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ACCELERATED COMMUNICATION

Isolation and Expression of a cDNA Coding for Rat Kidney Cytosolic Cysteine Conjugate β-Lyase

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SUMMARY

The role of rat kidney cysteine conjugate β-lyase in the production of nephrotoxic thiols from S-cysteine conjugates of xenobiotics has been well established. However, the factors controlling the cellular distribution and substrate specificity of the enzyme have yet to be elucidated. As an approach to this we have isolated a cDNA for cysteine conjugate β-lyase from a rat kidney cDNA library, using a combination of immunological and hybridization screening. A full length cDNA was sequenced and its identity was confirmed by deduced molecular weight, deduced amino acid composition, the presence of a consensus pyridoxal phosphate (PLP) binding site in the deduced amino acid sequence, kidney-specific expression of the corresponding mRNA, and the expression of β-lyase and glutamine transaminase K activities in tissue culture cells transfected with the cDNA. The cDNA coded for a protein of 48 kDa containing the sequence Ser-Ala-Gly-Lys-Ser-Phe, which corresponds closely to the PLP binding site in other PLP-containing enzymes. Use of the cDNA to detect β-lyase mRNA sequences in rat liver and kidney RNA demonstrated that expression was kidney specific and that the mRNA size (2.1 kilobases) was in good agreement with the size of the cDNA. When the cDNA was inserted into the expression vector pUS1000 and transfected into COS-1 tissue culture cells, a 7–10-fold increase in cytosolic β-lyase and glutamine transaminase K activities could be detected. The use of β-lyase cDNA for the elucidation of the mechanism of action of this enzyme and for the development of *in vitro* systems to examine xenobiotic cysteine conjugate toxicity is discussed.

A major route of detoxication for many halogenated xenobiotics is by conjugation to the tripeptide glutathione. Subsequent metabolic processing of such conjugates yields the cysteine conjugate of the original chemical, which may then serve as a substrate for enzymes that express β-lyase activity (1). Attention has been directed towards the toxicological importance of these enzymes because β-lyase action can yield metabolites that are selectively toxic to the P_3 segment of the kidney proximal tubule (2–4). It has been shown that a combination of specific kidney uptake of xenobiotic metabolites (5, 6) and the proximal tubular location of a specific form of β-lyase (glutamine transaminase K) are causative factors in the tissue-selective toxicity of these chemicals (7, 8). Apart from the kidney, β-lyase activity is also found predominately in the liver and the gastrointestinal microflora. Evidence from animal and human studies (9–11) has indicated that the liver and kidney forms of β-lyase are different enzymes with different substrate specificities. There is also some evidence that in the kidney cytosolic and mitochondrial β-lyase activities may be due to different isoenzymes (12).

The mechanism of action of β-lyase enzymes with cysteine conjugates as substrates remains unclear. PLP-containing enzymes support several different reactions, including β-elimination (serine dehydratase), β-replacement (kynureninase), γ-elimination (cystathionase), and transamination (aspartate amino transferase and glutamine transaminase K). The majority of β- and γ-elimination enzymes and the majority of transaminases do not express adventitious β-lyase activity with cysteine conjugates. In contrast, both glutamine transaminase K (13) and kynureninase (14) show good β-lyase activity towards selected cysteine conjugates as substrates. These enzymes must therefore possess structural features that allow β-elimination to proceed yet distinguish these enzymes from other PLP-containing enzymes with similar catalytic functions. It is also of note that in the kidney some cysteine conjugates can be transaminated to their corresponding nontoxic keto acid rather than undergoing β-elimination of the toxic thiol group (13, 15). The substrate characteristics that govern the reaction pathway

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ABBREVIATIONS: PLP, pyridoxal phosphate; SDS, sodium dodecyl sulfate; AOAA, aminooxyacetic acid; kb, kilobases.

taken and the role of enzyme structure in this have yet to be examined. An understanding of the structure and function of β-lyase enzymes and their selectivity towards xenobiotic cysteine conjugates will assist in the prediction of chemical toxicity and of the site-specific nature of the resulting lesion.

The role of β-lyase in kidney cell toxicity has also been examined in cell culture, as an approach to the development of *in vitro* systems for the analysis of xenobiotic cysteine conjugate toxicity. Such studies have been hindered by the extremely low levels of β-lyase activity found in cell culture lines, compared with kidney slices or cell suspensions (16). It is now clear that for such systems to be of use in the future the balance of enzymes must be restored to *in vivo* levels. Gene transfer would provide one solution to this problem.

