of liver, kidney, lung, brain, heart, spleen, adrenal gland, placenta, small intestine, prostate, and testis, in order to clarify whether cDNAs for the two enzymes are due to allelic variation or derived from different genes.

MATERIALS AND METHODS

Materials—Liver, kidney, prostate, adrenal gland, and testis were obtained with informed consent from patients during biopsy or surgery of these tissues for pathological examination, and a placenta was obtained from a healthy volunteer after parturition. Total RNAs of the tissues (brain, lung, liver, heart, spleen, and small intestine) of non-Japanese were purchased from Sawady Technology (Tokyo). The use of the human samples for this study had been approved by the University Ethics Committee. The cDNA for DBDH was kindly supplied by Dr. T. Nagase (Kazusa DNA Research Institute, Kisarazu) and the cDNAs for AKR1C1, AKR1C2, and AKR1C4 were previously prepared (*15, 16, 18*). *Eco*NI, *Eco*RI, and *Cfr*9I were purchased from New England Biolabs, Takara (Kusatsu), and Toyobo (Osaka), respectively. Anti-inflammatory drugs were obtained from Sigma Chemicals, and the other chemicals used in this study are as specified elsewhere (*5, 8, 12, 15, 16, 19*), unless otherwise noted.

Site-Directed Mutagenesis and Expression of Recombinant Enzymes—Two AKR1C3 proteins, K75M/M175I and K75E/M175I, were generated by the overlap-extension technique (*20*) using DBDH cDNA as the template and pairs of forward and reverse primers (Table I), as previously described (*19*). The entire coding regions were amplified with C3exF and C3exR, which contain *Eco*RI and *Cfr*9I restriction sites, respectively. The PCR products were ligated in pKK 223-3 plasmids at the restriction enzyme sites (*15*), and the nucleotide sequences of the constructs were confirmed by DNA sequencing (*12*). The wild-type and mutated cDNAs were expressed in *Escherichia coli* (JM109) cells as described (*15*). The recombinant enzymes were purified by ammonium sulfate fractionation, Sephadex G-100 filtration, anion-exchange chromatography on Q-Sepharose, and affinity chromatography on Matrex Red-A as described for the purification of hepatic AKR 1C1 (*8*).

Enzyme Assay—Dehydrogenase and reductase activities of the recombinant enzymes were assayed by recording the production and oxidation, respectively, of NADPH as described (*8*). The standard reaction mixture for the dehydrogenase activity consisted of 0.1 M potassium phosphate, pH 7.4, 0.25 mM $NADP^+$, 2 mM *S*-indan-1-ol, and enzyme, in a total volume of 2.0 ml. The reductase activity was determined with 2.0 ml of 0.1 M potassium phosphate, pH 7.0, containing 0.1 mM NADPH, carbonyl substrates, and enzyme. One unit of the enzyme activity was defined as the amount of the enzyme catalyzing the formation or oxidation of 1 μmol NADPH/min at 25°C. Kinetic constants and IC_{50} values are expressed as means of duplicate or triplicate determinations.

RT-PCR—Total RNA from biopsy samples (10 mg) was isolated using Isogen (Nippon Gene, Tokyo) and reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and an oligo $(dT)_{12-18}$ primer (Gibco BRL).

TABLE I. **Primers used in this study.**

Forward (f) and reverse (r) primers used for site-directed mutagenesis of DBDH

Primer	Sequence (mutated nucleotides are underlined)	Mutated codon	Paired primer	1st PCR product
DbK75Ef	5'-TGGCAGTGTGGAGAGAGAAG-3'	^{223}AAG→GAG	C3exR	768 bp
DbK75Er	5'-CTTCTCTCTCCACACTGCCA-3'	^{225}CTT→CTC	C3exF	240 bp
DbK75Mf	5'-TGGCAGTGTGATGAGAGAAG-3'	^{223}AAG→ATG	C3exR	768 bp
DbK75Mr	5'-CTTCTCTCATCACACTGCCA-3'	^{225}CTT→CAT	C3exF	240 bp
DbM175If	5'-AGCTGGAGATCATCCTCAAC-3'	^{523}ATG→ATC	C3exR	466 bp
DbM175Ir	5'-GTTGAGGATGATCTCCAGCT-3'	^{525}CAT→GAT	C3exF	562 bp

Primers used for amplification of the entire coding regions of DBDH and AKR1C3 cDNAs

