Characterization of a cDNA for the Unexpressed Form of Cytochrome P-450g from the (−g) Rat and Differentiation of Its mRNA from That of the (+g) Phenotype Using Specific Oligoprobes†

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ABSTRACT: Our laboratory recently isolated a cDNA for cytochrome P-450g (IIC13), a male-specific, highly polymorphic P-450 isozyme, from livers of the high phenotype (+g) of Sprague-Dawley rats [McClellan-Green et al. (1989) *Biochemistry 28*, 5832–5839]. Hybridization studies using a specific oligonucleotide probe for P-450 (+g) indicated that equivalent amounts of P-450g mRNA were present in livers of both the high and low phenotypes (+g and −g) of male Sprague-Dawley, Fischer (inbred −g), or ACI (inbred +g) rats. In the present study, we isolated one full-length and one nearly full-lengh cDNA clone coding for the unexpressed form of cytochrome P-450g from a cDNA library constructed from mRNA from a (−g) male Sprague-Dawley rat. The longest cDNA had an open reading frame of 1473 nucleotides which coded for a 490 amino acid polypeptide of M_r 55839. Although the 5′-noncoding leader sequence and the 3′-noncoding region were unchanged, the coding sequence of the (−g) phenotype differed from that of the cDNA isolated from the (+g) phenotype by nine bases changes. These base changes would result in seven amino acid differences between the protein sequences for the two phenotypes. Two specific oligonucleotide probes for (+) P-450g and (−) P-450g containing three base differences between the (+g) and (−g) sequences hybridized differentially to mRNA from the (+g) and (−g) phenotypes. These data demonstrate unequivocally that these base changes occurred in vivo in the mRNAs for P-450 (−g) and that the phenotypic differences in hepatic levels of P-450g are due to the expression of a variant mRNA for P-450 (−g) containing nine base mutations.

Cytochrome P-450g (IIC13) is a male-specific member of the P-450IIC family of monooxygenases (Nebert et al., 1989). Cytochrome P-450g (P-450g)[1] is of particular interest because it is highly polymorphic (expressed at high, low, and perhaps intermediate levels) in livers of outbred strains of male Long Evans and Sprague-Dawley (CD) rats, at high levels in the inbred ACI strain, and at low levels in the inbred Fischer strain (Bandiera et al., 1986; McClellan-Green et al., 1987; Rampersaud et al., 1987). A number of polymorphisms of P-450 have been reported in humans, and these polymorphisms have been shown to contribute to interindividual variations in drug metabolism in humans (Guengerich et al., 1987). The mechanisms responsible for these polymorphisms are an active area of research.

Our laboratory recently isolated and sequenced a cDNA for P-450g from livers of the high phenotype (+g) of male Sprague-Dawley rats (McClellan-Green et al., 1989). However, hybridization studies using a specific oligonucleotide probe complementary to a unique sequence in P-450g cDNA indicated that the levels of P-450g mRNA were identical in livers of the high (+g) and low (−g) phenotypes of male Sprague-Dawley, ACI, and Fischer rats. These hybridization studies verified results of our earlier studies, which found no differences in the levels of translatable mRNA for P-450g in the two phenotypes (McClellan-Green et al., 1987). The results of these two studies suggest the possibility that the (−g) phenotype might be the result of a defective sequence, e.g., deletions, nonsense mutations, or mutations within the mRNA which produce a defective, unstable protein. Alternatively, mutations resulting in abnormal splicing of the mRNA could not be ruled out.

The present study was undertaken to elucidate the molecular mechanism for the polymorphism by attempting to isolate and characterize a cDNA from a (−g) rat liver library using specific oligoprobes for P-450g. Two cDNAs, one full-length and one nearly full-length, were isolated from a cDNA library constructed from mRNA from a (−g) male Sprague-Dawley rat. Sequencing of these cDNAs revealed significant differences from the P-450 (+g) cDNA. Specific oligonucleotide probes were synthesized in a region of variability between the two sequences and used to verify differences in the mRNA from livers of (+g) and (−g) phenotypes.