In this study we have isolated a cDNA corresponding to the mRNA for rat kidney cytosolic cysteine conjugate β-lyase (glutamine transaminase K). Confirmation that the cDNA does indeed code for β-lyase has been obtained from the tissue distribution of mRNA synthesis, the deduced enzyme molecular weight and amino acid composition, the presence of a PLP binding site in the amino acid sequence, and expression of β-lyase activity after transfection of the cDNA into tissue culture cells.

Materials and Methods

Isolation and sequencing of cDNA clones. A Wistar rat kidney cDNA library in the expression vector λgt11 (kindly supplied by Dr. M. Muekler, Washington University School of Medicine) was screened (17) for β-lyase-expressing phage clones by using a desorbed monospecific sheep polyclonal antibody (7) to purified rat kidney cytosolic cysteine conjugate β-lyase. From a total of 2×10^5 plaques a single positive clone, λβl-1.0, was obtained and purified through two additional rounds of screening at lower plaque density. Phage DNA was prepared using a modified potassium acetate-SDS precipitation method (18), and the 0.75-kb cDNA insert from λβl-1.0 was excised by digestion with *Eco*RI. The insert was purified and subcloned into the plasmid vector pGEM-7Zf(+) (Promega, Southampton, UK) to form the recombinant plasmid pβl-1.0. Plasmid DNA was prepared (QIAGEN Midi Prep; DIAGEN GmbH, Hilden, Germany) and the cDNA insert from pβl-1.0 was excised by double digestion with *Eco*RI and *Ava*II to produce a 0.65-kb cDNA fragment lacking polyadenosine sequences. The DNA fragment was purified by gel electrophoresis and was extracted using Geneclean (Stratech Scientific, Luton, UK), according to the manufacturer's instructions. This cDNA probe was radiolabeled using [α-^{32}P]dCTP (3000 Ci/mmol; ICN Biomedicals, Inc.) and a random priming kit (Amersham International, Amersham, UK), according to the manufacturer's instructions. After chromatography to remove unincorporated nucleotides (Sephadex G-50 NICK columns; Pharmacia Biosystems Ltd., Milton Keynes, UK), the probe was used to screen a Sprague Dawley rat kidney 5′ stretch cDNA library in λgt10 (Clonetech Laboratories, Inc., Palo Alton, CA). From 2×10^5 plaques, 17 positive clones were obtained. After three rounds of further plaque purification at lower plaque density, small-scale recombinant phage DNA preparations were made from the 17 clones (18). The cDNA inserts were sized by agarose gel electrophoresis after digestion of the phage DNAs with *Eco*RI. A clone, λβl-2.0, that contained the largest cDNA insert (1.9 kb) was taken for further analysis. The 1.9-kb cDNA insert was removed by partial digestion with *Eco*RI (as shown in Fig. 1, the cDNA contains an internal *Eco*RI site, giving two fragments if digested to completion), purified, and subcloned into the plasmid vector pGEM-7Zf(+) to give the recombinant clone pβl-2.0. Recombinant plasmids containing the two separate *Eco*RI subfragments were also constructed (pβl-2.1 and pβl-2.2) for sequencing purposes. Plasmid DNA was prepared as before and the cDNA inserts in pβl-1.0, pβl-2.0, pβl-2.1, and pβl-2.2 were sequenced using the method of Sanger *et al.* (19), as modified for plasmid double-stranded DNA sequencing (Sequenase version 2.0; United States Biochemicals, Cleveland, OH). Sequencing from the plasmid vectors was performed using the appropriate vector-specific oligonucleotide primers. Where necessary, additional internal oligonucleotide primers (synthesized on an Applied Biosystems 381A DNA synthesizer) were used to complete the sequence (see Fig. 1). DNA sequence assembly, analysis, and translation were performed using the Gene Jockey 1.1 software package (Biosoft Ltd., Cambridge, UK). Comparison with the EMBL DNA sequence database was performed using the Wisconsin Genetics Computer Group DNA analysis software.

Northern blot analysis of mRNA. Total RNA was prepared from rat liver and kidney samples (20), electrophoresed in a 1% (w/v) agarose gel containing 2.2 M formaldehyde, and transferred to a nitrocellulose membrane (21). β-Lyase mRNA transcripts were detected by hybridization of the blot with the cDNA insert from pβl-1.0, prepared and radiolabeled as described above.

Expression of β-lyase cDNA in COS-1 cells. The full length cDNA insert from pβl-2.0 was recloned into the expression vector pUS1000 (22), placing transcription of the cDNA under control of the strong immediate early promotor of human cytomegalovirus. Amplification in monkey COS-1 cells (23) was ensured by the presence of a simian virus 40 origin of replication in the vector. The 1.9-kb β-lyase cDNA was inserted into the vector *Eco*RI site in both the sense and antisense orientations, to produce the recombinant plasmids pUSβl-2.3 and pUSβl-2.4, respectively. COS-1 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum, in 5% CO_2 in air at 37°. DNA transfection of subconfluent cells was performed using DEAE-dextran (24), scaled up to 25-cm^2 or 75-cm^2 tissue culture flasks (Greigner Labortechnik Ltd., Dursley, UK).

