

Molecular Cloning and Expression of a 2-Arylpropionyl-Coenzyme A Epimerase: A Key Enzyme in the Inversion Metabolism of Ibuprofen

CHRISTINE REICHEL, ROLAND BRUGGER, HOLGER BANG, GERD GEISSLINGER, and KAY BRUNE

Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen/Nürnberg, D-91054 Erlangen, Germany

Received September 3, 1996; Accepted December 13, 1996

SUMMARY

The 2-arylpropionic acid derivatives, including ibuprofen, are the most widely used anti-inflammatory analgesic cyclooxygenase inhibitors. The (–)-*R*-enantiomer, which is inactive in terms of cyclooxygenase inhibition, is epimerized *in vivo* via the 2-arylpropionyl-coenzyme A (CoA) epimerase to the cyclooxygenase-inhibiting (+)-*S*-enantiomer. The molecular biology of the epimerization pathway is largely unknown. To clarify this mechanism, the sequence of the 2-arylpropionyl-CoA epimerase was identified, and the enzyme cloned and expressed. A cDNA clone encoding the 2-arylpropionyl-CoA epimerase was isolated from a rat liver cDNA library. The nucleotide and the deduced amino acid sequence of this enzyme was determined. Significant amino acid sequence similarity was found between the rat epimerase and carnitine dehydratases from *Caenorhab-*

ditis elegans (41%) and *Escherichia coli* (27%). A bacterial expression system (*E. coli* strain M15[pREP4]) was used to express the epimerase protein, representing up to 20–30% of the total cellular *E. coli* protein. The expression of the epimerase was confirmed with Western blots using specific anti-epimerase antibodies and by measuring the rate of inversion of (*R*)-ibuprofenoyl-CoA. Northern blot analysis revealed a prominent 1.9-kb mRNA transcript in different rat tissues. In addition to its obvious importance in drug metabolism, the homology of the epimerase with carnitine dehydratases from several species suggests that this protein, which up to now has only been characterized as having a role in drug transformation, has a function in lipid metabolism.

The 2-arylpropionic acids, including IBU, are a subset of nonsteroidal anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs are widely used in the treatment of pain, fever, and inflammation. A characteristic feature of these therapeutic agents is their inhibition of COXs, key enzymes in prostaglandin biosynthesis (1). Clinically, most 2-arylpropionic acids are administered as racemates. An important pathway in the metabolism of 2-arylpropionic acids is the chiral inversion in which the (–)-*R*-enantiomers, which are inactive in terms of COX inhibition, are inverted to the COX-inhibiting (+)-*S* form (2, 3). Chiral inversion has been investigated in cellular homogenates (4, 5), whole isolated cells (6, 7), and whole perfused organs (8) including lung (9) and kidney (10). The mechanism of chiral inversion involves three metabolic steps (Fig. 1). The thioesterification of (–)-(*R*)-IBU (Fig. 1A) with CoA (11) via an adenylate intermediate (12) is catalyzed by a microsomal and mitochondrial enzyme, which we recently characterized as long-chain acyl-

CoA synthetase (13). The unidirectional formation of the respective adenylates with (–)-*R*-enantiomers is the stereoselective step of inversion. In the following reaction (Fig. 1B), the resulting (–)-(*R*)-ibuprofenoyl-CoA thioester is epimerized (14, 15) via a cytosolic and mitochondrial enzyme (16, 5), the 2-arylpropionyl-CoA epimerase. Hydrolysis of the thioesters (Fig. 1C) occurs in a nonstereoselective manner (17). The rate of inversion is substance-dependent and species-dependent (18). Tissue specific inversion of 2-arylpropionic acids may have therapeutic and toxicological implications, such as tissue-specific activity and toxicity.

The 2-arylpropionyl-CoA epimerase was recently isolated from the cytosolic and mitochondrial fraction of rat liver and has been characterized biochemically (16). We raised polyclonal antibodies against the epimerase and analyzed the relationship between the inversion activity in various tissues and the tissue distribution of the protein in guinea pigs and rats (5). Furthermore, three internal peptides of the epimerase were identified (5). To facilitate research into the physiological and pharmacological function of the enzyme, it was necessary to identify the gene structure of the 2-arylpropio-

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 353/A9) and by Graduiertenkolleg/Schmerzforschung.

ABBREVIATIONS: IBU, ibuprofen; PCR, polymerase chain reaction; (*R*)-IBU-CoA, (*R*)-ibuprofenoyl-coenzyme A thioester; DIG, digoxigenin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; COX, cyclooxygenase; IPTG, isopropylthiogalactoside; TBS-T, Tris-buffered saline/Tween 20; bp, base pair(s); kb, kilobase pair(s).

