Characterization of cDNA Clones Encoding Rabbit and Human Serum Paraoxonase: The Mature Protein Retains Its Signal Sequence[†,‡]

Christopher Hassett,[§] Rebecca J. Richter,[∥,⊥] Richard Humbert,[∥,⊥,#] Christine Chapline,[○] John W. Crabb,[○] Curtis J. Omiecinski,[§,#] and Clement E. Furlong[*,∥,⊥,#]

Department of Environmental Health, School of Public Health and Community Medicine, Department of Genetics, Division of Medical Genetics, School of Medicine, and Dana Program in Ecogenetics, University of Washington, Seattle, Washington 98195, and The W. Alton Jones Cell Science Center, Lake Placid, New York 12946

Received April 5, 1991; Revised Manuscript Received July 1, 1991

ABSTRACT: Serum paraoxonase hydrolyzes the toxic metabolites of a variety of organophosphorus insecticides. High serum paraoxonase levels appear to protect against the neurotoxic effects of organophosphorus substrates of this enzyme [Costa et al. (1990) *Toxicol. Appl. Pharmacol. 103*, 66–76]. The amino acid sequence accounting for 42% of rabbit paraoxonase was determined by (1) gas-phase sequencing of the intact protein and (2) peptide fragments from lysine and arginine digests. From these data, two oligonucleotide probes were synthesized and used to screen a rabbit liver cDNA library. A clone was isolated and sequenced, and contained a 1294-bp insert encoding an open reading frame of 359 amino acids. Northern blot hybridization with RNA isolated from various rabbit tissues indicated that paraoxonase mRNA is synthesized predominately, if not exclusively, in the liver. Southern blot experiments suggested that rabbit paraoxonase is coded by a single gene and is not a family member of closely related genes. Human paraoxonase clones were isolated from a liver cDNA library by using the rabbit cDNA as a hybridization probe. Inserts from three of the longest clones were sequenced, and one full-length clone contained an open reading frame encoding 355 amino acids, four less than the rabbit paraoxonase protein. Each of the human clones appeared to be polyadenylated at a different site, consistent with the absence of the canonical polyadenylation signal sequence. Of potential significance with respect to the paraoxonase polymorphism, the derived amino acid sequence from one of the partial human cDNA clones differed at two positions from the full-length clone. Amino-terminal sequences derived from purified rabbit and human paraoxonase proteins suggested that the signal sequence is retained, with the exception of the initiator methionine residue [Furlong et al. (1991) *Biochemistry* (preceding paper in this issue)]. Characterization of the rabbit and human paraoxonase cDNA clones confirms that the signal sequences are not processed, except for the N-terminal methionine residue. The rabbit and human cDNA clones demonstrate striking nucleotide and deduced amino acid similarities (greater than 85%), suggesting an important metabolic role and constraints on the evolution of this protein.

Polymorphic genes encoding human biotransformation enzymes which result in variable rates of metabolism of certain drugs and xenobiotics have been identified. Examples of polymorphic enzymes include cytochrome P450 isozymes which hydroxylate the antihypertensive drug debrisoquine and the anticonvulsant mephenytoin (Kalow, 1987), an *N*-acetyltransferase which metabolizes arylamine and hydrazine compounds (Weber, 1987), the glutathione transferase μ isozyme which conjugates glutathione to electrophilic compounds (Seidegard et al., 1988), and serum cholinesterase which metabolizes the anesthetic succinylcholine (Brown et al., 1981).

Paraoxonase, like serum cholinesterase, demonstrates a substrate-dependent polymorphism in human populations [see Geldmacher-von Malinckrodt and Diepgen (1988) for review]. Some paraoxonase substrates, such as phenylacetate and chlorpyrifos oxon, are hydrolyzed with the same turnover number by both allelic forms of the enzyme, whereas paraoxon is hydrolyzed slowly by one allelic form and rapidly by the other (LaDu et al., 1986; Furlong et al., 1989, Smolen et al., 1991). It has been suggested that high serum levels of paraoxonase may be protective against poisoning by organophosphate substrates of this enzyme (Omenn, 1987; LaDu & Eckerson, 1984; Furlong et al., 1988, 1989). Experiments with animal systems support this hypothesis (Main, 1956; Costa et al., 1990).

One of our aims is to determine the molecular basis for the paraoxonase polymorphism observed in humans. Because rabbits have very high levels of paraoxonase (Costa et al., 1987), we first purified and partially sequenced rabbit paraoxonase (Furlong et al., 1991). The protein sequence data were used to design oligonucleotide probes which permitted the isolation of a rabbit cDNA. The rabbit clone was subsequently used as a probe to isolate human paraoxonase cDNAs. This report describes these cloning experiments and presents the structural characterization of rabbit and human paraoxonase.

Materials and Methods

Protein Purification. Paraoxonase was purified through the DEAE-cellulose fractionation step as described previously (Furlong et al., 1991). Paraoxonase was further purified by

[†] Supported by NIH Grants GM-15253 (Center Grant), ES-05194 (to C.E.F.), GM-32281, ES-04696, and ES-04978 (to C.J.O.), and EY-06603 and CA-37589 (to J.W.C.) and by a grant from the Charles A. Dana Foundation.

[‡] The nucleotide sequences in this paper have been submitted to the GenBank/EMBL Data Bank under Accession Numbers M63011, M63012, M63013, and M63014.

* Address correspondence to this author at the Department of Genetics, SK-50, University of Washington, Seattle, WA 98195.

[§] Department of Environmental Health, School of Public Health and Community Medicine, University of Washington.

[∥] Department of Genetics, University of Washington.

[⊥] Division of Medical Genetics, School of Medicine, University of Washington.

[#] Dana Program in Ecogenetics, University of Washington.

[○] The W. Alton Jones Cell Science Center.

0006-2960/91/0430-10141$02.50/0

high-performance chromatography on a 5-μm Vydac C_{18} column.

Protein Digests and Peptide Purification. Paraoxonase was pyridylethylated and succinylated as described by Crabb et al. (1988). Pyridylethylated HPLC-purified paraoxonase was fragmented at lysyl residues with endoproteinase Lys-C (Crabb et al., 1986). Pyridylethylated, succinylated DEAE-purified paraoxonase was cleaved at arginyl residues with trypsin (Crabb et al., 1986). Peptides were purified by narrow-bore reverse-phase HPLC using an Applied Biosystems Model 130 HPLC system.

Protein/Peptide Sequencing. Intact paraoxonase and fractionated peptides were sequenced with an Applied Biosystems gas-phase sequencer (Model 470) and an on-line phenylthiohydantoin amino acid analyzer (Model 120) using the 03RPTH sequencer program and the manufacturer's recommended program and solvents for the PTH analyzer (Crabb et al., 1988). Phenylthiocarbamyl (PTC) amino acid analysis was performed according to West and Crabb (1990) using an Applied Biosystems automatic system (Models 420H/130/920).

Oligonucleotide Synthesis. DNA probes and primers were synthesized with an Applied Biosystems DNA synthesizer using phosphoramidite chemistry.