C3exF	5'-CCGAATTCATGGATTCCAAACAGCAGTGT-3'	(corresponds to nucleotides 1-21 of the two cDNAs, and includes a boxed *Eco*R I site above the initiation codon)
C3exR	5'-AACCCGGGTTAATATTCATCTGAATATGGAT-3'	(complementary to nucleotides 972-950 of the two cDNAs, and includes a boxed *Cfr*9 I site below the stop codon)

Primers used for amplification of cDNA fragments for DBDH and AKR1C3

Primer	Sequence	Paired primer	Product expected (positions)
C3f	5'-AGCCAGGTGAGGAACTTTC-3'	C3r	560 bp (368-927 of the two cDNAs)
C3r	5'-ATCACTGTTAAAATAGTGGAG-3'	C3f	
C3rA	5'-ACTGGGTCCTCCAAGAGC-3'	C3f	352 bp (368-719 of DBDH cDNA)
C3rB	5'-ACTGGGTCCTCCAAGAGG-3'	C3f	352 bp (368-719 of AKR1C3 cDNA)

The cDNAs were subjected to PCR in 25 μl of 10 mM Tris-HCl, pH 8.3, containing 2.5 mM $MgCl_2$, 10 mM KCl, 200 μM deoxyribonucleotide triphosphates, 500 nM primers (Table I), and AmpliTag DNA polymerase Stoffel Fragment (1 unit, PE Applied Systems). The amplification consisted of an initial denaturation step at 95°C for 10 min; 30 cycles of denaturation (94°C/1 min), annealing (57°C/1 min), and extension (72°C/2 min); and a final extension step at 72°C for 10 min. The common forward primer, C3f, was designed to be annealed specifically with both cDNAs for DBDH and AKR1C3, and the reverse primer, C3rB, was specific for AKR1C3 cDNA among several similar cDNAs for human 3αHSDs and its related proteins. Another reverse primer, C3rA, should also have been annealed with the cDNAs for AKR1C1 and AKR1C2 in addition to the DBDH cDNA, but under the conditions of PCR with C3f and C3rA the expected 352-bp fragment was not amplified for the cDNAs encoding AKR1C1, 1C2, and 1C4 (data not shown). Thus, the primer pair of C3f and C3rA was used to specifically amplify DBDH cDNA, and that of C3f and C3rB was used for AKR1C3 cDNA. To further confirm which of mRNAs for DBDH cDNA and AKR1C3 is expressed, another PCR with a group-specific primer pair (C3f and C3r) was performed under the same conditions except for the annealing step (61°C/1 min), and then the products were digested at 37°C for 3 h by *Eco*NI (10 units), the site of which is present at position 705 only in the AKR1C3 cDNA. As the positive control cDNA for AKR1C3, a DNA fragment with the sequences of C3f and C3rB at its 5′- and 3′-ends was prepared by the PCR (the annealing step: 50°C/1 min) with the two primers and DBDH cDNA as the template. With each sample, β-actin cDNA was co-amplified as an internal control using the specific primers (Takara). The PCR products were analyzed by agarose electrophoresis and visualized with ethidium bromide. In a separate experiment, the cDNAs prepared from the total RNAs of liver, kidney, and lung were subjected to PCR with C3exF and C3exR primers and with *Pfu* DNA polymerase (Stratagene), and the products were subcloned into pT7Blue-3 plasmids (Novagene) as described by the manufacturer. Clones were selected by PCR with the group-specific primer pair, and the nucleotide sequences of their cDNA inserts were determined.

Other Methods—Products in the reaction mixture of PG D_2 reduction and 9α,11β-PG F_2 oxidation were analyzed by thin-layer chromatography as described (*6*). Protein concentration was determined using bovine serum albumin as the standard by the method of Bradford (*21*). SDS-polyacrylamide gel electrophoresis on a 12.5% slab gel and isoelectric focusing on a 7.5% polyacrylamide disc gel were performed as described by Laemmli (*22*) and Hara *et al.* (*23*), respectively.