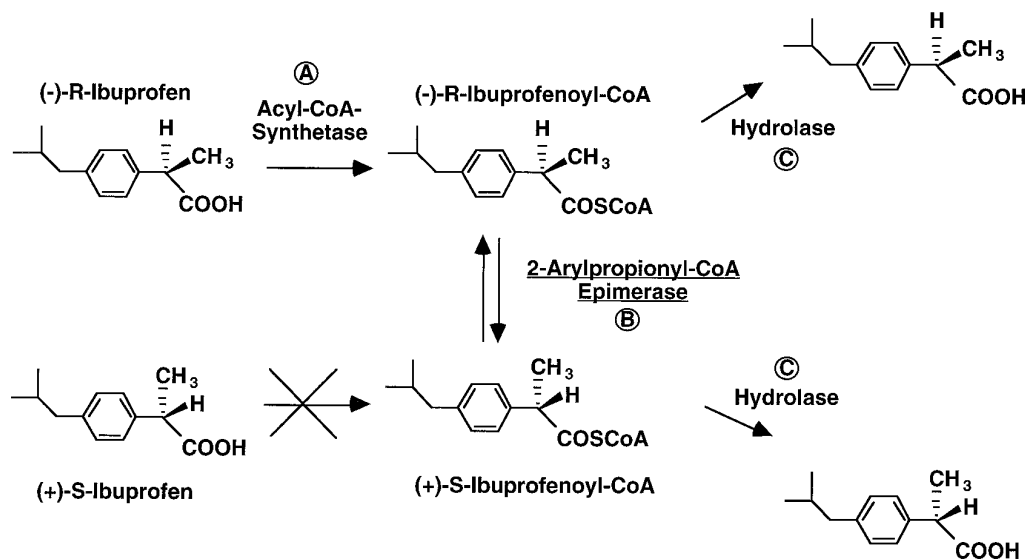


Fig. 1. Proposed mechanism of chiral inversion of 2-arylpropionic acids.

nyl-CoA epimerase. We describe the cloning of the rat 2-arylpropionyl-CoA epimerase and its sequencing and expression in *Escherichia coli*.

Materials and Methods

Drugs. IBU enantiomers were kindly supplied by Pharma Trans Sanaq AG (Basel, Switzerland). The optical purity of the enantiomers exceeded 98.5%. (-)-(*R*)-IBU-CoA was synthesized as described by Brugger *et al.* (13). All standard chemicals were purchased from Sigma Chemie (Deisenhofen, Germany).

Library screening and cloning. 2-arylpropionyl-CoA epimerase antibodies were obtained and purified as described previously (5). Approximately 6.0×10^5 plaques from a commercially available λ gt11 cDNA library constructed from rat liver RNA (Clontech Laboratories, Palo Alto, CA) were transferred onto nitrocellulose membranes (Schleicher & Schuell, Keene, The Netherlands) and screened with the polyclonal antibody against 2-arylpropionyl-CoA epimerase. The hybridization and washing conditions were performed as described by the manufacturer (Clontech). Eleven positive phage clones were isolated, and their DNAs were purified by using the plate lysate method (19). The inserts were amplified by PCR, with the λ gt11 5'-AGCAAGTTCAGCCTGGTTAAGT-3' and 5'-TTATGAGTATTCTTCACAGG-3' insert screening amplicon (Clontech). The reaction profile was as follows: denaturation at 94° for 30 sec, annealing at 55° for 30 sec, and extension at 72° for 60 sec, each for 35 cycles. Amplified inserts were partially sequenced. The DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) was used for the preparation of the samples for sequence analysis on the ABI Model 373A DNA Sequencer (Applied Biosystems). One clone included the information from peptide 1 (LALDL) and peptide 2 (IYARLSGFGQSGIFSKVA) of the three peptide sequences obtained as described previously (5). The insert (1.5 kb) of this clone was subcloned into the *Eco*RI site of the puc19 vector (Pharmacia, Freiburg, Germany) and sequenced on both strands by primer walking (19). Primer (+305), 5'-CCAGGCTGAGTGGATT-3'; primer (+749), 5'-GGAAGTCCCCAGCCAGA-3'; primer (+1045), 5'-GAGGTGCTTAAAGACTATGG-3'; primer (-1237), 5'-TACTGTTTCATTCACATT-3'; primer (-655), 5'-TTGTGTAGAAAGGTGCCC-3'; primer (-289), 5'-GATTGTCCTGCCGTAG. The positive clone contained a full-length open reading frame coding for a 361-amino-acid deduced protein.

Expression and activity. The expression vector pQE30 and the *E. coli* host strain M15[pREP4] were obtained from Qiagen (Chats-

worth, CA). The 5'- and 3'-end of the cDNA was modified by PCR amplification, with primers incorporating the desired sequences, which included a *Bam*HI site in front of the starting methionine codon and a *Sac*I site 3' behind the termination codon. The PCR product was ligated directly into the pCRII vector (TA Cloning Kit; Invitrogen, Leek, The Netherlands) and transformed into One Shot competent cells (Invitrogen). After *Bam*HI/*Sac*I digestion, the resulting insert was cloned into the *Bam*HI/*Sac*I-digested pQE30 vector. The resulting construct was transformed into *E. coli* M15 cells. Protein synthesis was induced by the addition of 1 mM IPTG. The 2-arylpropionyl-CoA epimerase activity of the recombinant protein was determined in the enzyme fractions by incubation of the chemically synthesized (-)-(*R*)-IBU-CoA thioesters and subsequent stereoselective high pressure liquid chromatography analysis of IBU after alkaline hydrolysis as described previously (20, 13).

Western blot analysis. *E. coli* cells were prepared according to standard methods as described by the manufacturer (Qiagen). Samples were resolved on a 10% SDS gel and transferred onto nitrocellulose membranes (Schleicher & Schuell). All subsequent steps were conducted at room temperature. Filters were blocked for 1 hr with TBS-T (20 mM Tris base, 137 mM NaCl, HCl to pH 7.6, and 0.1% Tween 20) containing 5% nonfat dried milk (BioRad, München, Germany). Blots were rinsed in TBS-T and then incubated (1 hr) with the epimerase antibodies diluted in TBS-T (0.005 μ g/ml). After extensive washing in TBS-T, immunoblots were incubated with peroxidase-linked anti-rabbit antibody (Sigma) diluted 1:2000 in TBS-T. Immunoreactivity was detected according to standard methods (21). Blots were stained with ponceau S solution (Sigma) to monitor the efficiency of the protein transfer. Prestained protein molecular weight markers (Sigma) were used to estimate the apparent molecular weight of proteins.

