Molecular cloning and expression of a cDNA for human kidney cysteine conjugate β-lyase

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Abstract Kidney cysteine conjugate β-lyase (glutamine transaminase K, kyneurenine aminotransferase, EC 2.6.1.64) metabolises the cysteine conjugates of certain halogenated alkenes and alkanes to form reactive metabolites which can produce nephrotoxicicity and neurotoxicicity in experimental animals and man. Using a combination of hybridisation screening and PCR techniques we have isolated a full-length cDNA for human kidney cysteine conjugate β-lyase. Comparison of the deduced amino acid sequence with that of the rat enzyme indicated an 82% overall similarity, with 90% similarity around the pyridoxal phosphate binding site, many of the changes being conservative in nature. Expression of the cDNA in Cos-1 cells resulted in the production of a cytosolic enzyme which showed both cysteine conjugate β-lyase and glutamine transaminase K activity. Preliminary mapping of the gene for human cysteine conjugate β-lyase by PCR analysis of genomic DNA from human–rodent hybrid cells indicated that it is located on human chromosome 9.

Key words: Cysteine conjugate β-lyase; Glutamine transaminase K; Kyneurenine amino transferase; Nephrotoxicity; Neurotoxicity; Human chromosome 9

1. Introduction

Many halogenated xenobiotics are detoxified by conjugation to glutathione [1]. Subsequent metabolic processing yields the cysteine conjugate of the original chemical which may then serve as a substrate for enzymes that express β-lyase activity [1]. In rat and human kidney this activity is expressed by a specific form of cysteine conjugate β-lyase (glutamine transaminase K, GTK, EC 2.6.1.64) resulting in the production of metabolites which in the rat are toxic to the P3 segment of the renal proximal tubule [2]. In man, however, while abnormality of kidney function has been observed in workers exposed to the dry-cleaning fluid perchloroethylene [3], nephrotoxicity similar to that in the rat has not been reported following exposure to halogenated compounds which can be metabolised via the cysteine conjugate β-lyase pathway. Rather, excessive exposure in man to halogenated alkenes can result in neurotoxicity and neurodegeneration [4,5]. The reason for this difference in response between rat and man is not yet clear. It is known that cysteine conjugate β-lyase/GTK is present in brain [6] where its function may be related to ammonia metabolism [6]. However, the finding that the enzyme also possesses kyneurenine amino transferase (KAT) activity [7] raises the possibility that it may play a role in neurotransmission via modulation of the NMDA receptor [8]. Thus the neurotoxicity of some halogenated alkenes in man may be related to the specific localisation of cysteine conjugate β-lyase/GTK/KAT in specific neurons and the localised effects of toxic metabolite production. As an approach to studying this enzyme in man we now report the isolation and characterisation of a cDNA clone containing the complete coding region of human kidney cysteine conjugate β-lyase.

2. Materials and methods

2.1. Hybridisation screening of a human kidney cDNA library

A human kidney 5′-stretch cDNA library in λgt10 (HL1123a, Clontech Laboratories Inc., Palo Alto, USA) was screened by hybridisation with a probe derived from the full-length rat kidney cysteine conjugate β-lyase cDNA described previously [9]. A total of 1×10^6 plaques were screened under stringent conditions of hybridisation. Phage DNA from 9 positive plaques was digested with EcoRI and the cDNA insert sizes assessed by gel electrophoresis. The cDNA inserts were extracted and re-cloned in the plasmid vector pGEMzf(+) for sequencing and further analysis. DNA sequencing on both strands was performed by the dideoxy chain termination method using the Sequenase version 2.0 system (Amersham Life Science, Amersham, UK). The largest (1.5 kb) human β-lyase cDNA fragment containing an open reading frame was named phβl-1. Further screening of the library by hybridisation to phβl-1did not produce a full-length cDNA clone (expected minimum size 1.7 kb) or one containing the 5′ end of the cDNA. Interestingly, one of the clones from the original screening contained an intronic sequence prior to nucleotide 231 of the phβl-1 cDNA sequence, with a splice acceptor site at that position.