RESULTS AND DISCUSSION

Properties of Recombinant DBDH and AKR1C3—The recombinant DBDH was purified to homogeneity on SDS-polyacrylamide gel electrophoresis, with an overall yield of 12% (3.8 mg) from the extract of the *E. coli* cells cultured in a 1 liter of medium. The enzyme exhibited high dehydrogenase activity for *trans*-1,2-dihydrobenzene-1,2-diol and *S*-isomers of alicyclic alcohols, although the K_m values are much higher than those of the recombinant AKR1C1 (*e.g.* K_m for *S*-indan-1-ol of 50 μM), 1C2 (490 μM) and 1C4 (146 μM) (*15, 16, 18*). It also oxidized some 3α-hydroxy-androstanes at low rates, but did not act towards bile acids (Table II), which is quite different from the substrate specificity reported with the crude recombinant AKR1C3 preparation (*13*). To compare the properties with those of AKR1C3, we prepared two recombinant proteins, K75M/M175I and K75E/M175I, which correspond to the amino acid sequences deduced from the cording regions of the AKR1C3 gene (*13*) and its cDNA (*10*), respectively. The homogeneous K75M/M175I (2.8 mg) and K75E/M175I (3.9 mg) obtained by the purification showed the identical molecular mass of 36 kDa with that of the wild-type DBDH, but had lower pI values of 8.3 and 8.1, respectively, than pI 8.5 of the wild-type enzyme (Fig. 1), because of loss of the basic Lys and its replacement with the acidic Glu. The two AKR1C3 enzymes showed substrate specificities similar to that of DBDH. The three recombinant enzymes exhibited about 120% of the $NADP^+$-linked activity with 2 mM NAD^+ as the coenzyme, but their K_m values for NAD^+ (0.2-0.4 mM) were much higher than those for $NADP^+$ (1.0-1.2 μM). In addition, the inhibitor sensitivity of DBDH was also the same as those of K75M/M175I and K75E/M175I (Table III). The results clearly indicate that DBDH and AKR1C3 are identical in their catalytic properties, *i.e.*, the substitutions of the two residues at positions 75 and 175 between the two enzymes do not affect the binding of

TABLE II. **Comparison of substrate specificity between the recombinant DBDH and AKR1C3.**

Substrate	DBDH			K75M/M175I			K75E/M175I		
	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}\cdot mM^{-1}$)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}\cdot mM^{-1}$)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}\cdot mM^{-1}$)
Oxidation									
S-Indan-1-ol	2,800	23	8.2	2,300	21	9.5	2,300	14	6.1
S-1,2,3,4-Tetrahydronaphth-1-ol	1,400	35	25	2,000	39	20	2,100	28	13
trans-1,2-Dihydrobenzene-1,2-diol	4,200	5.2	1.2	3,600	5.1	1.4	4,300	3.9	0.9
5α-Androstan-3α-ol-17-one[a]	–[c]	(0.05)	–	–	(0.01)	–	–	(0.02)	–
5α-Androstane-3α,17β-diol	17	0.6	35	12	0.4	33	15	0.3	20
9α,11β-PG F_2	6.8	6.4	941	5.4	6.5	1,200	7.7	3.3	430
Bile acids[b]	–	0	–	–	0	–	–	0	–
Reduction									
PG D_2	0.6	0.6	1,000	0.6	0.8	1,300	0.7	0.7	1,000
5α-Androstan-17β-ol-3-one[a]	–	(0.1)	–	–	(0.1)	–	–	(0.1)	–

The dehydrogenase and reductase activities towards the substrates were determined at pH 7.4 and 7.0, respectively. [a]The values in parentheses were calculated with the activities for the substrates (50 μM). [b] Chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid, and cholic acid were tested at concentrations of 10-50 μM. [c]Not determined because of low activity.

substrates and inhibitors. The two residues are not involved in the substrate and coenzyme binding sites of the tertiary structure of rat liver 3αHSD (*24*), which shows about 70% sequence identity with DBDH and AKR1C3. The chenodeoxycholic acid dehydrogenase activity of AKR1C3 reported in the previous study (*13*) might be caused by the use of the crude enzyme preparation, because *E. coli* contains bile acid 7α-HSD (*25, 26*).

During the course of this study, Lin *et al.* (*27*) cloned a cDNA for 3α(17β)-HSD from a human prostate cDNA library and suggested that the encoded enzyme is identical to AKR1C3 or a structural allele because of their close homology. This cDNA differs by two nucleotides at position 855 and in the 3′-untranslated region from DBDH cDNA, but its deduced amino acid sequence is identical to that of DBDH. Although the recombinant 3α(17β)-HSD exhibits dehydrogenase activities for both 3α- and 17β-hydroxy-5α-androstanes and testosterone with the k_{cat} values of less than 0.6 min^{-1} (*27*), DBDH exhibited higher dehydrogenase activity for 5β-androstanes with 3α- and/or 17β-hydroxy groups than for their 5α-isomers (Table IV). Thus, DBDH is 3α(17β)-HSD that prefers 5β-androstanes to their 5α-isomers.