Library Screening and Subcloning. (A) Rabbit. A λgt11 cDNA library constructed from the pooled livers of male and female New Zealand white rabbits was obtained from Clontech (Palo Alto, CA). The library was screened as described previously (Hassett & Omiecinski, 1987; Hassett et al., 1989) by using the oligonucleotide probes described under Results. The rabbit insert was subcloned into pUC13 with *Escherichia coli* DH5α as host (BRL, Gaithersburg, MD).

(B) Human. A λgt11 human liver cDNA library derived from an adult female was also obtained from Clontech. This library was screened with the 952-bp *Bst*XI restriction fragment from rabbit paraoxonase cDNA. Inserts were subcloned in pSK(+) Bluescript plasmid vector and used to transform XL1-Blue cells (Stratagene, La Jolla, CA).

DNA Sequence Analysis. (A) Rabbit. The insert cDNA was sequenced directly in pUC13 by using the forward and reverse universal plasmid primers and the 17-base paraoxonase-specific primer. Insert DNA was subcloned into the vector in both orientations, relative to the multiple cloning site. Unique *Bam*HI and *Hin*dIII restriction sites in the insert DNA and in the vector cloning region allowed deletion constructs to be engineered which facilitated sequence analysis of both strands from the universal primers. Each DNA strand was sequenced at least three times.

(B) Human. The nucleotide sequence of the human DNA clones was determined in the plasmid by using primers complementary to the T3 and T7 promoters of the vector. Additionally, 11 oligonucleotide primers were synthesized for sequencing on the basis of the derived human and rabbit sequences.

DNA was sequenced by using the dideoxy termination method (Sanger et al., 1977) and Sequenase Version 2.0 (U.S. Biochemicals, Cleveland, OH), as described previously (Hassett & Omiecinski, 1990). Sequence analysis and database searches were performed with either GENEPRO (Riverside Scientific Enterprises, Bainbridge Island, WA) or Intelligenetics (Palo Alto, CA) software and databases, which included GenBank and EMBL DNA databases and the PIR protein database.

Northern Blot Analysis. RNA was isolated (Omiecinski et al., 1985) from the liver, lung, kidney, and testes of two New Zealand White rabbits. Twenty micrograms of total RNA from each organ was size-fractionated in a 6% formaldehyde/1.15% agarose gel and transferred to a GeneScreen Plus nylon membrane as per the manufacturer's directions (Du Pont/NEN, Boston, MA). A 438-bp *Bam*HI fragment isolated from the 3′ region of the rabbit paraoxonase cDNA was radiolabeled (Hassett & Omiecinski, 1990) and used as a hybridization probe. The membrane was washed at 45 °C in 0.1× SSC/0.1% SDS (1× SSC: 1.5 M NaCl, 0.15 M sodium citrate) and exposed overnight to X-ray film in the presence of two intensifying screens. The size of the in vivo RNA transcript was estimated by using an RNA ladder standard (BRL, Gaithersburg, MD).

Southern Blot Analysis. Peripheral white blood cell DNA was extracted and isolated from 5 mL of whole blood withdrawn from a single rabbit and processed essentially as described (Blin & Stafford, 1976). Twenty micrograms of DNA was digested with *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, or *Xho*I, size-fractionated on a 0.85% agarose gel, and transferred to a nylon membrane as described previously (Hassett et al., 1989). The Southern blot was incubated with a radiolabeled 419-bp fragment isolated from the rabbit paraoxonase cDNA (*Eco*RI/*Bam*HI fragment). The blot was washed in a final solution of 0.1× SSC/0.1% SDS at 50 °C and exposed to X-ray film for 6 days in the presence of two intensifying screens. Drigest III (Pharmacia, Piscataway, NJ) was employed as a molecular size standard.

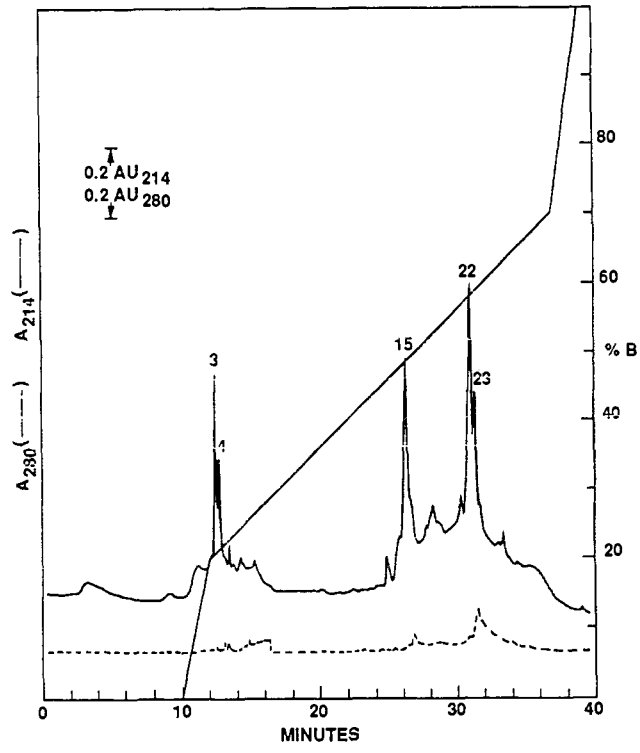

FIGURE 1: Reverse-phase HPLC purification of rabbit paraoxonase. Paraoxonase was purified through the DEAE-Trisacryl M step (87 μg) and fractionated by reverse-phase HPLC on a 5-μm Vydac C18 column. Solvent A was 0.1% trifluoroacetic acid in H_2O, and solvent B was 84% acetonitrile containing 0.09% trifluoroacetic acid.

RESULTS

Purification of Rabbit Paraoxonase. Rabbit paraoxonase, purified through the DEAE-Trisacryl M step as described in the preceding paper, was further purified by high-performance reverse-phase liquid chromatography (Figure 1). Peak 15 contained only homogeneous paraoxonase while peaks 22 and 23 contained both paraoxonase and apolipoprotein A1 (de-

Table I: Rabbit Paraoxonase Protein/Peptide Sequence[a]

protein/peptide	sequence	position
amino terminus	AKLTALTLLGLGLALFDGQKS-FQT	2–26
lysyl peptides	S-FQTRFNVHREVTPVELPN-NL	22–44
	L/PSVNDIVAVGPEHFYA	163–180
	IHVYEK	245–250
	SLDFNTLVDNISVDPV	261–276
	NPPASEVLRIQDIL	298–311
	ALY-ELSQAN	350–359
arginyl peptides	FNVHR	28–32
	VVAEGFDFANGINISPDGKYVYIAELLAHKI-VY	215–248
	IFYYDPKNPPASEVLR	291–306
	IQDILSKEPKV-VAYAE	307–323

[a] Cycles where no residue was assigned are shown as dashes. Tentative assignments are underlined. The single assignment which differed from the deduced sequence shown in Figure 3 is italicized. Sequences that were used for probe design are shown in bold type.