2.2. 5′-RACE amplification and cloning

The missing 5′ end of the human β-lyase cDNA sequence was isolated using the 5′-rapid amplification of cDNA ends (5′-RACE) procedure [10] with human kidney 5′-RACE-ready cDNA (Clontech Laboratories Inc.). A primary PCR amplification was performed using a 3′ oligonucleotide primer corresponding to nucleotides 468 to 483 of phβl-1, and the 5′ anchor primer provided with the Clontech Inc kit. After 30 rounds of amplification using the manufacturer's recommended procedure, an aliquot of the primary reaction was used in a second PCR amplification using a nested 3′ oligonucleotide primer corresponding to nucleotides 358 to 377 of phβl-1 and the anchor primer provided with the kit. PCR products were sized by electrophoresis, extracted, and subcloned into the TA Vector pCRII (Invitrogen R & D Systems Europe Ltd, Abingdon, UK) for DNA sequencing. The longest overlapping human β-lyase cDNA fragment (0.4 kb) which included the putative start of the coding sequence was named phβl-2.

2.3. RT-PCR amplification of full-length human kidney β-lyase cDNA

Total RNA was extracted from a human kidney sample using RNAzol B (Biogenesis Ltd, Poole, UK), as per the manufacturer's instructions. First strand cDNA synthesis was performed with reverse transcriptase (Life Technologies, Paisley, UK), according to the manufacturer's recommended procedure, using a 3′ oligonucleotide primer corresponding to nucleotides 1,480 to 1,499 of phβl-1. PCR amplification of the first strand product was performed using a second, nested 3′ primer corresponding to nucleotides 1,338 to 1,357 and a 5′ primer corresponding to nucleotides 30 to 45 of phβl-2. The 1.3 kb PCR

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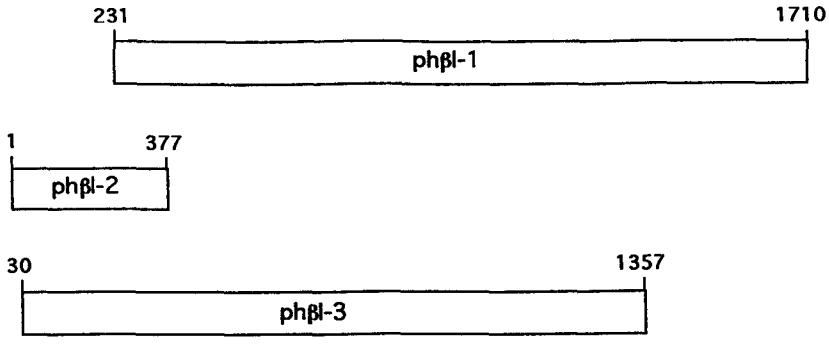
(a)



(b)

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1                                                    GGGGTGAAGCGGCCCAGGTGAGGCTCGCC      HUMAN

30 ATG GCC AAA CAG CTG CAG GCC CGA AGG CTA GAC GGG ATC GAC TAC AAC CCC TGG GTG GAG TTT GTG AAA      HUMAN
   Met Ala Lys Gln Leu Gln Ala Arg Arg Leu Asp Gly Ile Asp Tyr Asn Pro Trp Val Glu Phe Val Lys 23
    •  Thr  •  Arg  •   •   •   •   •   •   •   •   •   •   •  Gln  •  Leu  •   •   •   •  Gly  •      RAT

99 CTG GCC AGT GAG CAT GAC GTC GTG AAC TTG GGC CAG GGC TTC CCG GAT TTC CCA CCA CCA GAC TTT GCC      HUMAN
   Leu Ala Ser Glu His Asp Val Val Asn Leu Gly Gln Gly Phe Pro Asp Phe Pro Pro Pro Asp Phe Ala 46
    •  Thr Lys  •  Tyr  •   •   •   •   •   •   •   •   •   •   •  Ser  •   •   •   •   •   •      RAT

168 GTG GAA GCC TTT CAG CAC GCT GTC AGT GGA GAC TTC ATG CTT AAC CAG TAC ACC AAG ACA TTT GGT TAC      HUMAN
   Val Glu Ala Phe Gln His Ala Val Ser Gly Asp Phe Met Leu Asn Gln Tyr Thr Lys Thr Phe Gly Tyr 69
   Thr Gln  •   •   •  Gln  •  Thr  •   •  Asn  •   •   •   •   •   •  Arg Ala  •   •   •      RAT