Since hydroxysteroids, however, were not good substrates for DBDH, PGs were tested as the endogenous substrates, and 9α,11β-PG F_2 was found to be highly and specifically oxidized by the recombinant enzyme (Table IV). In the reverse reaction, PG D_2 was also efficiently reduced. The oxidized and reduced products were identified as PG D_2 and 9α,11β-PG F_2, respectively, by thin-layer chromatography. The K75M/M175I and K75E/M175I enzymes showed the same reactivity for the PGs, indicating again that the catalytic properties of DBDH and AKR1C3 are identical. The K_m of DBDH for 9α,11β-PG F_2 at the optimal pH of 10.0 is low compared to 138 μM of AKR1C1 (*16*) and 208 μM of AKR1C2 (*5*), and the k_{cat}/K_m value is much higher than those of AKR 1C1 (51 $min^{-1}\cdot mM^{-1}$) and AKR 1C2 (120 $min^{-1}\cdot mM^{-1}$). Although both K_m and k_{cat} values of DBDH decreased at a physiological pH, the k_{cat}/K_m value is also greater than the value (5 $min^{-1}\cdot mM^{-1}$) of AKR1C2 determined at 37°C and pH 7.0. For PG D_2, the k_{cat}/K_m value of DBDH at pH 6.0 is higher than those of AKR1C1 (240 $min^{-1}\cdot mM^{-1}$) and AKR1C2 (177 $min^{-1}\cdot mM^{-1}$), and the K_m value is much lower than those of AKR1C1 (20 μM), AKR1C2 (79 μM), and PG D_2 11-ketoreductases (10–200 μM) from other animal tissues (*28–30*). Thus, the PGs are good endogenous substrates for DBDH and AKR1C3.

The inhibitor sensitivity of DBDH is distinct from those of AKR1C1, 1C2, and 1C4 (*8, 16, 31*). In particular, the present enzyme is characterized by the inhibition by anti-inflammatory drugs such as suprofen, flurbiprofen, and ketoprofen, which did not have such high inhibitory potency toward AKR 1C1, 1C2, and 1C4 (more than 80% activity was retained on addition of 5 μM flurbiprofen or 10 μM concentrations of other drugs). Flufenamic acid, suprofen, flurbiprofen, and ketoprofen were competitive inhibitors

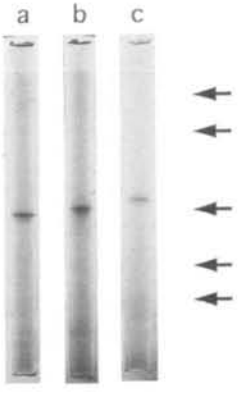

Fig. 1. **Isoelectric focusing of the wild-type and mutated DBDH.** About 5 μg of the enzymes (a, DBDH; b, K75M/M175I; c, K75E/M175I) were run on 7.5% polyacrylamide disc gels containing 0.1% ampholyte (pH 3.5–10), and stained for protein with Coomassie Brilliant Blue G. Arrows indicate the positions of marker proteins with pI of 4.9, 6.4, 8.3, 9.7, and 10.6 from the top.

TABLE III. **Comparison of inhibitor sensitivity between the recombinant DBDH and AKR1C3.**

Inhibitor	Concentration (μM)	% Inhibition		
		DBDH[a]	K75M/M175I	K75E/M175I
Flufenamic acid	1	82 (0.2)	80	64
Suprofen	1	55 (0.8)	56	53
Flurbiprofen	2	56 (1.4)	70	57
Indomethacin	5	54 (4.1)	57	60
Ursodeoxycholic acid	5	54 (4.2)	55	51
Ketoprofen	10	53 (7.1)	53	52
Chenodeoxycholic acid	50	57	60	55
Ibuprofen	100	58	51	51
Betamethasone	100	56	51	40
1,10-Phenanthroline	100	24	28	25

[a]The value in the parenthesis represents IC_{50} value (μM) for the inhibitor.