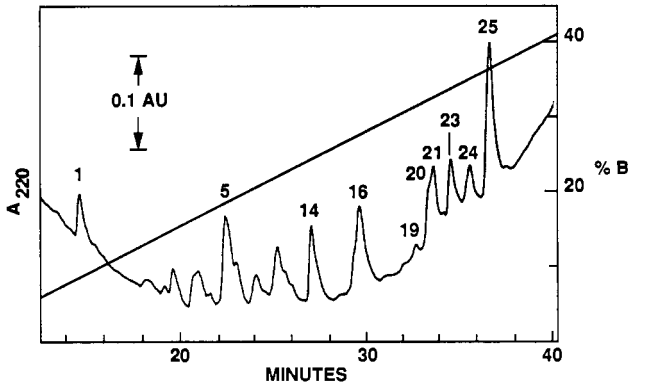

FIGURE 2: HPLC purification of lysine peptides from paraoxonase. Peptides resulting from the cleavage of pyridylethylated paraoxonase (~78 μg) with endopeptidase Lys-C were purified by narrow-bore reverse-phase HPLC with a linear gradient of buffer A (0.1% TFA) to 50% buffer B (85% acetonitrile, 0.005% TFA) run over 40 min.

termined by sequence analysis).

Peptide Generation. RP-HPLC-purified pyridylethylated paraoxonase was digested with endoproteinase Lys-C, and the resulting peptides were purified by narrow-bore RP-HPLC (Figure 2) as described under Materials and Methods. In addition, paraoxonase (200 μg) purified by DEAE-Trisacryl M chromatography (preceding paper) was succinylated and then subjected to arginyl-specific cleavage with trypsin, and the resulting peptides were purified by narrow-bore RP-HPLC. Since the intact paraoxonase had not been RP-HPLC-purified prior to digestion, peptides from apolipoprotein A1 were also identified by sequence analysis (data not shown).

Gas-Phase Protein Sequencing. Unequivocal amino acid sequencing data were obtained from rabbit paraoxonase, four arginyl peptides, and six lysyl peptides (Table I). Residues 16–20 of the amino terminus of rabbit paraoxonase (Phe-AspGlyGlnLys) allowed the design of the 15-base oligonucleotide 5′-TTY GAY GGN CAR AAR-3′[1] with 64-fold redundancy (Table I, Figure 3). A 17-base oligomer (5′-GGR TCR TAR TAR AAD AT-3′) with 48-fold redundancy was designed as a complement to the nucleotide sequence encoding residues 1–6 of the arginine peptide IFYYDP (Table I, Figure 3). The wobble position of the proline codon was not used in the design of this oligomer.

Isolation and Sequence of Rabbit Paraoxonase cDNA. Approximately 400 000 plaques were screened from the rabbit cDNA library with the 15-base probe, yielding 35 potentially positive autoradiographic signals. Twenty-four of these phage were rescreened with the 17-base probe, and a plaque which hybridized to this oligomer was plated at a low density. This cDNA clone was screened a final time with the 15-base probe and once again showed positive hybridization. Phage DNA was purified, digested with *Eco*RI, and subcloned into pUC13.

DNA sequence analysis of the rabbit paraoxonase cDNA (RabPON, GenBank Assession Number M63011) identified an insert of 1294 bp, containing the entire protein coding sequence (Figure 3). Fifty-one nucleotides precede the methionine initiation codon, ATG, which begins an open reading frame coding for 359 amino acids. An amber stop codon, TAG, is followed by an additional 163 nucleotides of 3′ noncoding sequence. The ATG at position 1 is the likely start position since there is a stop codon beginning 15 nucleotides upstream from this ATG. No poly(A) signal or sequence was identified in this clone. The fragment containing this information was presumably deleted during library construction since multiple efforts to isolate this region from the original λ phage were unsuccessful.

Comparison between the Derived and Determined Amino Acid Sequences. The deduced amino acid sequence is shown in Figure 3. The sequence verified by gas-phase amino acid sequencing is presented in Table I. The verified protein sequence totaled 151 residues or about 42% of the rabbit paraoxonase protein sequence deduced from the cDNA clone. The one difference observed (i.e., Ile for Leu at position 164) may simply reflect a variant in the rabbit population.

Analysis of Paraoxonase mRNA Expression in Rabbit Tissues. Northern blot analysis performed with RNA isolated from four rabbit organs revealed the presence of paraoxonase-specific RNA in liver only. RNA isolated from lung, kidney, or testes did not hybridize to the paraoxonase cDNA probe (Figure 4). On the basis of this Northern blot and linear regression analysis, the molecular size estimate for the in vivo liver mRNA transcript was approximately 1400 bases. Pretreatment of animals with phenobarbital 16 h prior to sacrifice did not influence steady-state mRNA levels of liver paraoxonase (data not shown).

Evaluation of Rabbit Paraoxonase Gene Complexity. Southern-blotted rabbit genomic DNA was digested with five restriction endonucleases prior to electrophoresis and probed with the 400-bp *Eco*RI/*Bam*HI fragment of the rabbit paraoxonase cDNA. In each restriction digest lane, only one hybridization band was observed (Figure 5). These data suggest that rabbit paraoxonase protein is probably encoded by a single gene, and not a member of a family of closely related genes.

Isolation and Sequence of Human Paraoxonase cDNAs. Approximately 300 000 plaques from the human cDNA library were screened with a 952-bp *Bst*XI radiolabeled fragment from the rabbit paraoxonase cDNA. From this library screen, 41 plaques were identified, and the three longest clones were