Sequence data analysis. Analysis of nucleic acid and protein sequence data was performed with the Lasergene software package (DNASTAR, supported by SFB 263) or the IBI/Pustell DNA and Protein Sequence Analysis System (International Biotechnologies, Inc., New Haven, CT).

Northern blot analysis. Total RNA was prepared from tissues using guanidinium isothiocyanate (22). The 1086-bp *Bam*HI/*Sac*I fragment incorporating the full-length 2-arylpropionyl-CoA epimerase cDNA was DIG-labeled with DIG-11-deoxyuridine triphosphate (Boehringer, Mannheim, Germany) during PCR and hybridized to a rat multiple-tissue Northern blot. The hybridization and washing conditions were constructed as described by the manufacturer (Boehringer).

Results

Molecular cloning and sequencing. Approximately 6×10^5 recombinants were screened with the polyclonal antibodies, and 11 positive clones were obtained. The inserts of the immunopositive reacting clones were amplified by PCR and partially sequenced. One of the clones showed a 100% amino acid sequence identity with peptide 1 and peptide 2 when compared with our previously obtained peptide sequences (5). This clone was subcloned into the *EcoRI* site of puc19 vector and sequenced on both strands. The nucleotide sequence, which was termed p-EP1, is presented in Fig. 2.

Nucleotide and deduced amino acid sequence of the rat 2-arylpropionyl-CoA epimerase. The p-EP1 cDNA from rat liver cytosol is shown in Fig. 2, along with the deduced amino acid sequence. The nucleotide sequence consists of 1498 bp, including a 48-bp sequence at the 5'-noncoding region, followed by an open reading frame of 1083 bp and 367 bp in the 3'-noncoding region. The ATG codon at +1 does not consist of the typical consensus sequence for eukaryotic initiation sites, as proposed by Kozak (23). The size of the protein and the similarity of the sequence obtained with peptide 1 at position 94–108 leads to the conclusion that this

ATG is the start codon. The poly(A)⁺ tail of 9-adenine residues could be identified in the 3'-noncoding region. The hexamer (AATAAA) at position 1462 is usual for a polyadenylation signal (24). The size of epimerase mRNA is 1.9 kb, as presented below. It is approximately 400 bp longer than the cDNA sequence. Because the sequence includes the entire coding region, the 400 missing bases must correspond to the 5'-noncoding region.

The protein is predicted to have a molecular mass of 43,870 Da. In agreement with reported findings (5, 16), we found that the homogeneous protein in the SDS-PAGE has a molecular mass of approximately 42 kDa. As shown in Fig. 2, the amino acid sequence and the sequences of the three endoproteinase LysC-digested peptides from the purified rat epimerase matched identically those deduced from the cDNA sequence. Database searches revealed homologies in the amino acid sequences between the epimerase amino acid sequence and the *Caenorhabditis elegans* and *E. coli* carnitine dehydratases amounting to 41% and 27%, respectively (Fig. 3). Several consensus sequences for protein kinase-mediated protein phosphorylation (25) were found. These include five consensus sites for protein kinase C-dependent