TABLE IV. **Specificity of DBDH for steroids and PGs.**

Substrate	K_m (μM)	k_{cat}[a] (min^{-1})	k_{cat}/K_m ($min^{-1}\cdot mM^{-1}$)
Oxidation			
5α-Androstan-3α-ol-17-one		(0.05)	
5β-Androstan-3α-ol-17-one	5.9	0.2	34
5α-Androstane-3α,17β-diol	17	0.6	35
5β-Androstane-3α,17β-diol	9.5	2.4	260
5α-Androstan-17β-ol-3-one		(na)	
5β-Androstan-17β-ol-3-one	1.6	6.4	250
Testosterone		(na)	
5α-Pregnane-3α,20α-diol		(0.02)	
5β-Pregnane-3α,20α-diol		(0.07)	
Other pregnanes[b]		(na)	
9α,11β-PG F_2 (at pH 10)	81	220	2,700
9α,11β-PG F_2	6.8	6.4	940
PG $F_{2\alpha}$		(0.2)	
Other PGs[b]		(na)	
Reduction			
5α-Androstan-17β-ol-3-one		(0.1)	
5β-Androstan-17β-ol-3-one	1.6	0.6	350
5α-Androstane-3,17-dione		(0.1)	
5β-Androstane-3,17-dione	2.2	1.0	450
PG D_2 (at pH 6)	1.1	1.4	1,270
PG D_2	0.6	0.6	1,000

The dehydrogenase and reductase activities towards the substrates were determined at pH 7.4 and 7.0, respectively, except that the activities for the PGs were also assayed at the indicated pH. [a]The values in parentheses were calculated with the activities for androstanes (50 μM), pregnanes (20 μM), and PGs (50 μM). na, no activity was detected. [b]Pregnanes (20 μM): 5α-Pregnan-3β-ol-20-one, 5α- and 5β-pregnan-3α-ol-20-ones, and 5α- and 5β-pregnan-20α-ol-3-ones. PGs (50 μM): PG A_1, PG A_2, PG B_1, PG D_1, and PG D_2.

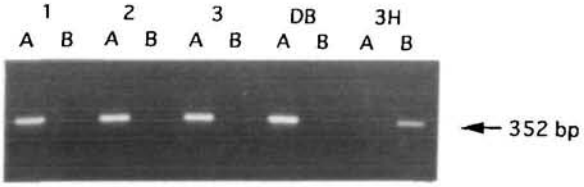

Fig. 2. **RT-PCR analysis for expression of mRNAs for DBDH and AKR1C3.** PCR with the primer pair of C3f and C3rA (A) or C3f and C3rB (B) was performed for the representative cDNA samples of three liver specimens (1, 2, 3), and the control cDNAs (10 pg) for DBDH (DB) and AKR1C3 (3H).

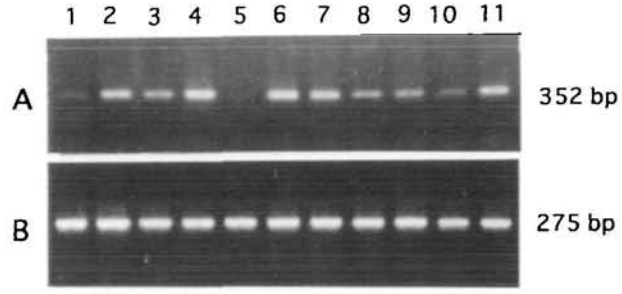

Fig. 3. **Expression of mRNA for DBDH in human tissues.** Samples of brain (1), lung (2), heart (3), liver (4), spleen (5), adrenal gland (6), kidney (7), placenta (8), small intestine (9), prostate (10), and testis (11) were analyzed by RT-PCR with the primer pair of C3f and C3rA (Panel A) and the specific primers for β-actin cDNA (Panel B). The very low signals of the DNA fragment detected in the spleen sample are not reproduced well in the photograph. No DNA fragment with the expected size was amplified by RT-PCR with the specific primers (C3f and C3rB) for AKR1C3 cDNA (data not shown).