[1] As recommended by the Nomenclature Committee of the International Union of Biochemistry, nucleotides are abbreviated as follows: R = purine; Y = pyrimidine; N = A, T, G, or C; D = G, A, or T.

```
RabPON                                                  CGG CCC   -46

    AGC CCG TGG TGC TCG CGC CGG TCC AGC CTT TAG TCT GCC CTG ACC    -1
HuPON1                                                  CCC CCG ACC    -1

    ATG GCT AAA CTG ACA GCG CTC ACG CTC TTG GGG CTG GGA TTG GCA     45
    Met Ala Lys Leu Thr Ala Leu Thr Leu Leu Gly Leu Gly Leu Ala
    Met Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala          15
    ATG GCG AAG CTG ATT GCG CTC ACC CTC TTG GGG ATG GGA CTG GCA

    CTC TTC GAT GGA CAG AAG TCT TCT TTC CAA ACA CGA TTT AAT GTT     90
    Leu Phe Asp Gly Gln Lys Ser Ser Phe Gln Thr Arg Phe Asn Val
    Leu Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala          30
    CTC TTC AGG AAC CAC CAG TCT TCT TAC CAA ACA CGA CTT AAT GCT

    CAC CGT GAA GTA ACT CCA GTG GAA CTT CCT AAC TGT AAT TTA GTT    135
    His Arg Glu Val Thr Pro Val Glu Leu Pro Asn Cys Asn Leu Val
    Leu Arg Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val          45
    CTC CGA GAG GTA CAA CCC GTA GAA CTT CCT AAC TGT AAT TTA GTT

    AAA GGG ATT GAC AAT GGT TCT GAA GAC TTG GAA ATA CTG CCC AAT    180
    Lys Gly Ile Asp Asn Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn
    Lys Gly Ile Glu Thr Gly Ser Glu Asp Met Glu Ile Leu Pro Asn          60
    AAA GGA ATC GAA ACT GGC TCT GAA GAC ATG GAG ATA CTG CCT AAT

    GGA CTG GCT TTC ATC AGC GCC GGA TTA AAA TAT CCT GGA ATA ATG    225
    Gly Leu Ala Phe Ile Ser Ala Gly Leu Lys Tyr Pro Gly Ile Met
    Gly Leu Ala Phe Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys          75
    GGA CTG GCT TTC ATT AGC TCT GGA TTA AAG TAT CCT GGA ATA AAG

    AGC TTT GAC CCT GAT AAG CCT GGA AAG ATA CTT CTA ATG GAC CTG    270
    Ser Phe Asp Pro Asp Lys Pro Gly Lys Ile Leu Leu Met Asp Leu
    Ser Phe Asn Pro Asn Ser Pro Gly Lys Ile Leu Leu Met Asp Leu          90
    AGC TTC AAC CCC AAC AGT CCT GGA AAA ATA CTT CTG ATG GAC CTG

    AAT GAG AAA GAC CCA GTA GTA TTG GAA CTG AGC ATT ACT GGA AGT    315
    Asn Glu Lys Asp Pro Val Val Leu Glu Leu Ser Ile Thr Gly Ser
    Asn Glu Glu Asp Pro Thr Val Leu Glu Leu Gly Ile Thr Gly Ser         105
    AAT GAA GAA GAT CCA ACA GTG TTG GAA TTG GGG ATC ACT GGA AGT

    ACA TTT GAT TTA TCT TCA TTT AAC CCT CAT GGG ATT AGC ACA TTC    360
    Thr Phe Asp Leu Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe
    Lys Phe Asp Val Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe         120
    AAA TTT GAT GTA TCT TCA TTT AAC CCT CAT GGG ATT AGC ACA TTC

    ACA GAT GAA GAT AAT ATC GTC TAC CTG ATG GTG GTG AAC CAT CCA    405
    Thr Asp Glu Asp Asn Ile Val Tyr Leu Met Val Val Asn His Pro
    Thr Asp Glu Asp Asn Ala Met Tyr Leu Leu Val Val Asn His Pro         135
    ACA GAT GAA GAT AAT GCC ATG TAC CTC CTG GTG GTG AAC CAT CCA

    GAT TCC AAG TCC ACA GTG GAG TTG TTT AAA TTC CAA GAA AAA GAA    450
    Asp Ser Lys Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Lys Glu
    Asp Ala Lys Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Glu Glu         150
    GAT GCC AAG TCC ACA GTG GAG TTG TTT AAA TTT CAA GAA GAA GAA

    AAA TCA CTT TTG CAT CTG AAA ACC ATC AGA CAC AAG CTT CTG CCT    495
    Lys Ser Leu Leu His Leu Lys Thr Ile Arg His Lys Leu Leu Pro
    Lys Ser Leu Leu His Leu Lys Thr Ile Arg His Lys Leu Leu Pro         165
    AAA TCG CTT TTG CAT CTA AAA ACC ATC AGA CAT AAA CTT CTG CCT

    AGT GTG AAT GAC ATT GTC GCT GTG GGA CCT GAA CAC TTT TAT GCT    540
    Ser Val Asn Asp Ile Val Ala Val Gly Pro Glu His Phe Tyr Ala
    Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu His Phe Tyr Gly         180
    AAT TTG AAT GAT ATT GTT GCT GTG GGA CCT GAG CAC TTT TAT GGC

    ACC AAT GAT CAC TAT TTT ATT GAC CCT TAC TTA AAA TCC TGG GAA    585
    Thr Asn Asp His Tyr Phe Ile Asp Pro Tyr Leu Lys Ser Trp Glu
    Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Gln Ser Trp Glu         195
    ACA AAT GAT CAC TAT TTT CTT GAC CCC TAC TTA CAA TCC TGG GAG

    ATG CAT TTG GGA TTA GCG TGG TCA TTT GTT ACT TAT TAT AGT CCC    630
    Met His Leu Gly Leu Ala Trp Ser Phe Val Thr Tyr Tyr Ser Pro
    Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr Ser Pro         210
    ATG TAT TTG GGT TTA GCG TGG TCG TAT GTT GTC TAC TAT AGT CCA

    AAT GAT GTT CGA GTA GTG GCA GAA GGA TTT GAT TTT GCT AAC GGA    675
    Asn Asp Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn Gly
    Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn Gly         225
    AGT GAA GTT CGA GTG GTG GCA GAA GGA TTT GAT TTT GCT AAT GGA

    ATC AAC ATC TCA CCA GAC GGC AAG TAT GTC TAT ATA GCT GAA CTG    720
    Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
    Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu         240
    ATC AAC ATT TCA CCC GAT GGC AAG TAT GTC TAT ATA GCT GAG TTG
```

```
CTG GCT CAT AAG ATC CAT GTG TAT GAA AAG CAC GCT AAT TGG ACT   765
Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr
Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr        255
CTG GCT CAT AAG ATT CAT GTG TAT GAA AAG CAT GCT AAT TGG ACT

TTA ACT CCA TTG AAG TCC CTC GAC TTT AAC ACT CTT GTG GAC AAC   810
Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn
Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn        270
TTA ACT CCA TTG AAG TCC CTT GAC TTT AAT ACC CTC GTG GAT AAC

ATA TCC GTG GAT CCT GTG ACA GGG GAC CTT TGG GTT GGT TGT CAT   855
Ile Ser Val Asp Pro Val Thr Gly Asp Leu Trp Val Gly Cys His
Ile Ser Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His        285
ATA TCT GTG GAT CCT GAG ACA GGA GAC CTT TGG GTT GGA TGC CAT

CCC AAT GGC ATG CGA ATC TTC TAC TAT GAC CCA AAG AAT CCT CCT   900
Pro Asn Gly Met Arg Ile Phe Tyr Tyr Asp Pro Lys Asn Pro Pro
Pro Asn Gly Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro        300
CCC AAT GGC ATG AAA ATC TTC TTC TAT GAC TCA GAG AAT CCT CCT

GCA TCA GAG GTG CTT CGA ATC CAG GAC ATT TTA TCC AAA GAG CCC   945
Ala Ser Glu Val Leu Arg Ile Gln Asp Ile Leu Ser Lys Glu Pro
Ala Ser Glu Val Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro        315
GCA TCA GAG GTG CTT CGA ATC CAG AAC ATT CTA ACA GAA GAA CCT

AAA GTG ACA GTG GCT TAT GCA GAA AAT GGC ACT GTG TTA CAG GGC   990
Lys Val Thr Val Ala Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly
Lys Val Thr Gln Val Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly        330
AAA GTG ACA CAG GTT TAT GCA GAA AAT GGC ACA GTG TTG CAA GGC

AGC ACG GTG GCC GCT GTG TAC AAA GGG AAA ATG CTG GTT GGC ACC  1035
Ser Thr Val Ala Ala Val Tyr Lys Gly Lys Met Leu Val Gly Thr
Ser Thr Val Ala Ser Val Tyr Lys Gly Lys Leu Leu Ile Gly Thr        345
AGT ACA GTT GCC TCT GTG TAC AAA GGG AAA CTG CTG ATT GGC ACA

GTG TTC CAC AAA GCT CTC TAC TGT GAG CTC TCA CAG GCC AAT TAG  1080
Val Phe His Lys Ala Leu Tyr Cys Glu Leu Ser Gln Ala Asn ***         359
Val Phe His Lys Ala Leu Tyr Cys Glu Leu ***                         355
GTG TTT CAC AAA GCT CTT TAC TGT GAG CTC TAA CAG ACC GAT TTG

CAC CCG TGC CGC GGA CAC TGG CAC CCA CGA TTT CAA CTG CTT GCC  1125
CAC CCA TGC CAT AGA AAC TGA GGC CAT TAT TTC AAC CGC TTG CCA

GGC CAC ATT CTT GGG GCC ACA GTG CCC TCG GCG GGA TGA TGG ACA  1170
TAT TCC GAG GAC CCA GTG TTC TTA GCT GAA CAA TGA ATG CTG ACC

ACC CTA AAT TTG ACA TCA ACT GCA TCG CAG CCT AGA GTG GAT ATG  1215
CTA AAT GTG GAC ATC ATG AAG CAT CAA AGC ACT GTT TAA CTG GGA

AAG AGT AGG GCT TTT TGA GCG TGA ATT C                        1243
GTG ATA TGA TGT GTA GGG CTT TTT TTT GAG AAT ACA CTA TCA AAT  1260

CAG TCT TGG AAT ACT TGA AAA CCT CAT TTA CCA TAA AAA TCC TTC  1305

TCA CTA AAA TGG ATA AAT CAG TTA AAA AAA AA                   1337
```