237 CCA CCA CTG ACG AAG ATC CTG GCA AGT TTC TTT GGG GAG CTG CTG GGT CAG GAG ATA GAC CCG CTC AGG      HUMAN
   Pro Pro Leu Thr Lys Ile Leu Ala Ser Phe Phe Gly Glu Leu Leu Gly Gln Glu Ile Asp Pro Leu Arg 92
    •   •   •   •  Asn Val  •   •   •   •   •   •  Lys  •   •   •   •   •  Met  •   •   •  Thr      RAT

308 AAT GTG CTG GTG ACT GTT GGT GGC TAT GGG GCC CTG TTC ACA GCC TTC CAG GCC CTG GTG GAC GAA GGA      HUMAN
   Asn Val Leu Val Thr Val Gly Gly Tyr Gly Ala Leu Phe Thr Ala Phe Gln Ala Leu Val Asp Glu Gly 115
    •   •   •   •   •   •  Ala  •   •   •   •   •  Arg  •   •   •   •   •   •   •   •      RAT

375 GAC GAG GTC ATC ATC ATC GAA CCC TTT TTT GAC TGC TAC GAG CCC ATG ACA ATG ATG GCA GGG GGT CGT      HUMAN
   Asp Glu Val Ile Ile Ile Glu Pro Phe Phe Asp Cys Tyr Glu Pro Met Thr Met Met Ala Gly Gly Arg 138
    •   •   •   •   •  Met  •   •  Ala  •   •   •   •   •   •   •   •   •   •   •  Cys      RAT

444 CCT GTG TTT GTG TCC CTG AAG CCG GGT CCC ATC CAG AAT GGA GAA CTG GGT TCC AGC AGC AAC TGG CAG      HUMAN
   Pro Val Phe Val Ser Leu Lys Pro Gly Pro Ile Gln Asn Gly Glu Leu Gly Ser Ser Ser Asn Trp Gln 161
    •   •   •   •  Thr  •   •   •  Ser  •  Ala Pro Lys  •  Lys  •   •  Ala  •  Asn Asp  •   •      RAT

513 CTG GAC CCC ATG GAG CTG GCC GGC AAA TTC ACA TCA CGC ACC AAA GCC CTG GTC CTC AAC ACC CCC AAC      HUMAN
   Leu Asp Pro Met Glu Leu Ala Gly Lys Phe Thr Ser Arg Thr Lys Ala Leu Val Leu Asn Thr Pro Asn 184
    •   •   •  Ala  •   •   •  Ser  •   •   •  Pro  •   •   •  Ile  •   •   •   •   •   •      RAT

582 AAC CCC CTG GGC AAG GTG TTC TCC AGG GAA GAG CTG GAG CTG GTG GCC AGC CTT TGC CAG CAG CAT GAC      HUMAN
   Asn Pro Leu Gly Lys Val Phe Ser Arg Glu Glu Leu Glu Leu Val Ala Ser Leu Cys Gln Gln His Asp 207
    •   •   •   •   •   •   •   •   •  Met  •   •   •   •   •   •  Asn  •   •   •   •   •      RAT

651 GTG GTG TGT ATC ACT GAT GAA GTC TAC CAG TGG ATG GTC TAC GAC GGG CAC CAG CAC ATC AGC ATT GCC      HUMAN
   Val Val Cys Ile Thr Asp Glu Val Tyr Gln Trp Met Val Tyr Asp Gly His Gln His Ile Ser Ile Ala 230
    •   •   •   •  Ser  •   •   •   •   •   •  Leu  •   •   •   •   •   •   •  Val  •   •   •      RAT

720 AGC CTC CCT GGC ATG TGG GAA CGG ACC CTG ACC ATC GGC AGC GCC GGC AAG ACC TTC AGC GCC ACT GGC      HUMAN
   Ser Leu Pro Gly Met Trp Glu Arg Thr Leu Thr Ile Gly Ser Ala Gly Lys Thr Phe Ser Ala Thr Gly 253
    •   •   •   •   •   •  Asp  •   •   •   •   •   •   •   •   •   •  Ser  •   •   •   •      RAT