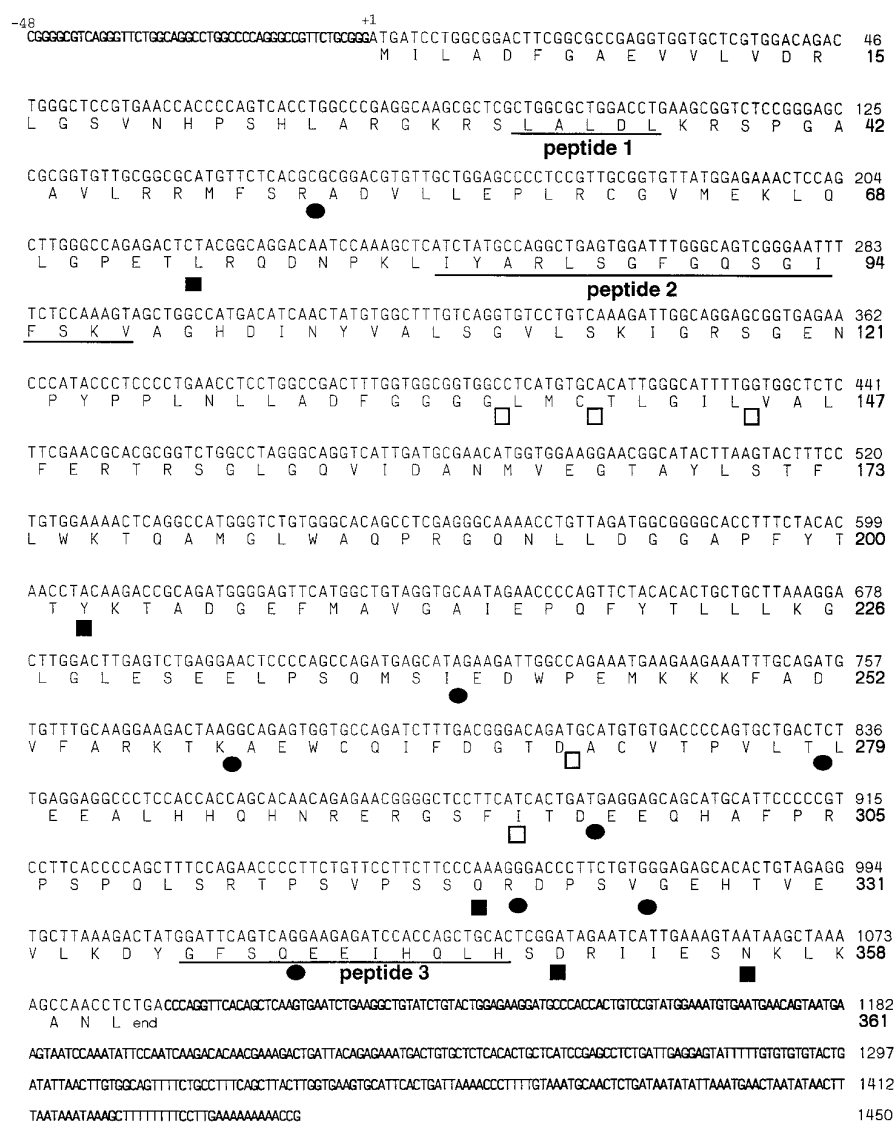


Fig. 2. Nucleotide and deduced amino acid sequence of 2-arylpropionyl-CoA epimerase cDNA. Right, numbering of the nucleotides. End, the stop codon flanking the open reading frame. Five putative sites of phosphorylation by protein kinase C (■) and eight putative phosphorylation sites for casein kinase II (●) are indicated. Additionally, five putative myristoylation sites (□) are shown. The three peptides obtained are underlined.

Rat	24	HLARGK-RSLALDLKRSPGA	AVLRMF	SRADVLL	EPLRCG	VMEKLQ	LGPET	73
<i>C.elegans</i>	42	RLNRGK-TMKQLDLKNP	EDIKKVR	DLQTS	VDVLLD	PYRPGT	LEKMGLDPST	90
<i>E.coli</i>	60	QLSRRLNHALSLNIFK	DEGREAF	LKLMET	TDIFIE	ASKGPA	FARRGITDEV	110
Rat	74	LRQDNPKLIYARLS	SGFGQSGI	--FSKVAG	HDINYVAL	SGVLSKI	-GRSGEN	121
<i>C.elegans</i>	91	LWNNNKGLIICKIS	GYGQTGR	--MSQET	GHDINYVAL	SGMLPT	FSGVNATR	140
<i>E.coli</i>	111	LWQHNP	KLVIAHLSGFGQY	GTEEYTNL	PAYNTIAQ	AFSGYLIQN	-G-DVDQ	159
Rat	122	PYPPLNLLAD	FGGGGLMCTL	GILVAL	FERTRSGL	GQ--VIDAN	MVEGTAYL	170
<i>C.elegans</i>	141	PWPPANMLAD	FAGGGLSAAF	GILSAIYAR	SHNG-GKG	CLLD	CSMTEGVAYL	190
<i>E.coli</i>	160	PMPAF	PPYTADY-FSGL	TATTAAL	AALHKVRET	GKGE--SIDIAM	YEVMLRM	207
Rat	171	STFL--WKTQ	AMGLWAQPR	GQNL	LDGGAPFY	T--TYK	TADG---EFMAV	212
<i>C.elegans</i>	191	SSFVQHYD	QPNLFTD-K	-YALF	SGECPIYR	--TYK	TKDD---KFAVAV	221
<i>E.coli</i>	208	GQYF---MMDY	FN--GGEM	CPRMSK	GKDPY	YAGCGLYK	CADGYIVMELVGI	253
Rat	213	GATEPQFY	TLLLKGLGLE	SEELPS	QMSIEDW	-----PEM	KKKFADVFARK	257
<i>C.elegans</i>	222	GAVEPKFY	QNLFK-----L	---LNVDG	-----R	---	DLFVNP	257
<i>E.coli</i>	254	TQIEEC	FKDIGLAHL-LG	TPHIE	EGTQLIHRI	EC	PGPLVEEKLDAWLATH	303
Rat	258	TKAEWCQIF	DGTDACVTP	VLTLEE				281
<i>C.elegans</i>	258	GQ-E-C-----	CVTPVLDIHE					271
<i>E.coli</i>	304	TIAEVKER	FAELNIACAK	VLTVPE				327

Fig. 3. A comparison of the deduced amino acid sequence of the rat epimerase, *C. elegans*, and *E. coli* carnitine dehydratase. The alignment was performed with the DNAsis program DNASTAR, Madison, WI). Shading, amino acid identities.

protein kinase phosphorylation and eight consensus sites for casein kinase II-dependent phosphorylation (Fig. 2). Furthermore, the epimerase amino acid sequence has five potential myristoylation sites (26).

Expression and activity of rat 2-arylpropionyl-CoA epimerase. To examine the activity of the cloned 2-arylpropionyl-CoA epimerase, we generated an expression construct that contained the complete coding sequence of the p-EP1 cDNA cloned from the pCRII vector into the pQE30 expression vector. This construct was transfected into *E. coli* M15 cells, and protein synthesis was induced by the addition of IPTG. As shown in Fig. 4A, the epimerase vector pQE30 selectively induced the synthesis of a markedly stained protein of about 43 kDa. No comparable heterologous protein was noticeable in noninduced cultures containing the vector construct. The difference from the molecular mass of 42 kDa determined for the isolated protein (5, 16) could be explained by the addition of six histidines at the amino-terminal region, which are a part of the vector construct. No comparable heterologous protein was detectable in noninduced cultures

containing the epimerase vector. The induced protein was analyzed for both presence and activity of epimerase. To determine the identity of the protein obtained, polyclonal antibodies against epimerase (Fig. 4B) and (MRGS)His antibodies (data not shown) were used in Western blots. The antisera detected a protein of approximately 43 kDa corresponding to the markedly stained protein in the SDS gel. To measure enzyme activity, the enzyme fractions were incubated with the chemically synthesized (-)-(R)-IBU-CoA thioester and analyzed by stereoselective high pressure liquid chromatography. The specific activity of the epimerase after 1 hr in the soluble fraction of the *E. coli* extracts containing the expressed rat epimerase was 2.6 nmol/min/mg (Table 1). This demonstrates that recombinant rat epimerase is expressed in a catalytically active form.