Enzyme activity of β-lyase in transfected cells. Transfected COS-1 cells were removed from the flasks by using 0.125% trypsin in 1 mM EDTA, centrifuged, and washed twice with ice-cold phosphate-buffered saline. Cell pellets were resuspended in 10 mM Tris·HCl, pH 7.5, containing 0.25 M sucrose, freeze-thawed twice, and sonicated twice for 10 sec with a 30-sec cooling interval. After centrifugation at 3000 × *g* for 5 min the cytosol was removed and stored at −70°. Glutamine transaminase K activity in the cytosolic extracts was measured as described previously (25), and cysteine conjugate β-lyase activity was measured using both *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine as substrates (9). Inhibition of β-lyase activity was carried out using AOAA at a concentration of 0.1 mM in the assay (26). Protein concentration was measured by the method of Lowry *et al.* (27). Western blot analysis of β-lyase apoprotein in cytosol samples was performed by electrophoresis of samples in a 10% SDS-polyacrylamide gel and electrophoretic transfer of the proteins to a nitrocellulose membrane. β-Lyase was detected using the desorbed monospecific polyclonal anti-β-lyase antibody (7). Development was with rabbit anti-sheep IgG conjugated to biotin (Sera-lab, Granley Down, UK) and a streptavidin-biotinylated horseradish peroxidase complex (Amersham International).

Results

Characterization of β-lyase cDNA. DNA sequence analysis of the cDNA inserts in pβl-1.0 and pβl-2.0, combined with restriction enzyme mapping, demonstrated that their sequences overlapped. As shown in Fig. 1, the insert in pβl-2.0 contains a cDNA representing nucleotides 1–1867 of the β-lyase cDNA (Fig. 2), whereas the pβl-1.0 insert represents an incomplete cDNA copy comprising nucleotides 1245–1892 plus a 97-nucleotide polyadenosine tail. Whether the first nucleotide of pβl-2.0 corresponds to the first nucleotide of the *in vivo* mature mRNA remains to be determined. Analysis of the combined DNA sequence of the pβl-1.0 and pβl-2.0 inserts (Fig. 2) indi-

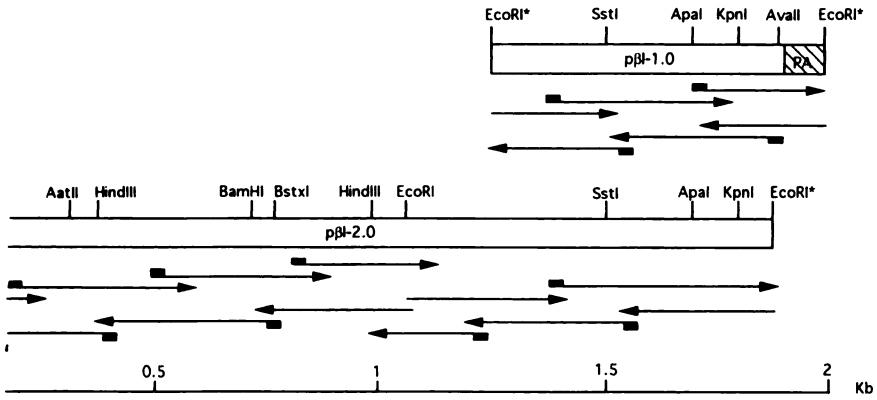

Fig. 1. Restriction enzyme maps of the overlapping β-lyase clones pβI-1.0 and pβI-2.0. *, *Eco*RI sites in the cloning linkers. *Hatched area*, the polyadenosine tail in pβI-1.0. *Arrows*, directions and extent of the DNA-sequencing reactions. *Solid boxes*, internal oligonucleotide primers.