FIGURE 3: Nucleotide and deduced amino acid sequences of RabPON and HuPON1 cDNAs. The rabbit sequences are presented in normal font in the upper lines; the human sequences are italicized in the lower lines. Alignment begins at the initiation codon ATG, which is arbitrarily designated position 1. Nucleotides preceding this codon are assigned negative numbers. The regions used for oligomer construction in the rabbit sequence are identified by an overline. Amino acid differences between RabPON and HuPON1 are boxed. Potential N-glycosylation sites are shown in bold type.

characterized by DNA sequencing (GenBank Assession Numbers: HuPON1, M63012; HuPON2, M63013; HuPON3, M63014). The DNA sequence of the these clones indicated that only HuPON1 was full length (Figure 3). This 1337-bp cDNA, including a 9-base poly(A) tail at the 3′ end, contained an open reading frame of 1065 bases that predicted a 355 amino acid protein. Clones HuPON2 and HuPON3 have 5′ termini starting 62 and 96 nucleotides downstream from the 5′ end of clone HuPON1, respectively. The nucleotide sequences of clones HuPON1 and HuPON2 predict a methionine at position 55 and glutamine at position 192, while clone HuPON3 predicts a protein with a leucine (TTG) at position 55 and an arginine (CGA) at position 192. The former substitution results in the loss of a restriction site (*Nla*III) in HuPON3, while the latter substitution creates *Alw*I and *Sau*3A sites in HuPON3.

Comparison of Rabbit and Human Paraoxonase Sequences. Alignment of rabbit and human cDNA coding regions revealed an 86% identity (Figure 3). The protein sequences deduced from these clones indicated an 85% identity, which increases to 88.7% when conservative amino acid substitutions are considered (Figure 3). The deduced rabbit amino acid sequence contains five potential N-glycosylation sites, whereas the human sequence predicts four possible N-glycosylation sites.

Discussion

The most difficult step in isolating a cDNA clone for human serum paraoxonase has been obtaining sufficient pure enzyme from which to obtain a protein sequence that in turn could be used to design oligomer probes for library screening. We overcame this problem by purifying and partially sequencing paraoxonase from rabbits, which have much higher levels of paraoxonase than humans and for which an activity stain was developed (preceding paper). These sequence data were used to design oligonucleotide probes which enabled the isolation of a rabbit paraoxonase cDNA. The rabbit clone was used to isolate corresponding human liver cDNA clones.

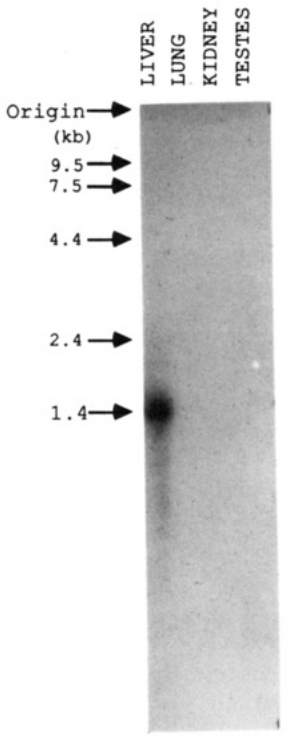

FIGURE 4: Northern blot analysis of rabbit RNA. Total RNA was isolated from the liver, lung, kidney, and testes of untreated rabbits and size-separated in an agarose/formaldehyde gel. Following tranfer to a nylon membrane, the blot was probed with the RabPON cDNA. Molecular size standards are shown in the left margin. A single hybridization band is observed only in liver, suggesting an in vivo transcript of approximately 1.4 kb.

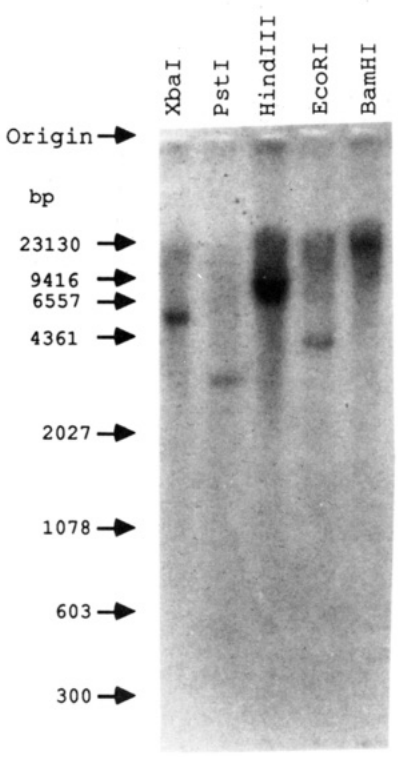

FIGURE 5: Southern blot analysis of rabbit genomic DNA. Twenty micrograms of DNA was digested under excess conditions with each of the indicated restriction enzymes, size-separated in an agarose gel, and transferred to a nylon membrane which was hybridized with a radioactive RabPON cDNA fragment. Molecular size markers are shown in the left margin.

The full-length rabbit and human clones demonstrate extensive conservation in nucleotide and deduced amino acid sequences despite the evolutionary distance separating these species. Although the predicted length of the two proteins differs by four amino acids, no gaps were required for the alignment of these sequences. A region of absolute conservation between the two sequences is observed from amino acids 213 to 275. Furthermore, within these 63 residues, three of the four predicted N-glycosylation sites common to the two proteins occur. Although the rabbit and human paraoxonase clones demonstrate significant conservation, other genes or proteins related to paraoxonase were not identified in database searches, despite the fact that many sequences have been described for proteins which perform similar catalytic functions (e.g., esterase activity). On the basis of the unreported partial peptide sequence from human paraoxonase, Gan et al. (1991) also did not identify closely related sequences in database searches.