Northern blot analysis. Approximately 10 μ g of total RNA was extracted from each of four rat tissues, electrophoresis was performed under denatured conditions, and the samples were transferred onto a positively charged nylon membrane and hybridized successively with the 1086-bp

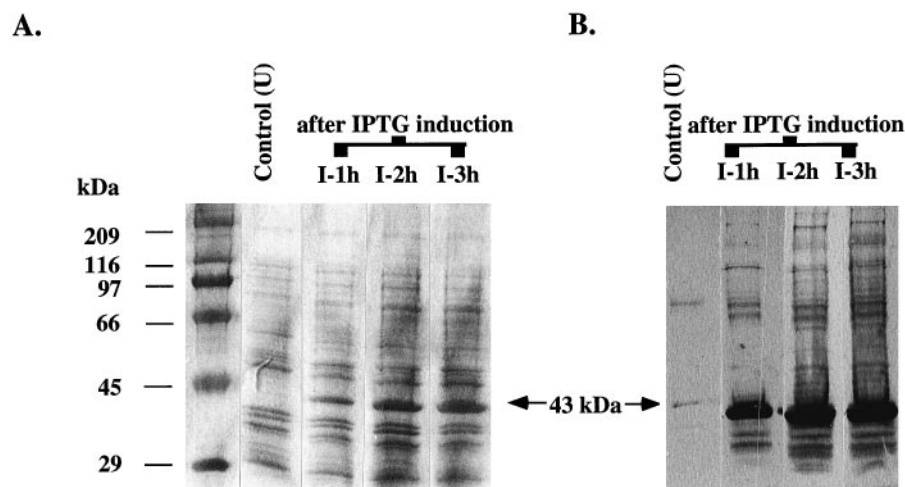


Fig. 4. Expression of rat 2-arylpropionyl-CoA epimerase in *E. coli*. A, SDS-PAGE analysis (Coomassie blue stained) of the recombinant protein in cell supernatants. The noninduced (control; U) and induced (I) supernatants of the expression culture are shown at different times. Left, Mobility of prestained protein molecular weight markers. B, The same fractions derived from expression culture were subjected to SDS-PAGE and immunoblotted with anti-epimerase antibodies.

TABLE 1

Comparison of the specific enzyme activities from rat liver homogenate and the cell supernatants of the recombinant protein from *E. coli*

	Total protein	Specific activity
	mg/ml	nmol/min/mg
Crude liver homogenate	2.94	0.88
Purified cytosolic epimerase from rat liver	0.005	3710
M15-PQE30 control (without IPTG induction)	10.0	—
Recombinant protein (60 min after IPTG induction)	10.0	2.6

*Bam*HI/*Sac*I-fragment (see Materials and Methods). Fig. 5 shows that a single prominent hybridizing band of 1.9 kb was detected in all tissues. A strong band of rat epimerase mRNA was detected in the liver and kidney. A very low expression was observed in the heart and brain.

Discussion

A 42-kDa epimerase has been isolated from the cytosolic and mitochondrial fraction of rat liver and biochemically characterized (16). Recently, we have raised a polyclonal antibody against this epimerase and analyzed the relation between the inversion activity in various tissues and the tissue distribution of the protein in guinea pigs and rats (5). Additionally, amino acid sequence analysis of three internal peptides of the epimerase revealed homology with regions of the enzymes involved in the lipid metabolism.

In the present investigation, we succeeded in isolating a cDNA clone coding for the cytosolic rat liver 2-arylpropionyl-CoA epimerase. The clone consists of a nucleotide sequence of 1498 bp, including an open reading frame of 1083 bp coding for the epimerase. Database searches using the deduced amino acid sequence for p-EP1 revealed a high degree of homology of the obtained protein sequence with the sequence

of the L-carnitine dehydratase from the higher eukaryont *C. elegans* (41% identity) (27) and with the sequence of the carnitine operon oxidoreductase (28) from *E. coli* (27% identity). The observed homology to proteins of the carnitine metabolism possibly indicates an as yet undefined physiological role of the epimerase. This hypothesis is supported by similar biochemical mechanisms of both the carnitine dehydratase and the epimerase. The function of the carnitine dehydratase in the carnitine pathway is the specific reversible dehydration of L-carnitiny-CoA to crotonobetainyl-CoA (compare Fig. 6A) (29). Thus, a common feature of 2-arylpropionyl-CoA-epimerase and carnitine dehydratase is the affinity for CoA-linked thioester substrates. The formation of the thioester leads to an acidic α carbon atom. This is an important prerequisite for the interaction with a basic moiety in the active site of the epimerase and for the following proton abstraction from the substrate. In experiments to determine the mechanism of proton exchange at the active site, Chen *et al.* (15) and Baillie *et al.* (30) used (–)-(R)-[2-²H]2-(4-isobutylphenyl)propionyl-CoA as substrate for the epimerase enzyme. After inversion, the (+)-S-enantiomer was completely depleted of the α deuterium, which indicates that the hydrogen atom of the new C–H bond is derived from the solvent protons. Obviously, ibuprofenoyl-CoA has no hydroxyl group to take part in the second step of a dehydratase reaction, which involves the release of a water molecule followed by formation of a double bond (Fig. 6 A). However, in line with previously discussed mechanisms of chiral inversion, a proposed carnitine dehydratase/epimerase could catalyze the first step of the dehydratase reaction, the linkage of the hydrogen atom of (–)-(R)-ibuprofenoyl-CoA to a base in the active site of the enzyme (Fig. 6 B). This would lead to an alteration of the methyl group configuration. The resulting carbanion may be stabilized by solvent protons in a nonstereospecific manner.