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1                                                AAACTGACCAAGGAGTATGATCAATCCCGTCCAGCCTCCGAG
43  CCTGCAGCCGTTTGGTCATGGTGAGCTGCTTCAGCTAACAATTGCACTGACAGTGCTCTTGAGCCAA
110 GTTGCTTCTGGGCGGAAGTAGTCCATCTAGGGCTCGGCCTCTTTAAAGAAACAGACTTCTGCAACCT
177 TGGGACTACGTTTGGGGTCGCCGGCTATTGGACGGAGCAGCGCAATTGTTAGCTGAAGCAGCTCACC
    Met Thr Lys Arg Leu Gln Ala Arg Arg Leu Asp Gly Ile Asp Gln Asn Leu   17
244 ATG ACC AAA CGG CTG CAG GCT CGG AGG CTG GAC GGG ATT GAT CAA AAC CTC
    Trp Val Glu Phe Gly Lys Leu Thr Lys Glu Tyr Asp Val Val Asn Leu Gly   34
295 TGG GTG GAG TTT GGC AAA CTG ACC AAG GAG TAT GAC GTC GTG AAC TTG GGT
    Gln Gly Phe Pro Asp Phe Ser Pro Pro Asp Phe Ala Thr Gln Ala Phe Gln   51
346 CAG GGC TTC CCT GAC TTC TCG CCT CCG GAC TTT GCA ACG CAA GCT TTT CAG
    Gln Ala Thr Ser Gly Asn Phe Met Leu Asn Gln Tyr Thr Arg Ala Phe Gly   68
397 CAG GCT ACC AGT GGG AAC TTC ATG CTC AAC CAG TAC ACC AGG GCA TTT GGT
    Tyr Pro Pro Leu Thr Asn Val Leu Ala Ser Phe Phe Gly Lys Leu Leu Gly   85
448 TAC CCA CCA CTG ACA AAC GTC CTG GCA AGT TTC TTT GGC AAG CTG CTG GGA
    Gln Glu Met Asp Pro Leu Thr Asn Val Leu Val Thr Val Gly Ala Tyr Gly  102
499 CAG GAG ATG GAC CCA CTC ACG AAT GTG CTG GTG ACA GTG GGT GCC TAT GGG
    Ala Leu Phe Thr Arg Phe Gln Ala Leu Val Asp Glu Gly Asp Glu Val Ile  119
550 GCC TTG TTC ACA CGC TTT CAG GCC CTG GTG GAT GAA GGA GAT GAG GTC ATC
    Ile Met Glu Pro Ala Phe Asp Cys Tyr Glu Pro Met Thr Met Met Ala Gly  136
601 ATC ATG GAA CCT GCT TTT GAC TGT TAT GAA CCC ATG ACA ATG ATG GCT GGA
    Gly Cys Pro Val Phe Val Thr Leu Lys Pro Ser Pro Ala Pro Lys Gly Lys  153
652 GGT TGC CCT GTG TTC GTG ACT CTG AAG CCG AGC CCT GCT CCT AAG GGG AAA
    Leu Gly Ala Ser Asn Asp Trp Gln Leu Asp Pro Ala Glu Leu Ala Ser Lys  170
703 CTG GGA GCC AGC AAT GAT TGG CAA CTG GAT CCT GCA GAA CTG GCC AGC AAG
    Phe Thr Pro Arg Thr Lys Ile Leu Val Leu Asn Thr Pro Asn Asn Pro Leu  187
754 TTC ACA CCT CGC ACC AAG ATC CTG GTC CTC AAC ACA CCC AAC AAC CCT TTA
    Gly Lys Val Phe Ser Arg Met Glu Leu Glu Leu Val Ala Asn Leu Cys Gln  204
805 GGA AAG GTA TTC TCT AGG ATG GAG CTG GAG CTG GTG GCT AAT CTG TGC CAG
    Gln His Asp Val Val Cys Ile Ser Asp Glu Val Tyr Gln Trp Leu Val Tyr  221
856 CAG CAC GAT GTC GTG TGC ATC TCT GAT GAG GTC TAC CAG TGG CTG GTC TAT
    Asp Gly His Gln His Val Ser Ile Ala Ser Leu Pro Gly Met Trp Asp Arg  238
907 GAC GGG CAC CAG CAC GTC AGC ATC GCC AGC CTC CCT GGC ATG TGG GAT CGG
    Thr Leu Thr Ile Gly Ser Ala Gly Lys Ser Phe Ser Ala Thr Gly Trp Lys  255
958 ACC CTG ACC ATC GGC AGT GCA GGC AAA AGC TTC AGT GCC ACT GGC TGG AAG
    Val Gly Trp Val Met Gly Pro Asp Asn Ile Met Lys His Leu Arg Thr Val  272
1009 GTG GGC TGG GTC ATG GGT CCA GAT AAC ATC ATG AAG CAC CTG AGG ACA GTG
    His Gln Asn Ser Ile Phe His Cys Pro Thr Gln Ala Gln Ala Ala Val Ala  289
1060 CAC CAG AAT TCT ATC TTC CAC TGC CCC ACC CAG GCC CAG GCT GCA GTA GCC
    Gln Cys Phe Glu Arg Glu Gln Gln His Phe Gly Gln Pro Ser Ser Tyr Phe  306
1111 CAG TGC TTT GAG CGG GAG CAG CAA CAC TTT GGA CAA CCC AGC AGC TAC TTT
    Leu Gln Leu Pro Gln Ala Met Glu Leu Asn Arg Asp His Met Ile Arg Ser  323
1162 TTG CAG CTG CCA CAG GCC ATG GAG CTG AAC CGA GAC CAC ATG ATC CGT AGC
    Leu Gln Ser Val Gly Leu Lys Leu Trp Ile Ser Gln Gly Ser Tyr Phe Leu  340
1213 CTG CAG TCA GTG GGC CTC AAG CTC TGG ATC TCC CAG GGG AGC TAC TTC CTC
    Ile Ala Asp Ile Ser Asp Phe Lys Ser Lys Met Pro Asp Leu Pro Gly Ala  357
1264 ATT GCA GAC ATC TCA GAC TTC AAG AGC AAG ATG CCT GAC CTG CCC GGA GCT
    Glu Asp Glu Pro Tyr Asp Arg Arg Phe Ala Lys Trp Met Ile Lys Asn Met  374
1315 GAG GAT GAG CCT TAT GAC AGA CGC TTT GCC AAG TGG ATG ATC AAA AAC ATG
    Gly Leu Val Gly Ile Pro Val Ser Thr Phe Phe Ser Arg Pro His Gln Lys  391
1366 GGC TTG GTG GGC ATC CCT GTC TCC ACA TTC TTC AGT CGG CCC CAT CAG AAG
    Asp Phe Asp His Tyr Ile Arg Phe Cys Phe Val Lys Asp Lys Ala Thr Leu  408
1417 GAC TTT GAC CAC TAC ATC CGA TTC TGT TTT GTC AAG GAC AAG GCC ACA CTC
    Gln Ala Met Asp Glu Arg Leu Arg Lys Trp Lys Glu Leu Gln Pro Stop      423
1468 CAG GCC ATG GAT GAG AGA CTG CGC AAG TGG AAA GAG CTC CAA CCC TGA GGA
1519 GGCTGCCCTCAGCCCCACCTCGAACACAGGCCTCAGCTATGCCTTAGCACAGGGATGGCACTGGAGG
1586 GCCCAGCTGTGTGACTGCGCATGTTTCCAGAAAAGAGGCCATGTCTTGGGGGTTGAAGCCATCCTTT
1653 CCCAGTGTCCATCTGGACTATTGGGTTGGGGGCCAGTTCTGGGTCTCAGCCTACTCCTCTGTAGGTT
1720 GCCTGTAGGGTTTTGATTGTTTCTGGCCTCTCTGCCTGGGGCAGGAAAGGGTGGAATATCAGGCCCG
1787 GTACCACCTTAGCCCTGCCGAGGCTCTGTGGCTTCTCTACATCTTCTCCTGTGACCTCAGGATGTTG
1854 CTACTGTTCCTAATAAAGTTTTAAGTTATTAGGACCCTCAn
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Fig. 2. Nucleotide and deduced amino acid sequences of the rat kidney cytosolic cysteine conjugate β-lyase cDNA. *Underlined nucleotide sequences*, the two 70-nucleotide inverted repeats. *Bold type*, cryptic branch site and splice acceptor nucleotide sequences. The PLP binding site, Ser-Ala-Gly-Lys-Ser-Phe (residues 244–249), is also *underlined*.