Comparison of the deduced protein sequences from RabPON and HuPON1 cDNAs to the amino-terminal sequences determined by gas-phase sequencing of the intact proteins reveals a unique feature of paraoxonase. Both the rabbit and human enzymes retain their signal sequences, with only the amino-terminal methionine residues cleaved. Database searches indicate that the N-terminal sequences of rabbit and human paraoxonase show similarity to other protein secretion signal sequences (Figure 6). Conservation of specific amino acids is apparent, but particularly interesting are the conserved three amino acid residues LAL. An inspection of other published signal sequences (Watson, 1984) indicates that many of these also contain this sequence in the hydrophobic core region.

We are unaware of other examples where typical nonmutant signal sequences are retained in mature, secreted proteins. Cleavage of signal sequences appears to follow certain rules (von Heijne, 1983). Both the human and rabbit sequences possess a positively charged amino terminus commonly found in signal sequences, as well as a 9-residue hydrophobic core starting at position 9. In eukaryotes, cleavage typically occurs 5–6 residues from the C-terminal boundary of the hydrophobic core, which would predict a cleavage site for the paraoxonase proteins following residue 22 or 23. Furthermore, accurate processing is thought to exclude certain residues at the −1 and −3 positions, relative to the cleavage site (von Heijne, 1986). On the basis of the cleavage site positions predicted above, the −3 position would be occupied in the paraoxonase protein by Gln_{20} or Lys_{21} (rabbit) or by His_{20} or Gln_{21} (human). These are "forbidden" residues in the −3 position and may explain why the signal sequence of paraoxonase is retained.

The function of the retained signal sequence is unknown. The "hydrophobic head" of paraoxonase may be important for interaction with the high-density lipoprotein particle with which it is intimately associated. Detergents are required to dissociate paraoxonase from apolipoprotein A1 (Furlong et al., preceding paper; Gan et al., 1991). Hydrophobicity analyses (Figure 7) clearly show the hydrophobic amino termini of rabbit and human paraoxonases, as well as considerable hydrophobic character in the remainder of the proteins.

Comparison of the full-length HuPON1 cDNA with the two human partial clones reveals two interesting features. First, two nucleotide substitutions result in amino acid differences between clones HuPON1 and HuPON2 vs HuPON3. It is not known if either of these substitutions accounts for the differences observed between high- and low-activity paraoxonase allelic forms. In this regard, it is of interest to compare the two amino acid substitutions predicted from clone HuPON3 with the orthologous positions predicted from the

	MAKLTALTLLGLGLALFDGQKSSFQTR	Rabbit Paraoxonase
	MAKLIALTLLGMGLALFRNHQSSYQTR	Human Paraoxonase
	MQMSPALTCLVLGLALVFGEGSAVHHP	Plasminogen Activator inhibitor-1 precursor, Human[1]
	MAPRTLLLLLSGALALTQTWARSHSMR	HLA alpha chain precursor, clone pHLA, Human[2]
4	MAPRTLILLLSGALALTETWAGSHSMR	HLA alpha chain precursor, cw3, Human[3]
4	MAPRTLLLLLAGALTLKDTQAGSHSMR	RLA alpha chain precursor histocompatibility antigen, Rabbit[4]
	MAPCTLLLLLAAALAPTQYRAGPHSLR	H-2 k-d alpha chain precursor class 1 antigen[5]
	MAKLLALSLSFCFLLLGGCFALREQPQ	Legumin A precursor Garden pea[6]
	MGKKSHICCFSLLLLLFAGLASGHQVL	α amylase 2-precursor Barley[7]
	MAAATTTTSRPLLLSRQQAAASSLQCR	Fructose 1, 6 bisphosphatase precursor wheat[8]
3	TAPRTVLLLLSAALALTETWAGSHSMR	CHLA-81 α chain precursor histocompatibility antigen chimpanzee[9]
2	ASRLTLLTLLLLLLAGDRASSNPNATS	Complement C1 inhibitor precursor - Human[10]
3	ATKTFALLLLSLFLAVGLGEKKEGHFS	Gastric inhibitor polypeptide precursor Human[11]
	MRMLLHLSLLALGAAYVYAIPTEIPTS	Interleukin 5 Human[12]
	MQMSPALTCLVLGLTLVFGEGSAVHHP	Plasminogen activator inhibitor-1 precursor Human[13]
2	MQKLLKCSRLVLALALILVLESSVQGY	Secretory granule proteoglycan core protein precursor-Human[14]
	MAGPPRLLLLPLLLALARGLPGALAAQ	T cell surface glycoprotein CD7 precursor Human[15]
2	EAPIVLLLLLWLALAPTPGSASSEAPP	Ribophorin I precursor Rat[16]

FIGURE 6: Comparison of the amino-terminal signal sequence regions of rabbit and human paraoxonases with similar signal sequences found in searching the DNA/protein databases. Numbers appearing to the left of the sequences indicate the residue position. Other sequences being at the first residue. Footnotes: (1) Pannekoek et al., 1986; Ginsburg et al., 1986; (2) Malissen et al., 1982; (3) Sodoyer et al., 1984; (4) Tykocinski et al., 1984; (5) Kvist et al., 1983; Lalanne et al., 1983; (6) Lycett et al., 1984; (7) Knox et al., 1987; (8) Raines et al., 1988; (9) Meyer et al., 1988; (10) Bock et al., 1986; (11) Takeda et al., 1987; (12) Azuma et al., 1986; (13) Strandberg et al., 1988; (14) Stevens et al., 1988; (15) Aruffo & Seed, 1987; (16) Harnik-Ort et al., 1987.