Interestingly, epimerase activity was located in both cytosol and mitochondria of rat liver cells. Although mitochondria contain some genetic information, most of the mitochondrial proteins are encoded by nuclear genes and synthesized in the cytosol. Proteins that are destined to be transported across the mitochondrial membrane are usually synthesized with an amino-terminal signal sequence that is proteolytically removed. A discrepancy in the amino acid composition of the cytosolic epimerase and its mitochondrial counterpart was shown by Shieh and Chen (16) and was the first indication for the existence of a signal peptide. It is generally assumed that signal peptides have highly degenerate primary sequences and that their group-specific properties reflect a hydrophobic structure (31). The first 20 amino acids of the amino-terminal region of the epimerase revealed a strong hydrophobic structure (data not shown) as determined by the Kyte and Doolittle method (32), which had no similarity to other amino acid sequences. Furthermore, the possible myristoylation modifications in the epimerase nucleotide sequence (consensus sites; see Fig. 2) might play a key role in membrane association of the modified protein. Thus, the results support the existence of a signal sequence and, consequently, suggest the hypothesis of cytosolic formation and subsequent transport into mitochondria.

The discrepancy of the relatively low specific activity of the expressed enzyme compared with the purified liver protein needs clarification. Epimerase cDNA was expressed in *E. coli*

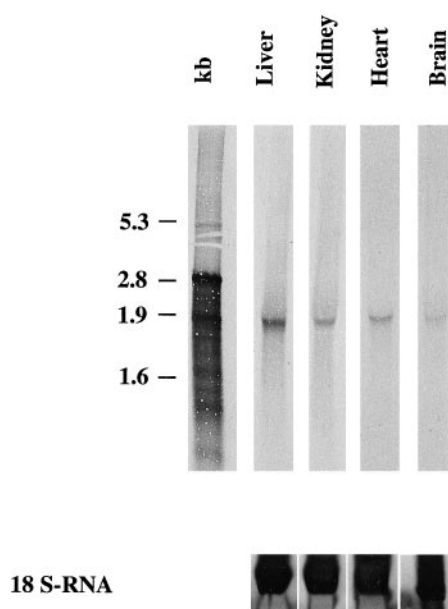


Fig. 5. Northern blot analysis of poly(A)⁺ RNA from various rat tissues. The blots were hybridized with DIG-labeled *Bam*HI/*Sac*I cDNA fragment of p-EP1. An RNA ladder from 1.6 to 5.3 kb in size (Boehringer) is used as a size marker.

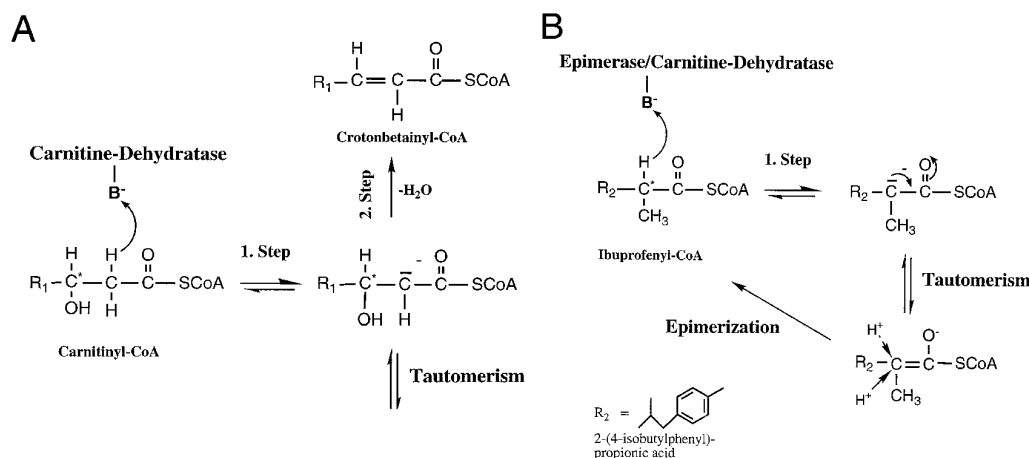


Fig. 6. A, The dehydration reaction of the carnitine dehydratase in the carnitine pathway. R_1 , $N(CH_3)_3-CH_2$. B, Proposed mechanism of the metabolic inversion of 2-arylpropionic acids.

because, in contrast to other microorganisms (33), no epimerase activity was detectable in the bacterial expression system. In the expression experiments, the highest activity of the enzyme as measured by the epimerization of $(-)-(R)$ -IBU-CoA was found 1 hr after IPTG induction. The recombinant enzyme showed a specific activity of 2.6 nmol/min/mg in the cell supernatants of *E. coli* after 1 hr (Table 1). The enzyme activity decreased over the time, but the level of the recombinant protein increased until 3 hr after IPTG induction. A possible explanation is be that the enzyme needs to be post-translationally processed. The consensus sites for post-translational phosphorylation (compare Fig. 2) suggests that the activity of the epimerase is regulated by further modifications. Moreover, most large amounts of the recombinant protein may be in an incorrectly folded state. Taken together, the lack of post-translational processing and an incorrect folding are possible reasons for the relatively low specific activity of the expressed enzyme compared with the purified liver protein.