789 TGG AAG GTG GGC TGG GTC CTG GGT CCA GAT CAC ATC ATG AAG CAC CTG CGG ACC GTG CAC CAG AAC TCC      HUMAN
   Trp Lys Val Gly Trp Val Leu Gly Pro Asp His Ile Met Lys His Leu Arg Thr Val His Gln Asn Ser 276
    •   •   •   •   •   •  Met  •   •   •  Asn  •   •   •   •   •   •   •   •   •   •   •      RAT

858 GTC TTC CAC TGC CCC ACG CAG AGC CAG GCT GCA GTA GCC GAG AGC TTT GAA CGG GAG CAG CTG CTC TTC      HUMAN
   Val Phe His Cys Pro Thr Gln Ser Gln Ala Ala Val Ala Glu Ser Phe Glu Arg Glu Gln Leu Leu Phe 299
    Ile  •   •   •   •   •   •  Ala  •   •   •   •   •  Gln Cys  •   •   •   •   •  Gln His  •      RAT

927 CGC CAA CCC AGC AGC TAC TTT GTG CAG TTC CCG CAG GCC ATG CAG CGC TGC CGT GAC CAC ATG ATA CGT      HUMAN
   Arg Gln Pro Ser Ser Tyr Phe Val Gln Phe Pro Gln Ala Met Gln Arg Cys Arg Asp His Met Ile Arg 322
   Gly  •   •   •   •   •   •  Leu  •  Leu  •   •   •   •  Glu Leu Asn  •   •   •   •   •      RAT

996 AGC CTA CAG TCA GTG GGC CTG AAG CCC ATC ATC CCT CAG GGC AGC TAC TTC CTC ATC ACA GAC ATC TCA      HUMAN
   Ser Leu Gln Ser Val Gly Leu Lys Pro Ile Ile Pro Gln Gly Ser Tyr Phe Leu Ile Thr Asp Ile Ser 345
    •   •   •   •   •   •   •  Leu Trp  •  Ser  •   •   •   •   •   •  Ala  •   •   •      RAT

1065 GAC TTC AAG AGG AAG ATG CCT GAC TTG CCT GGA GCT GTG GAT GAG CCC TAT GAC AGA CGC TTC GTC AAG      HUMAN
   Asp Phe Lys Arg Lys Met Pro Asp Leu Pro Gly Ala Val Asp Glu Pro Tyr Asp Arg Arg Phe Val Lys 368
    •   •   •  Ser  •   •   •   •   •   •   •  Glu  •   •   •   •   •   •  Ala  •      RAT

1134 TGG ATG ATC AAG AAC AAG GGC TTG GTG GCC ATC CCT GTC TCC ATC TTC TAT AGT GTG CCA CAT CAG AAG      HUMAN
   Trp Met Ile Lys Asn Lys Gly Leu Val Ala Ile Pro Val Ser Ile Phe Tyr Ser Val Pro His Gln Lys 391
    •   •   •   •   •  Met  •   •   •  Gly  •   •   •   •  Thr  •  Phe  •  Arg  •   •   •   •      RAT

1203 CAC TTT GAC CAC TAT ATC CGC TTC TGT TTT GTG AAG GAT GAA GCC ACG CTC CAG GCC ATG GAC GAG AAG      HUMAN
   His Phe Asp His Tyr Ile Arg Phe Cys Phe Val Lys Asp Glu Ala Thr Leu Gln Ala Met Asp Glu Lys 414
   Asp  •   •   •   •   •   •   •   •   •   •   •   •  Lys  •   •   •   •   •   •   •   •  Arg      RAT

1272 CTG CGG AAG TGG AAG GTG GAA CTC TAG CCCTGAAGTCACGCCTTGGCCCTGACATCCCCACATGCCCGCAGAGATCCTCTTT      HUMAN
   Leu Arg Lys Trp Lys Val Glu Leu Stop                                                        422
    •   •   •   •   •  Glu Leu Gln Pro Stop                                                   423      RAT

1354 GAGTGTCTGTCTTTGTCCAGGTTTCAGACATTTCTAGGTTGGGGAAGATGCTATTGGGAAACCTCTTCTCCGTGACACAGAATGTTCTGGG      HUMAN
1445 TGGGAGCCGCCCTTCTTCATCTTAGAGAACCAAGTGCCTCCTGTCTGAAAGGTGAGGGTGGCCTGACCTGGGCCTCTCCCTGCCCCTCCAT      HUMAN
1536 AGGTGGGTTTGTAGGGTCTTGTGTTGCTTCTGGTCTCTCCAGGCTTGGCTGAGACGGACGGTAGACTTCCACCATGTACCGATCACATCCC      HUMAN
1627 AGCTCTGCATGGCCCCTGCTAAGGCTCAGGTATAACCTCACCTTCCCTGGCTCATCTTGGCCTTGGGGAGTTGCCTTTAGGTTG   1710      HUMAN
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product was purified by gel electrophoresis, extracted, cloned into pCR II for DNA sequencing and named phβl-3.