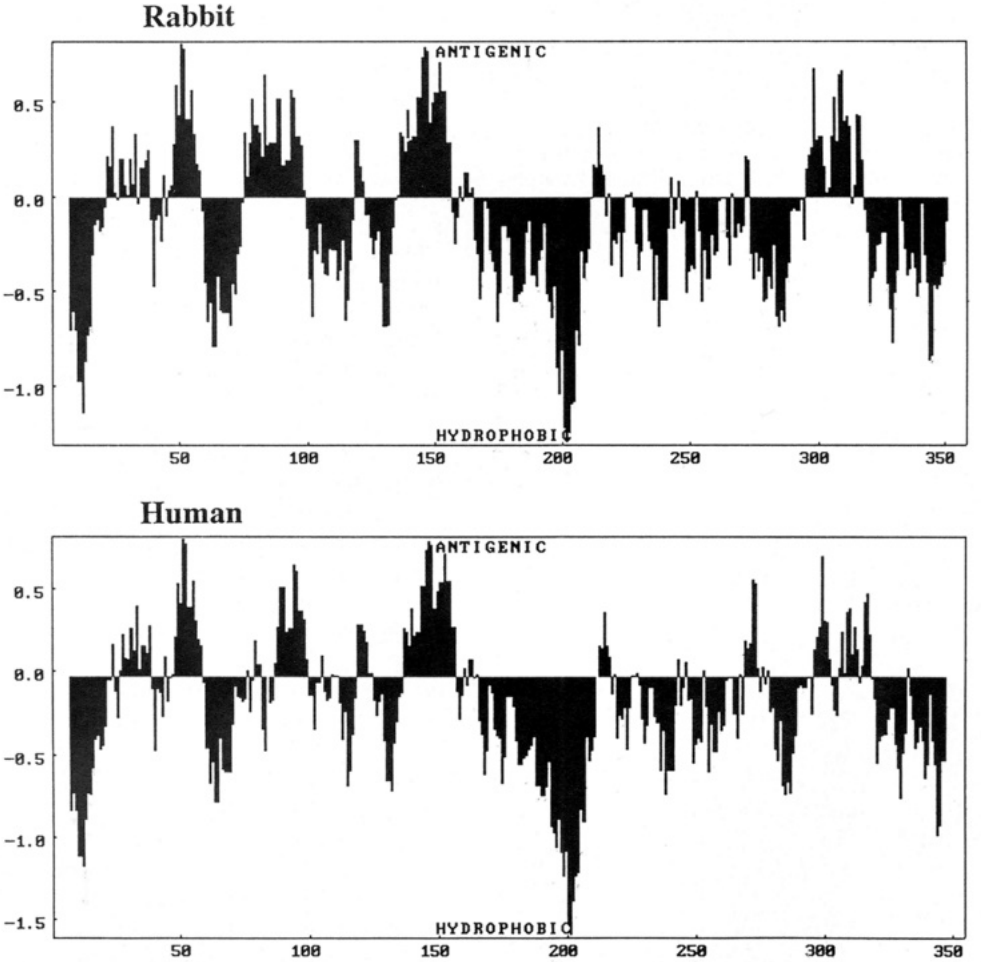

FIGURE 7: Hydrophobicity profiles of rabbit and human paraoxonase. The analysis was performed with GENEPRO software according to the Hopp and Woods (1981) algorithm with a window setting of 12.

rabbit cDNA. Amino acid 55 is a Leu in both sequences, whereas residue 192 is conservatively substituted (Lys in RabPON, Arg in HuPON3). It is tempting to speculate that since rabbits have high-activity paraoxonase, HuPON3 (which shares two similar and potentially important amino acids) might represent the high-activity allele genotype in the human. A gene frequency for the low-activity paraoxonase allele of 0.69 would predict a 43% probability that a given person would be a heterozygote (Furlong et al., 1989). Therefore, it is not unlikely that the individual from whom the human liver library was constructed was heterozygous for the paraoxonase allele, expressing mRNA for both high- and low-activity forms of the enzyme.

The importance of these amino acid changes could be examined by different approaches. Expressing the human cDNAs in vitro, or site-directed mutagenesis targeting the

```
HuPON1:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA   60
HuPON2:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA
HuPON3:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA
         ************************************************************

HuPON1:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC  120
HuPON2:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC
HuPON3:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC
         ************************************************************

HuPON1:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG  180
HuPON2:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG
HuPON3:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG
         ************************************************************

HuPON1:  AATACACTATCAAATCAGTCTTGGAATACTTGAAAACCTCATTTACCATAAAAATCCTTC  240
HuPON2:  AATACACTATCAAATCAGTCTTGGAAAAAAAAAAAAAA                       218
HuPON3:  AATACACTATCAAATCAGTCTTGGAATACTTGAAAACCTCATTTACCATAAAAATCCTTC
         ************************

HuPON1:  TCACTAAAATGGATAAATCAGTTAAAAAAAAA                             272
HuPON3:  TCACTAAAATGGATAAATCAGTTATGTCAATTGTCAGATATTAAATAACAGTGTGTGACC  300

HuPON3:  CCAAAAGTACTTACCCTAAAACATGTGTTGCCTGAAAGCACATGTGTGTATCGCTGCCTT  360

HuPON3:  GCCATGTCTTGTTCAGAAGACACAGGGGAGCAGGGTTAGCTCACGTGTCTTTAGAACTCC  420

HuPON3:  AGTACTCACCCAGGGACTCCAGTTCACAGGCCAGAAAACATATGCATTATGAAGTTCCCC  480

HuPON3:  TCTACTCCATGCACATAGTAAGTCTGACTATGGCAGTCAGACTTACTTACTCCCATTTTC  540

HuPON3:  CCTTCGATATATGACTTTTTCTCAGTAAATATTAACCTGAACTATTCCAAAAAAAAAAAA  600
HuPON3:  AAAAAAAAA                                                    609
```

FIGURE 8: Comparison of the 3′ noncoding portions of the human paraoxonase cDNAs. HuPON1, HuPON2, and HuPON3 are aligned and numbered beginning with the termination codon TAA, shown in bold type. Identical nucleotide residues in all sequences are indicated with an asterisk below the aligned residue. Potential polyadenylation signal sequences are underlined.

nucleotides encoding these amino acids, could reveal a concordant relationship with substrate-dependent metabolism and the human polymorphism. A more general approach for the identification of genetic alterations relevant to the paraoxonase polymorphism would be to sequence genomic DNA isolated from individuals characterized for high and low activity and to search for structural differences common to each group. Restriction site differences observed between the human sequences should also be useful in this regard.

A second observation in comparing the human clones is the different lengths of the 3′ untranslated regions, shown in Figure 8. The sequences are consistent with the existence of mRNAs which are polyadenylated at different sites. The canonical polyadenylation signal (AATAAA) is not found in any of these clones, although potential alternative poly(A) signal sequences are present. The probable polyadenylation signals CATAAA or ACTAAA (HuPON1), AATACA (HuPON2), and AGTAAA (HuPON3) are thought to be polyadenylated and cleaved inefficiently (Sheets et al., 1990). It may be relevant that Gieselman et al. (1989) found that individuals with arylsulfatase A pseudodeficiency had a point mutation of the polyadenylation signal of the arylsulfatase A gene, which resulted in a substantial reduction in the amount of normal message. The amount of arylsulfatase protein and arylsulfatase enzyme activity was reduced 90% in individuals with arylsulfatase A pseudodeficiency. A second mutation affecting a glycosylation site was present in individuals with arylsulfatase A pseudodeficiency, but was found not to affect enzyme activity. Variation up to 13-fold in paraoxonase/arylesterase enzyme activity between individuals with the same allozyme type (e.g., homozygous low paraoxonase activity) has been observed (Furlong et al., 1989), and the levels observed are stable over time. It remains to be determined whether variations in polyadenylation signals between individuals of a given allozyme type contribute to the observed stable differences in enzyme levels. Alterations in the 5′ regulatory region and stable differences in transcription factor levels could also contribute to or be responsible for these differences.

The physiological substrate for paraoxonase has not been identified.

ACKNOWLEDGMENTS

We thank Drs. A. G. Motulsky and G. S. Omenn for encouragement and stimulating discussions throughout this project. Oligonucleotide synthesis was carried out by Susan Ribiero in the Chemistry Department or by the University of Washington Molecular Pharmacology Facility.

REFERENCES

Aruffo, A., & Seed, B. (1987) *EMBO J. 6*, 3313–3316.

Azuma, C., Tanabe, T., Konishi, M., Kinashi, T., Noma, T., Matsuda, F., Yaoita, Y., Takatsu, K., Hammarstroem, L., Smith, C. I. E., Severinson, E., & Honjo, T. (1986) *Nucleic Acids Res. 14*, 9149–9158.

Blin, N., & Stafford, D. W. (1976) *Nucleic Acids Res. 3*, 2303–2308.

Bock, S. C., Skriver, K., Nielsen, E., Thogersen, H. C., Wiman, B., Donaldson, V. H., Eddy, R. L., Marrinan, J., Radziewjewska, E., Huber, R, Shows, T. B., & Magnusson, S. (1986) *Biochemistry 25*, 4292–4301.