cated the presence of a single 1269-nucleotide open reading frame initiated by an ATG codon embedded in a consensus translation start sequence. This open reading frame is preceded by a 243-nucleotide 5′ noncoding region containing in-frame stop codons and is followed by a 380-nucleotide 3′ noncoding region. Comparison of the complete sequence with the EMBL DNA sequence database indicated that the sequence was unique and had not been isolated previously. In the region of overlap between pβl-1.0 and pβl-2.0, only three nucleotide differences due to animal strain variation were observed. Of these differences only one was in the coding region, and it did not result in a change in amino acid sequence. The molecular mass of the β-lyase apoprotein deduced from the amino acid sequence was 47.8 kDa, in good agreement with the value obtained from polyacrylamide gel electrophoresis (48 kDa). The deduced amino acid composition agreed with that derived from complete acid hydrolysis of purified β-lyase enzyme (data not shown).[1] Examination of the deduced amino acid sequence for the presence of a consensus PLP binding site indicated that amino acids 244–249 have a strong resemblance to the PLP binding sites in other PLP-dependent enzymes (Fig. 3). Consideration of the combined coding capacity of pβl-1.0 and pβl-2.0 in terms of the deduced amino acid composition, polypeptide molecular weight, and the presence of a PLP binding site was taken as strong but not conclusive evidence that the cDNA sequence for rat kidney cytosolic β-lyase had been isolated. Also of interest, as shown in Fig. 2, is the presence of a 70-nucleotide inverted repeat in the 5′ end of the cDNA, the two elements of which are separated by 130 nucleotides of 5′ noncoding region. A cryptic branch site and splice acceptor have also been identified in this region. The role of this potential stem and loop structure in the synthesis or translation of β-lyase mRNA and the possibility of alternate splicing remain to be examined.