Materials and Methods

Animals. Strains of 8-week-old male and female rats were obtained as follows: Sprague-Dawley (CD) (Charles River, Kingston, NY), Fischer (Charles River, Raleigh, NC), and ACI (Harlan). Hepatic microsomes were prepared as previously described (Goldstein et al., 1982). Pieces of liver were frozen in liquid nitrogen and stored at −70 °C for extraction of mRNA.

Western Blots and Radioimmunoassay. Cytochrome P-450g was purified as described by Ryan et al. (1984). Immunospecific antibody to P-450g was purified as previously described by McClellan-Green et al. (1987) and recognized

† A preliminary report of this work was presented at the 73rd Annual Meeting of the Federation of American Societies for Experimental Biology (Yeowell et al., 1989).

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[1] Abbreviations: P-450, cytochrome P-450; CD, Sprague-Dawley (Charles River); SDS, sodium dodecyl sulfate; RIA, radioimmunoassay; SSC, saline–sodium citrate buffer.

only one polypeptide band on Western blots. SDS–polyacrylamide gel electrophoresis and Western blotting of hepatic microsomes were performed as previously described (Yeowell et al., 1985). Hepatic P-450g was measured in a double-antibody radioimmunoassay (RIA) similar to that previously described for P-450c and P-450d (Luster et al., 1983).

Isolation of Clones from a (–g) cDNA Library. Total RNA was isolated from the liver of a (–g) male CD rat (phenotyped by both Western blot analysis and RIA), using the guanidine hydrochloride method (Cox, 1968). Poly(A+) RNA was isolated by two passages over an oligo(dT)–cellulose column (Aviv & Leder, 1972). Double-stranded (ds) cDNA was synthesized by the method outlined by Watson and Jackson (1985). Following addition of *Eco*RI linkers to the double-stranded cDNA and size fractionation on a Sepharose 4B column, the longest fraction was ligated into λZAP (Stratagene, La Jolla, CA). The λZAP cDNA constructs were transfected into *Escherichia coli* BB4. The cDNA library was screened by using two specific oligoprobes complementary to the P-450 (+g) cDNA (c5126), one complementary to the 5′ end, and one complementary to bases 498–514 (McClellan-Green et al., 1989). Nine positive clones was isolated, transfected into XL1-Blue, and excised into the plasmid Bluescript, according to Stratagene's excision protocol. Three of these clones, one full-length (g353), one nearly full-length (g14), and one partial (g29), were sequenced. Screening of a cDNA library prepared by a similar method from the liver of a male Fischer rat yielded six positive clones, two of which were partially sequenced.

Sequence Analysis. The Bluescript plasmids containing the cDNA inserts g29, g14, and g353 were purified by CsCl gradients (Maniatis et al., 1982), and the ds cDNA inserts were sequenced by the dideoxy chain termination method of Sanger et al. (1977) using Sequenase kits (U.S. Biochemical Corp., Cleveland, OH). The full-length clone g353 (1720 nucleotides) and nearly full-length clone g14 (1709 nucleotides) were completely sequenced (100%) in both directions using a series of synthetic 20-mer primers based on the sequence of c5126 (+g) (McClellan-Green et al., 1989). The primers beginning at bases 1, 220, 520, 609, 760, 940, 1200, 1420, and 1600 were used to sequence in the 5′–3′ direction, and for the reverse (R) directions, sequences were read from primers at 1530R, 1410R, 1300R, 1100R, 940R, 695R, 510R, and 290R. The ends of the clones were also sequenced by using the Bluescript T3 primer and the M-13 universal primer.

The partial clone g29 was sequenced by analysis of ds DNA from *Exo*III deletion mutants (Erasabase, Promega). Two additional clones isolated from a cDNA library constructed from hepatic mRNA from a male Fischer rat (inbred –g strain) were partially sequenced to compare the 5′ and 3′ ends and verify the regions of variability with the (+g) and (–g) sequences. The recombinant PUC 13 plasmid containing c5126 was also purified by CsCl gradients and resequenced simultaneously with g353 through the regions of variability using the specific oligonucleotide primers.