Brown, S. S., Kalow, W., Pilz, W., Whittaker, M., & Waoronick, C. L. (1981) *Adv. Clin. Chem. 22*, 1–123.

Costa, L. G., Richter, R. J., Murphy, S. D., Omenn, G. S., Motulsky, A. G., & Furlong, C. E. (1987) in *Toxicology of Pesticides: Experimental, Clinical and Regulatory Perspectives* (Costa, L. G., Galli, C. L., & Murphy, S. D., Eds.) pp 262–266, Springer-Verlag, Heidelberg.

Costa, L. G., McDonald, B. E., Murphy, S. D., Omenn, G. S., Richter, R. J., Motulsky, A. G., & Furlong, C. E. (1990) *Toxicol. Appl. Pharmacol. 103*, 66–76.

Crabb, J. W., Armes, L. G., Carr, S. A., Johnson, C. M., Roberts, G. D., Bordoli, R. S., & McKeehan, W. L. (1986) *Biochemistry 25*, 4988–4993.

Crabb, J. W., Johnson, C. M., Carr, S. A., Armes, L. G., & Saari, J. C. (1988) *J. Biol. Chem. 263*, 18678–18687.

Furlong, C. E., Richter, R. J., Seidel, S. L., & Motulsky, A. G. (1988) *Am. J. Hum. Genet. 43*, 230–238.
Furlong, C. E., Richter, R. J., Seidel, S. L., Costa, L. G., & Motulsky, A. G. (1989) *Anal. Biochem. 180*, 242–247.
Furlong, C. E., Richter, R. J., Chapline, C., & Crabb, J. W. (1991) *Biochemistry* (preceding paper in this issue).
Gan, K. N., Smolen, A., Eckerson, H. W., & LaDu, B. N. (1991) *Drug Metab. Dispos. 19*, 100–106.
Geldmacher-von Mallinckrodt, M., & Diepgen, T. L. (1988) *Toxicol. Environ. Chem. 18*, 79–196.
Gieselman, V., Polten, A., Kreysing, J., & von Figura, K. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9436–9440.
Ginsburg, D., Zeheb, R., Yang, A. Y., Rafferty, U. M., Andreasen, P. A., Nielsen, L., Dano, K., Lebo, R. V., & Gelehrter, T. D. (1986) *J. Clin. Invest. 78*, 1673–1680.
Harnik-Ort, V., Prakash, K., Marcantonio, E., Colman, D. R., Rosenfeld, M. G., Adesnik, M., Sabatini, D. D., & Kreibich, G. (1987) *J. Cell. Biol. 104*, 855–863.
Hassett, C., & Omiecinski, C. J. (1987) *Biochem. Biophys. Res. Commun. 149*, 326–333.
Hassett, C., & Omiecinski, C. J. (1990) *Nucleic Acids Res. 18*, 1429–1434.
Hassett, C., Turnblom, S. M., DeAngeles, A., & Omiecinski, C. J. (1989) *Arch. Biochem. Biophys. 271*, 380–389.
Hopp, T. P., & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3824–3828.
Kalow, W. (1987) *Eur. J. Pharmacol. 31*, 633–641.
Knox, C. A. P., Sonthayanon, B., Chandra, G. R., & Muthukrishnan, S. (1987) *Plant Mol. Biol. 9*, 3–17.
Kvist, S., Roberts, L., & Dobberstein, B. (1983) *EMBO J. 2*, 245–254.
LaDu, B. N., & Eckerson, H. W. (1984) in *Genetic Variability in Response to Chemical Exposures* (Omenn, G. S., & Gelboin, H. V., Eds.) pp 167–175, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
LaDu, B. N., Piko, J. I., Eckerson, H. W., Vincent-Viry, M., & Seist, G. (1986) *Ann. Biol. Clin. 44*, 369–372.
Lalanne, J. L., Delarbre, C., Gachelin, G., & Kourilsky, P. (1983) *Nucleic Acids Res. 11*, 1567–1577.
Lycett, G. W., Croy, R. R. D., Shirsat, A. H., & Boulter, D. (1984) *Nucleic Acids Res. 12*, 4493–4506.
Main, A. R. (1956) *Can. J. Biochem. Physiol. 34*, 197–216.
Malissen, M., Malissen, B., & Jordan, B. R. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 893–897.
Meyer, W. E., Jonker, M., Klein, D., Ivanyi, P., van Seventer, G., & Klein, J. (1988) *EMBO J. 7*, 2765–2774.
Omenn, G. S. (1987) in *Toxicology of Pesticides: Experimental, Clinical and Regulatory Perspectives* (Costa, L. G., Galli, C. L., & Murphy, S. D., Eds.) pp 262–266, Springer-Verlag, Heidelberg.
Omiecinski, C. J., Walz, F. G., Jr., & Vlasuk, G. P. (1985) *J. Biol. Chem. 260*, 3247–3250.
Pannekoek, H., Veerman, H., Lambers, H., Diergaarde, P., Verweij, C. L., van Zonneveld, A. J., & van Mourik, J. A. (1986) *EMBO J. 5*, 2539–2544.
Raines, C. A., Lloyd, J. C., Longstaff, M., Bradley, D., & Dyer, T. (1988) *Nucleic Acids Res. 16*, 7931–7942.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Seidegard, J., Vorachek, W. R., Pero, R. W., & Pearson, W. R. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7293–7297.
Sheets, M. D., Ogg, S. C., & Wickens, M. P. (1990) *Nucleic Acids Res. 18*, 5799–5805.
Smolen, A., Eckerson, H. W., Gan, K. N., Hailat, N., & LaDu, B. N. (1991) *Drug Metab. Dispos. 19*, 107–112.
Sodoyer, R., Damotte, M., Delovitch, T. L., Trucy, J., Jordan, B. R., & Strachan, T. (1984) *EMBO J. 3*, 879–885.
Stevens, R. L., Avraham, S., Gartner, M. C., Bruns, G. A. P., Austen, K. F., & Weis, J. H. (1988) *J. Biol. Chem. 263*, 7287–7291.
Strandberg, L., Lawrence, D., & Ny, T. (1988) *Eur. J. Biochem. 176*, 609–616.
Takeda, J., Seino, Y., Tanaka, K. I., Fukumoto, H., Kayano, T., Takahashi, H., Mitani, T., Kurono, M., Suzuki, T., Tobe, T., & Imura, H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7005–7008.
Tykocinski, M. L., Marche, P. N., Max, E. E., & Kindt, T. J. (1984) *J. Immunol. 133*, 2261–2269.
von Heijne, G. (1983) *Eur. J. Biochem. 133*, 17–21.
von Heijne, G. (1986) *Nucleic Acids Res. 14*, 4682–4690.
Watson, M. E. E. (1984) *Nucleic Acids Res. 12*, 5145–5164.
Weber, W. W. (1987) *The Acetylator Genes and Drug Response*, Oxford University Press, New York.
West, K., & Crabb, J. W. (1990) in *Current Research in Protein Chemistry* (Villafranca, J. J., Ed.) pp 37–48, Academic Press, San Diego.