2.4. Expression of the human β-lyase cDNA in Cos-1 cells

The full-length human β-lyase cDNA insert from phβl-3 was excised with *Eco*RI and inserted into the expression vector pUS1000 [9]. The insert orientation relative to the CMV promoter of transcription in the vector was confirmed by digestion with *Apa*I (data not shown). Transfection of plasmid DNA into Cos-1 cells, preparation of cellular extracts, and measurement of both glutamine transaminase K and β-lyase enzyme activities was as described before [9].

2.5. Chromosome assignment of the human kidney β-lyase gene

Genomic DNA from a series of rodent-human somatic cell hybrids (a gift from Dr. Lesley Rooke, Imperial Cancer Research Fund, London, UK) was analysed for the presence of β-lyase sequences using a 3′ primer corresponding to nucleotides 468 to 483 of the phβl-1 sequence, and a 5′ primer corresponding to 16 nucleotides of intronic sequence 426 bases upstream of the splice site at nucleotide 231 of the sequence. PCR products were run on an agarose gel to separate the human-specific product from that of the rodent background. The authenticity of the PCR products was confirmed by Southern blotting and hybridisation to the full-length β-lyase cDNA.

3. Results and discussion

A composite sequence for human kidney β-lyase DNA was assembled from the overlapping clones phβl-1 and phβl-2 (Fig. 1a) and confirmed by sequencing of phβl-3. The open reading frame (Fig. 1b) codes for a polypeptide of 422 amino acids which has an overall similarity of 82% with rat kidney cytosolic β-lyase and a similarity of 90% in the region (amino acids 185 to 287) spanning the putative pyridoxal phosphate (PLP) binding site. The deduced subunit molecular weight of the human kidney β-lyase apoprotein is 47.9 kDa. Of the 78 amino acid sequence differences between the rat and human orthologues of this protein, 23 are conservative in nature.

The insert cDNA sequence from phβl-3 was recloned into a mammalian expression vector and transfected into Cos-1 cells. As shown in Fig. 2, a significant increase in both glutamine transaminase K and cysteine conjugate β-lyase activity was seen in cells transfected with the cDNA in the sense orientation relative to the promoter of mRNA synthesis. A preliminary estimate (data not shown) of the Km for the human β-lyase activity expressed in Cos-1 cells, using *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine as substrate, indicated that at 6.5 mM it corresponded closely with that measured for the rat enzyme expressed in the same cells (5.2 mM). This finding suggests that any species differences in kidney toxicity of halogenated alkenes between rat and man must be attributable to the amount of cysteine conjugate β-lyase enzyme in kidney proximal tubule cells, or other factors such as the uptake of cysteine conjugates into the cells, rather than to marked differences in kidney β-lyase enzyme kinetics or substrate specificity.

Analysis of genomic DNA from somatic cell hybrids for the presence of human kidney β-lyase sequences by PCR indicated that whereas a 0.7 kb band of rodent origin was obtained from all samples, only hybrid GM10611 which contained human chromosome 9 as the sole human genetic contribution, gave an additional PCR band of 1.0 kb. This latter band gave a strong signal when hybridised to the radiolabelled cDNA insert from phβl-3 (Fig. 3). An identical band was observed when total human genomic DNA was subjected to PCR using the same primers (data not shown) Experiments are in progress to confirm the presence of the human kidney β-lyase gene locus on chromosome 9 and to map its location. In conclusion, the availability of a cDNA for human kidney cysteine conjugate β-lyase/GTK/KAT will enable further study on β-lyase mediated toxicity of halogenated alkenes in man and the role of KAT in the modulation of neurotransmission via the NMDA receptor.