Northern blot analysis with the whole nucleotide sequence cDNA probe showed a single hybridizing band in rat liver, which demonstrates that this clone is derived from a single gene. Moreover, we observed single bands in other tissues, including heart, kidney, and brain. These results are consistent with the Western blot data obtained previously, which show that a single immunoreactive band of a 42-kDa protein is detectable in homogenates of liver, kidney, heart, and brain (5). A strong expression of epimerase mRNA and protein was observed in rat liver and kidney tissues, which reflects the physiological importance of the epimerase in these tissues. A very low level of expression of epimerase was detected in heart and brain. It cannot be excluded that other isoforms of epimerase may exist in these tissues, as described for long-chain acyl-CoA synthetase (34, 35), another key enzyme of stereoselective chiral inversion of $(-)-(R)$ -2-arylpropionic acids. Finally, our results may not be only of scientific importance, because prostaglandin endoperoxide synthase (COX) isozymes are target enzymes of 2-arylpropionic acids such as IBU (36). The $(+)-S$ -enantiomer of IBU inhibits the COX isoforms 2 orders of magnitude more potently than the respective $(-)-R$ -enantiomer. Chiral inversion of the $(-)-R$ - into the $(+)-S$ -enantiomer, which takes place *in vivo*, results in an increase in the COX-inhibiting $(+)-S$ -enantiomer (37). Malmberg and Yaksh (38) reported that the formalin-induced production of spinal prostaglandin E_2 in the subarach-

noid space adjacent to the lumbar spinal cord was unaffected after the systemic and intrathecal administration of $(-)-(R)$ -IBU in rats. In contrast, the $(+)-S$ -enantiomer lowered formalin-induced prostaglandin E_2 levels and showed a significant antinociceptive response. These results show that the $(-)-R$ -enantiomer itself does not possess relevant COX-inhibiting activity *in vivo* in the central nervous system at the spinal cord level. Moreover, these findings permit speculation that the affinity of $(-)-(R)$ -ibuprofenoyl-CoA to the epimerase in the brain may be relatively low. The weak signal detected in the brain by Western blot and Northern blot analyses supports this hypothesis.

In summary, we have described the isolation and expression of a cDNA for a 2-arylpropionyl-CoA epimerase that mediates a biocatalytic reactions with an as yet unknown physiological role. The significant homology to enzymes involved in carnitine metabolism suggests that the 2-arylpropionyl-CoA epimerase may play an important role in this aspect of fatty acid metabolism.

Further studies are necessary to clarify the physiological role of the 2-arylpropionyl-CoA epimerase. Inhibition experiments of $(-)-(R)$ -ibuprofenoyl-CoA epimerization with different CoA-activated carnitine metabolites could help to define the natural substrate. Moreover, the sequence data will make it possible to clone the human enzymes, which will help to clearly define the role of the epimerase in human tissues.

Acknowledgments

The authors thank Dr. M. Parnham and F. Beiche for reading the manuscript and helpful discussions. The excellent technical assistance of Mrs. S. Karafyllis and Mrs. B. García Alía is gratefully acknowledged.

References

- Kujubu, D. A., B. S. Fletcher, B. C. Varnum, R. W. Lim, and H. R. Herschman. TIS 10, a phorbol ester tumor promotor-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J. Biol. Chem.* **266**:12866–12872 (1991).
- Caldwell, J., A. J. Hutt, and S. Fournel-Gigleux. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochem. Pharmacol.* **37**:105–114 (1988).
- Hall, S. D., and O. Xiaotao. The role of coenzyme A in the biotransformation of 2-arylpropionic acids. *Chem. Biol. Interact.* **90**:235–251 (1994).
- Knadler, M. P., and S. D. Hall. Stereoselective arylpropionyl-CoA thioester formation *in vitro*. *Chirality* **2**:67–73 (1990).
- Reichel, C., H. Bang, K. Brune, G. Geisslinger, and S. Menzel. 2-arylpropionyl-CoA epimerase: partial peptide sequences and tissue localization. *Biochem. Pharmacol.* **50**:1803–1806 (1995).