Northern Blot Analysis of mRNA from the Two Phenotypes. Total RNA was isolated from livers of 13 individual male Sprague-Dawley rats by the guanidine hydrochloride method (Cox, 1968). Poly(A+) RNA was prepared from total RNA by one passage over oligo(dT)–cellulose columns (Aviv & Leder, 1972). In a second experiment, poly(A+) RNA was isolated by using Fast track mRNA kits (Invitrogen) from the livers of three additional male ACI rats, three male Fischer rats, and individual female CD, ACI, and Fischer rats. Poly(A+) RNA (5 μg) was electrophoresed in duplicate on 2.2 M formaldehyde–1% agarose gels (Maniatis et al., 1982), transferred overnight to Nytran membranes (Schleicher & Schuell) in 20 × SSC transfer buffer, and baked for 2 h and 80 °C. Liver microsomes from the male CD rats were phenotyped by Western blot and RIA analysis as described above.

Synthesis of Oligoprobes and Hybridization Conditions. Two oligoprobes for a region containing three base changes between the (+g) and (–g) cDNAs were synthesized on an Applied Biosystems 380B DNA synthesizer, purified on a NAP-5 column (Pharmacia), and labeled at the 5′ end with [γ-^{32}P]ATP to a specific activity of 1×10^8 cmp/μg (Zarbl et al., 1985). The (+g) probe was complementary to 5′-TCAAAAATCATACATGGCTT-3′ and the (–g) probe complementary to 5′-TAAAAAATTATACATGGGTT-3′. The blots were prehybridized for 2 h in 6 × SSC, 4 × Denhardts, 0.5% SDS, and 10 μg/mL sonicated salmon sperm DNA. On the basis of the estimated temperature of hybridization (Davis et al., 1986), hybridization was then carried out overnight at 47 °C for (+g) and at 42 °C for (–g) in 6 × SSC, 4 × Denhardts, 0.1% SDS, and 10 μg/mL sonicated salmon sperm DNA. Blots were then washed using 2 × SSC and 0.1% SDS 2–3 times for 5 min at room temperature, and then for (+g), 1 × 2 min at 37 °C and 1 × 2 min at 47 °C, and for (–g), 2 min at 42 °C. Total P-450g mRNA was measured by using a third oligoprobe (g2) corresponding to the anticoding strand (498th–517th nucleotides) of a region which did not vary between the P-450 (+g) and P-450 (–g) cDNAs. This probe was identical with that used in our earlier studies, and hybridization and washing conditions were identical with those described previously (McClellan-Green et al., 1989).

Results

Two clones, g14 and g353, isolated from a (–g) CD rat liver cDNA library, were sequenced completely in both directions by using a series of synthetic oligoprimers based on the cDNA sequence of c5126 (+g) (McClellan-Green et al., 1989). The cDNAs for g14 and g353 were essentially identical (1709 and 1720 bp, respectively) without the poly(A+) tail except that g14 had an additional AT at the start of the poly(A+) tail and did not contain the 11-base 5′ noncoding leader sequence or the AT of the initial methionine. In all other respects, the sequences of both clones were identical. A third partial clone, g29 (962 nucleotides), contained the 5′ end but lacked 748 bases at the 3′ end. This clone was otherwise identical with the first two clones. The full-length clone contained an open reading frame of 1473 nucleotides which coded for a protein of 490 amino acids of M_r 55 839.

A comparison of the nucleic acid and predicted amino acid sequences of g353 with c5126 (+g) (Figure 1) showed no difference in either the 11-base 5′ leader sequence or the 251-base 3′ noncoding sequence containing the consensus sequence for the polyadenylation signal. However, g14 and g353 differed from c5126 by nine base changes at nucleotides 65, 300, 539, 702, 709, 718, 801, 1013, and 1107. Two of the base changes were conservative, but from the remaining seven base changes, one would predict seven amino acid changes in the protein sequence for P-450 (–g). These predicted amino acid substitutions from (+g) to (–g) occur at the following positions: proline to leucine (22), serine to cysteine (180), phenylalanine to leucine (234), histidine to tyrosine (237), leucine to valine (240), cysteine to serine (338), and glutamic acid to aspartic acid (369). The sequence of the third partial clone g29 (965 nucleotides) showed the same changes as g353 in the first 965 nucleotides.