Expression of β-lyase mRNA. Northern blot analysis of rat liver and kidney mRNA using the radiolabeled 0.65-kb *Eco*RI-*Ava*II fragment from pβl-1.0 (Fig. 4) demonstrated that hybridizing mRNA sequences were present only in RNA from rat kidney and that the full length mRNA size was approximately 2.1 kb. This tissue specificity of mRNA expression was taken as further evidence that the β-lyase cDNA had been isolated. The result also suggests that the *in vivo* mature β-lyase mRNA may be longer than the combined sequence from pβl-1.0 and pβl-2.0. This would suggest either that the poly-

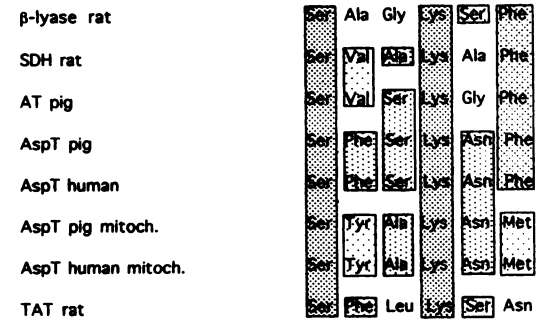

Fig. 3. Relationship between the putative β-lyase PLP binding site and the sites in other PLP-dependent enzymes. *Intensity of the box filling*, degree of amino acid conservation. *SDH*, serine dehydratase; *AT*, alanine aminotransferase; *AspT*, aspartate aminotransferase; *TAT*, tyrosine aminotransferase (EMBL database, 1992).

[1] J. Commandeur and N. Vermeulen, personal communication.

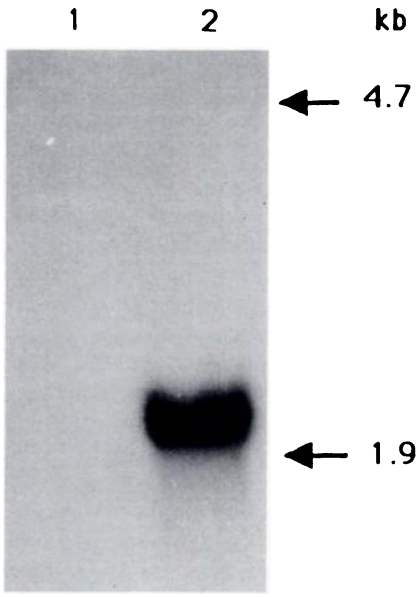

Fig. 4. Northern blot analysis of rat mRNA probed with the radiolabeled 0.65-kb *Eco*RI-*Ava*II cDNA fragment from pβl-1.0. *Lane 1*, 20 μg of liver total RNA; *lane 2*, 20 μg of kidney total RNA. *Arrows*, positions of the 28 S and 18 S ribosomal RNA markers.

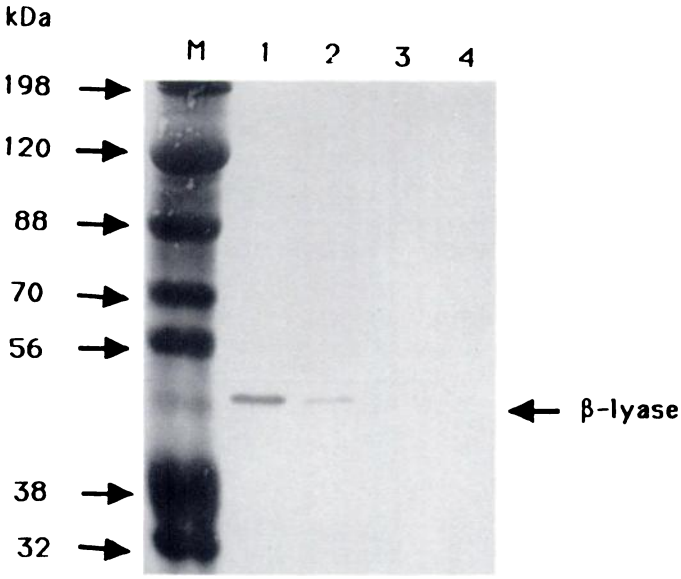

Fig. 5. Western blot analysis of cytosolic proteins (20 μg/lane) from monkey COS-1 cells transfected with rat cDNAs. *Lane 1*, purified rat kidney cytosolic β-lyase; *lane 2*, transfection with pUSβl-2.3 (sense orientation); *lane 3*, transfection with pUSβl-2.4 (antisense orientation); *lane 4*, control, no cDNA transfection. *Arrows*, position and size of the standard proteins (SDS-7B prestained markers; Sigma) (*lane M*).

adenosine tail is longer than 97 nucleotides *in vivo* and/or that the native 5′ noncoding end of the mRNA has not been isolated.