6. Menzel-Soglowek, S., G. Geisslinger, J. Mollenhauer, and K. Brune. Metabolic chiral inversion of 2-arylpropionates in rat H4IIE and human HEP G2 hepatoma cells. *Biochem. Pharmacol.* **43**:1487–1492 (1992).
7. Sanins, S. M., W. J. Adams, D. G. Kaiser, G. W. Halstead, and T. A. Baillie. Studies on the metabolism and chiral inversion of ibuprofen in isolated rat hepatocytes. *Drug Metab. Dispos.* **18**:527–532 (1990).
8. Cox, J. W., S. R. Cox, G. J. VanGiessen, and M. J. Ruwart. Ibuprofen stereoisomer hepatic clearance and distribution in normal and fatty *in situ* perfused rat liver. *J. Pharmacol. Exp. Ther.* **232**:636–641 (1985).
9. Hall, S. D., M. Hassanzadeh-Khayyat, M. P. Knadler, and P. R. Mayer. Pulmonary inversion of 2-arylpropionic acids: influence of protein binding. *Chirality* **4**:349–352 (1992).
10. Nakamura, Y., and T. Yamaguchi. Stereoselective metabolism of 2-phenylpropionic acid in rat. I. *In vitro* studies on the stereoselective isomerization and glucuronidation of 2-phenylpropionic acid. *Drug Metab. Dispos.* **15**:529–534 (1987).
11. Tracy, T. S., D. P. Wirthwein, and S. D. Hall. Metabolic inversion of (*R*)-ibuprofen. Formation of ibuprofenyl-coenzyme A. *Drug Metab. Dispos.* **21**:114–120 (1993).
12. Menzel, S., R. Waibel, K. Brune, and G. Geisslinger. Is the formation of *R*-ibuprofenyl-adenylate the first stereoselective step of chiral inversion? *Biochem. Pharmacol.* **48**:1056–1058 (1994).
13. Brugger, R., B. Garcia Alia, C. Reichel, S. Menzel, K. Brune, and G. Geisslinger. Isolation and characterization of rat liver microsomal *R*-ibuprofenyl-CoA synthetase. *Biochem. Pharmacol.* **52**:1007–1013 (1996).
14. Tracy, T. S., and S. D. Hall. Metabolic inversion of (*R*)-ibuprofen: epimerization and hydrolysis of ibuprofenyl-coenzyme A. *Drug Metab. Dispos.* **20**:322–327 (1992).
15. Chen, C. S., W. R. Shieh, P. H. Lu, S. Harrimann, and S. Y. Chen. Metabolic stereoisomeric inversion of ibuprofen in mammals. *Biochim. Biophys. Acta.* **1078**:411–417 (1991).
16. Shieh, W. R., and C. S. Chen. Purification and characterization of novel "2-arylpropionyl-CoA epimerases" from rat liver cytosol and mitochondria. *J. Biol. Chem.* **268**:3487–3493 (1993).
17. Knihinicki, R. D., R. O. Day, and K. M. Williams. Chiral inversion of 2-arylpropionic acids non-steroidal anti-inflammatory drugs-II. Racemization and hydrolysis of (*R*)- and (*S*)-ibuprofen-CoA thioesters. *Biochem. Pharmacol.* **42**:1905–1911 (1991).
18. Fournel, S., and J. Caldwell. The metabolic chiral inversion of 2-phenylpropionic acid in rat, mouse and rabbit. *Biochem. Pharmacol.* **35**:4153–4159 (1986).
19. Maniatis, T., E. F. Fritsch, and J. Sambrook. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).
20. Menzel-Soglowek, S., G. Geisslinger, and K. Brune. Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α 1-acid glycoprotein column. *J. Chromatogr.* **532**:295–303 (1990).
21. Harlow, E., and D. Lane. *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988).
22. Chomczynski, P., and N. Sacchi. Single step method of RNA extraction by guanidinium thiocyanate-phenol-chloroform. *Anal. Biochem.* **162**:156–169 (1987).
23. Kozak, N. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12**:857–872 (1984).
24. Proudfoot, N. F., and G. G. Brownlee. 3' non-coding region sequences in eukaryotic messenger RNA. *Nature (Lond.)* **263**:211–214 (1976).
25. Kennelly, P. J., and E. G. Krebs. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. *J. Biol. Chem.* **266**:15555–15558 (1991).
26. Milligan, G., M. Parenti, and A. I. Magee. The dynamic role of palmitoylation in signal transduction. *Trends. Biochem. Sci.* **20**:181–186 (1995).
27. Wilson, R., and P. Wohldman. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature (Lond.)* **368**:32–38 (1994).
28. Eichler, K., F. Bourgis, A. Buchet, H. P. Kleber, and M. A. Mandrand-Berthelot. Molecular characterization of the cai operon necessary for carnitine metabolism in *Escherichia coli*. *Mol. Microbiol.* **13**:775–786 (1994).
29. Jung, H., K. Jung, and H. P. Kleber. Synthesis of L-carnitine by microorganisms and isolated enzymes. *Adv. Biochem. Eng. Biotechnol.* **50**:21–44 (1993).
30. Baillie, T. A., W. J. Adams, D. G. Kaiser, L. S. Olanoff, G. W. Halstead, H. Harpootlian, and G. J. VanGiessen. Mechanistic studies of the metabolic chiral inversion of (*R*)-ibuprofen in humans. *J. Pharmacol. Exp. Ther.* **249**:517–523 (1989).
31. Schatz, G., and B. Dobberstein. Common principles of protein translocation across membranes. *Science (Washington D. C.)* **271**:1519–1526 (1996).
32. Kyte, J., and R. F. Doolittle. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**:105–132 (1982).
33. Hutt, A. J., A. Kooloobandi, and G. W. Hanlon. Microbial metabolism of 2-arylpropionic acids: chiral inversion of ibuprofen and 2-phenylpropionic acid. *Chirality* **5**:596–601 (1993).
34. Suzuki, H., Y. Kawarabayasi, J. Kondo, T. Abe, K. Nishikawa, S. Kimura, T. Hashimoto, and T. Yamamoto. Structure and regulation of rat long-chain acyl-CoA synthetase. *J. Biol. Chem.* **265**:8681–8685 (1990).
35. Fujino, T., and T. Yamamoto. Cloning and functional expression of a novel long-chain acyl-CoA synthetase expressed in brain. *J. Biochem.* **111**:197–203 (1992).
36. Meade, E. A., W. L. Smith, and D. L. DeWitt. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal antiinflammatory drugs. *J. Biol. Chem.* **268**:6610–6614 (1993).
37. Adams, S. S., P. Bresloff, and C. G. Mason. Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (–)-isomer. *J. Pharm. Pharmacol.* **28**:256–257 (1976).
38. Malmberg, A. B., and T. L. Yaksh. Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. *J. Neurosci.* **15**:2768–2776 (1995).

Send reprint requests to: Gerd Geisslinger, Ph.D., M. D., Department of Experimental and Clinical Pharmacology and Toxicology, Universitätsstr. 22, D-91054 Erlangen, Germany. E-mail: gg@macpost.pharmakologie.uni-erlangen.de