with respect to *S*-indan-1-ol, showing K_i values of 0.1, 0.6, 0.8, and 3.8 μM, respectively. Recent crystallographic studies of the AKR family proteins (ternary complexes with coenzyme and competitive inhibitors) have shown that the inhibitors with a free carboxylic acid bind to an anionic site delineated by the C4N of the nicotinamide coenzyme and the side chains of Tyr and His residues at the enzyme active site (*24, 32, 33*). Since the two active site residues are conserved in DBDH, the anti-inflammatory drugs may bind to the anion binding site. From pharmacological and pharmaceutical points of view, the high inhibitory potency of these drugs toward DBDH with PG D_2 11-ketoreductase activity is important, because, in addition to the well-known biological activity of PG D_2, 9α,11β-PG F_2 has been reported to exhibit various biological activities (*34-37*), and its levels are increased in bronchoalveolar lavage fluid, plasma and urine in patients with mastocytosis (*34*) and bronchial asthma (*38-40*).

mRNA Species Expressed in Liver and Other Tissues—The previous Northern blot hybridization analyses with cDNAs for DBDH (*17*) and 3α(17β)-HSD (*27*) as probes suggested the ubiquitous expression of its mRNA in human tissues. For AKR1C3, the expression of its mRNA in five tissues has also been shown by RT-PCR analysis (*13*). However, it remains possible that the probes used in the Northern blot hybridization cross-hybridize with structurally similar mRNAs for AKR1C1, 1C2, and 1C4 as well as AKR1C3 mRNA (*27*), and the primers used in the previous RT-PCR (*13*) would not discriminate the mRNAs for AKR1C3, 3α(17β)-HSD, and DBDH. Thus, it is still unknown whether mRNAs for AKR1C3, 3α(17β)-HSD, and DBDH are expressed from different genes in a tissue. Although cDNAs for 3α(17β)-HSD and DBDH could not be distinguished by PCR, we established a RT-PCR method which examined the expression of the two different mRNAs for AKR1C3 and DBDH [or 3α(17β)-HSD] in a tissue. Liver samples of 18 Japanese and 2 non-Japanese were analyzed by RT-PCR with two pairs of the specific primers (C3f and C3rA, and C3f and C3rB), and the representative results for the liver samples are shown in Fig. 2. The PCR with C3f and C3rA amplified the 352-bp DNA fragments for all the liver samples and DBDH cDNA, whereas in the PCR with C3f and C3rB the expected fragment was detected for the control AKR1C3 cDNA, but not for the liver samples. Another PCR with C3f and C3r amplified the expected 560-bp DNA fragments for all the liver samples, and none of the DNA fragments were digested by *Eco*NI (data not shown), indicating the expression of DBDH mRNA, not AKR1C3 mRNA, in the samples. Furthermore, the two RT-PCR analyses of 10 extra-hepatic tissues also showed that only the DBDH mRNA species was expressed, as shown in Fig. 3. The same results were obtained on analyses of two more kidneys and three more prostates. Since relatively high levels of the message were observed in liver, kidney, and lung, the entire coding regions of the cDNAs were amplified from the total RNAs of the three tissues by RT-PCR with C3exF and C3exR as the primers and *Pfu* DNA polymerase, and the cDNA inserts in all the positive clones were confirmed to have the sequence identical with that of DBDH cDNA by DNA sequencing. This again excludes the possibility that DBDH and AKR1C3 mRNAs are differently expressed depending on the tissue, and also indicates that mRNA for 3α(17β)-HSD, which is distinct from DBDH mRNA by one nucleotide in their coding regions, is not expressed at least in the three samples. The RT-PCR analyses of a total of 33 tissue samples of Japanese and non-Japanese and the sequencing of the cloned cDNAs strongly suggested that one mRNA species for DBDH was expressed regardless of individual difference and tissue specificity, in contrast to the isolation of AKR1C3 gene and cDNAs for AKR1C3 and 3α(17β)-HSD. We propose that DBDH cDNA may represent the principal AKR1C3 allele, and the other cDNAs and gene could be rare allelic variants or sequencing artifacts, although further analyses of both cDNAs and genomic DNAs with more samples will be necessary to establish the proposed genetic polymorphisms of AKR1C3.

Conclusion—This study indicates that DBDH and AKR1C3 are functionally identical and catalyze the oxido-reduction between PG D_2 and 9α,11β-PG F_2 more efficiently than that of 3α- or 17β-hydroxysteroids. This ability may be related to the ubiquitous tissue distribution of the enzyme's mRNA and its induction in several human cultured cells by glutathione-S-transferase inhibitors (*41* and Shiraishi, H., unpublished work). The principal gene for AKR1C3 in the human appears to have a coding region represented by DBDH cDNA, which suggests that the previously cloned cDNAs for the enzyme may be a rare allelic variant of this gene.

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