Heterologous expression of β-lyase cDNA. When cytosolic proteins from monkey COS-1 cells transfected with pUSβl-2.3 and pUSβl-2.4 were subjected to Western blot analysis (Fig. 5), a protein of 48 kDa reacting with the monospecific β-lyase antibody (7) was found only in those cells transfected with cDNA inserted into the vector in the sense orientation. Transfection with the antisense plasmid pUSβl-2.4 or with no cDNA did not result in the presence of a band on the Western blot. Analysis of glutamine transaminase K and β-lyase activ-

ities in extracts of control and transfected COS-1 cells (Fig. 6) demonstrated that, whereas a low level of activity could be detected in both untransfected cells and those transfected with the antisense plasmid pUSβl-2.4, the activity seen in cells transfected with pUSβl-2.3 was significantly (7–10-fold) higher. The level of enzyme activity was reproducible between experiments and was stable on storage. As shown in Fig. 6b, β-lyase activity in the extracts could be reduced by the PLP enzyme-specific inhibitor AOAA. Similar results for β-lyase expression (data not shown) were obtained using *S*-(1,2-dichlorovinyl)-L-cysteine as substrate. The levels of enzyme activity in the cytosolic extracts thus confirmed the result of the Western blot. The absence on the Western blot of β-lyase bands in lanes corresponding to the antisense or no-cDNA control transfections may have been due to either species specificity of the antibody or low sensitivity for small amounts of enzyme. The results of these transfection experiments were taken as conclusive proof that the cDNA for rat kidney cytosolic cysteine conjugate β-lyase had been isolated.

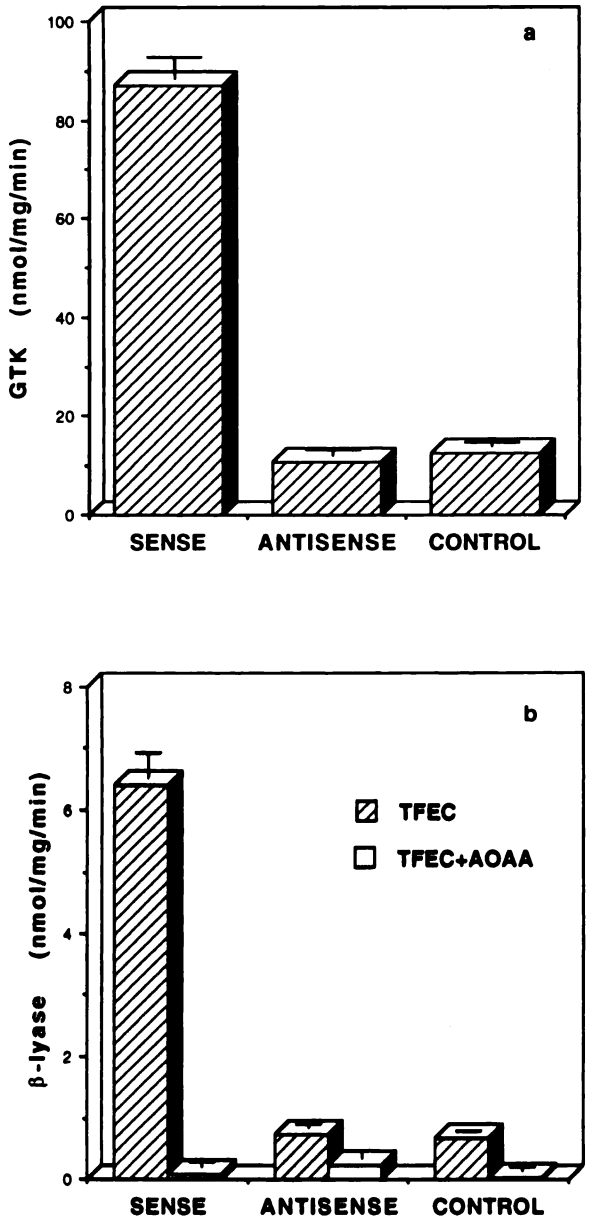

Fig. 6. Cytosolic enzyme activities in transfected COS-1 cells. a, Glutamine transaminase K activity; b, cysteine conjugate β-lyase activity with *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (*TFEC*) as substrate, with and without the inhibitor AOAA (0.1 mM). *Bars*, standard errors for replicate assays on different days (for glutamine transaminase K, eight experiments; for β-lyase, three experiments).