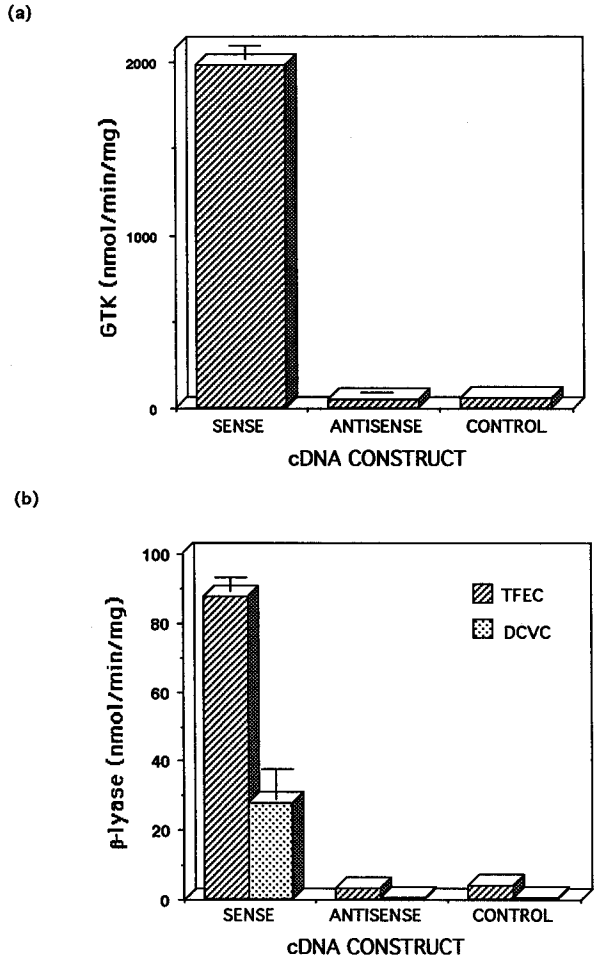

Fig. 2. Enzyme activity in cytosolic extracts of Cos-1 cells transfected with the human β-lyase cDNA (phβl-3). (a) Glutamine transaminase K, (b) Cysteine conjugate β-lyase activity using *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-(1.1.2.2-tetrafluoroethyl)-L-cysteine (TFEC) as substrates.

←

Fig. 1. Human kidney cysteine conjugate β-lyase cDNA. (a) Overlapping human β-lyase cDNAs referred to in the text, (b) sequence composite of the overlapping 5′ and 3′ cDNAs from phβl-2 and phβl-1, confirmed between nucleotides 30 and 1357 by subsequent sequencing of the cDNA insert from phβl-3. The primer sequences used to produce the full-length cDNA, phβl-3, by RT-PCR are underlined. The concensus pyridoxal phosphate binding site is boxed. Amino acid differences from rat kidney cytosolic cysteine conjugate β-lyase [9] are shown below the human sequence; •, indicates amino acid identity.

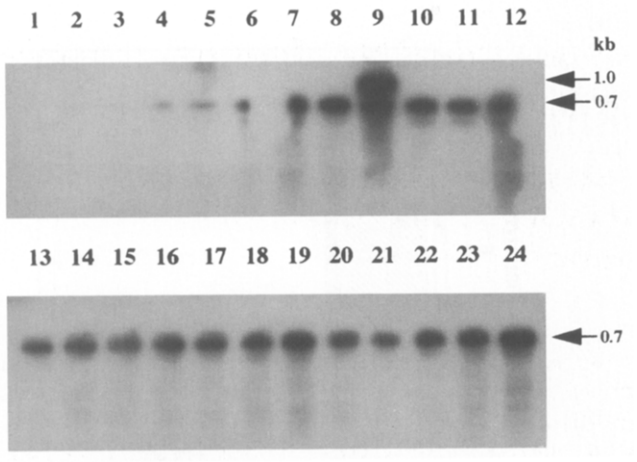

Fig. 3. Preliminary chromosomal assignment of human β-lyase. PCR products from amplification of rodent-human hybrid cell DNA using β-lyase specific primers were electrophoresed on an agarose gel, transferred to nylon membrane and hybridised to the human β-lyase cDNA probe (phβl-3). A human-specific band is seen only in lane 9 corresponding to the human chromosome 9-containing hybrid, GM10611.

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