Discussion

We have presented here evidence for the successful isolation of a cDNA for rat kidney cytosolic β-lyase. Analysis of the nucleotide sequence indicates that the 5′ noncoding region of the mRNA may be involved in the regulation of β-lyase expression via the formation of a secondary loop structure that may affect ribosome progression. Also of note is the possibility of alternative pre-mRNA splicing at the cryptic splice acceptor site. Whether this can result in the addition of extra coding sequence to the amino terminus of β-lyase, perhaps for mitochondrial targeting, remains to be determined. Analysis of the open reading frame in the cDNA indicates that it can code for a protein of 47.8 kDa. This is in good agreement with the presently accepted size of β-lyase from rats and humans (8, 28), although previous estimates have varied from 43 kDa to 51 kDa. A hydrophilicity plot (data not shown) of the amino acid sequence confirms the soluble nature of the deduced protein, with no evidence for membrane-anchoring or -spanning regions. The presence of a region of amino acid sequence similar to the conserved PLP binding sites in other transaminases confirms that the open reading frame codes for a PLP-dependent enzyme. We have also identified other conserved amino acids, such as lysine (residue 267), tyrosine (residue 216), and arginine (residue 387), that are postulated to play a role in the mechanism of action of PLP-dependent enzymes.

Analysis of β-lyase mRNA synthesis demonstrates that sequences hybridizing to the probe can be detected only in kidney RNA and are not present in RNA from rat liver. Because it has been shown previously (28, 29) that substantial glutamine transaminase activity can be found in liver (glutamine transaminase L), this result indicates that there is little sequence similarity between the kidney and liver forms of this enzyme. The result also indicates that there is very little sequence similarity with kynureninase, the major form of liver β-lyase activity, and confirms our previous findings[3] that the kidney β-lyase-specific antibody does not detect cross-reacting proteins in the liver cytosol. We have recently shown (30) that the level of β-lyase mRNA in rat kidney can be modulated by treatment of rats with *S*-pentochlorobutadienyl-L-cysteine at a dose of 3 mg/kg. The physiological regulation of β-lyase mRNA transcription by hormonal or other endocrine signals will be the subject of further study.

The question of whether rat kidney mitochondrial β-lyase is a separately coded isoenzyme or a modified version of the cytoplasmic form has yet to be resolved. As indicated above, this modification might occur through alternative splicing of β-lyase pre-mRNA, which could produce both mitochondrial targeting and the observed differences in substrate specificity. However, other possibilities to explain the kinetic differences, including PLP or keto acid availability, might be postulated. Our preliminary Southern blot studies on the structure and size of the gene for β-lyase in rat genomic DNA (data not shown)

[3] M. Macfarlane, G. G. Gibson, P. S. Goldfarb, and E. A. Lock, unpublished observations.

indicate that the gene is unique, with a maximum size of about 7.5 kb. No evidence for a second, closely related, DNA sequence that might code for a separate mitochondrial form of the enzyme could be found.

Final confirmation that a cDNA for β-lyase had been isolated comes from the transfection studies. Clearly the cDNA can be expressed in tissue culture cells, because increased apoprotein and enzyme activity can be detected. The similar increases in both glutamine transaminase K and β-lyase activities indicate that the heterologously expressed enzyme closely resembles the native form and is functionally normal. The sensitivity to inhibition by AOAA reflects that observed previously (26, 28) and confirms that expression of the rat cDNA in a foreign environment does not alter enzyme function. The level of enzyme activity detected in the cytosol of transfected COS-1 cells is similar to that observed in whole-kidney cytosol. In view of the cell-type specificity of β-lyase expression in the kidney, that value is probably an underestimate of the intracellular level in the proximal tubule. This would indicate that the level of expression in transfected COS-1 cells is relatively low, compared with the *in vivo* situation. Whether expression of β-lyase can be increased by manipulation of the 5′ noncoding region of the cDNA, by use of a different expression vector, or by improvement of the transfection efficiency is presently being investigated. It is of note, however, that the level of β-lyase in transfected COS-1 cells is ~60-fold higher than that in pig kidney LLC-PK1 cells, a cell line that has been investigated as a possible *in vitro* toxicity test system for cysteine conjugates (16). The possibility of increasing the sensitivity of such cells to cysteine conjugate toxicity by the introduction of β-lyase cDNA is being actively pursued. The availability of a cDNA for β-lyase and the demonstration of its heterologous expression will also aid studies on the mechanism of action of the enzyme, the importance of subcellular localization, the toxicological relationship between cytosolic and mitochondrially located activities (31), and the analysis of species differences.

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