Because the differences between c5126 and g353 constituted only nine single base changes, we resequenced both cDNAs

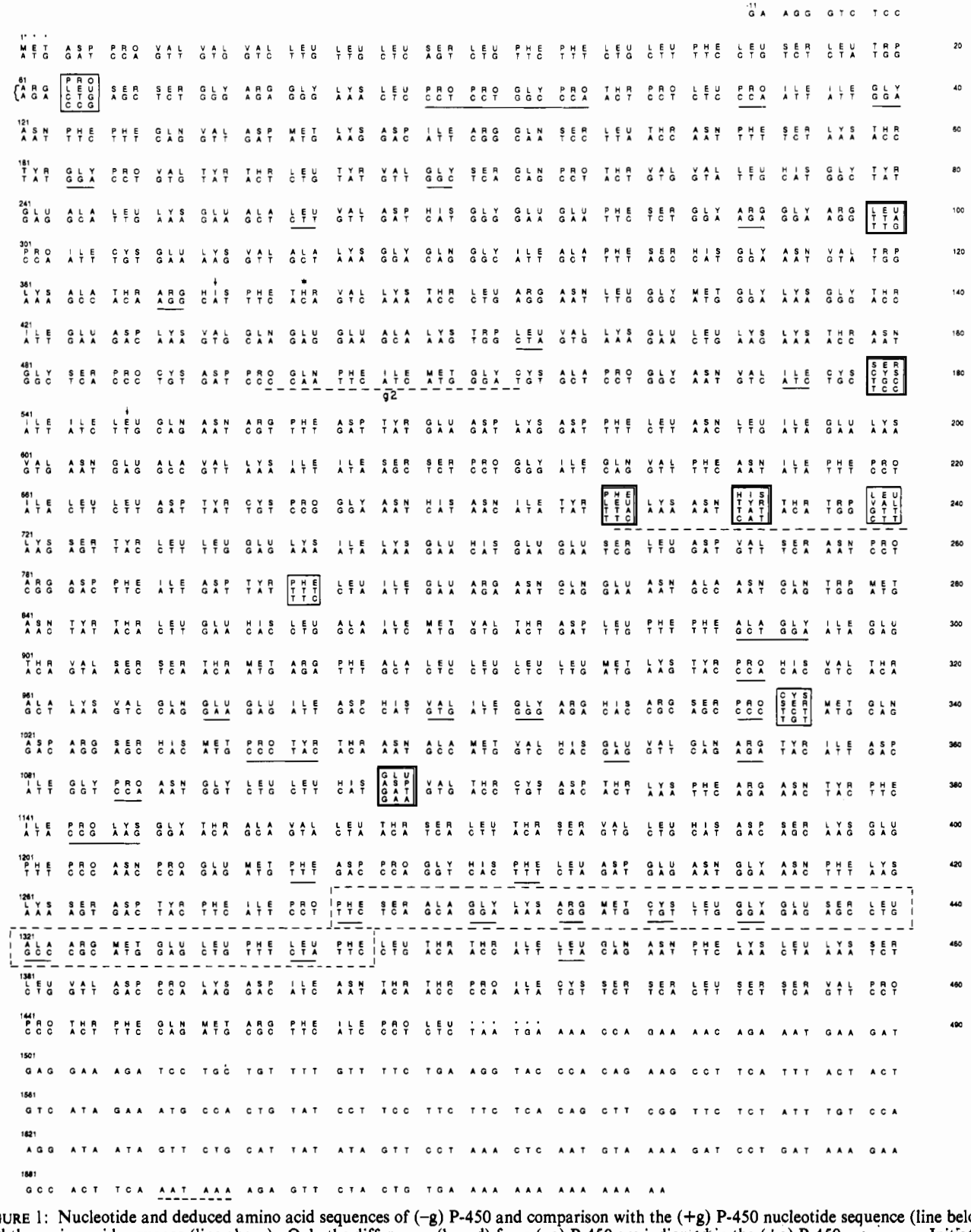

FIGURE 1: Nucleotide and deduced amino acid sequences of (−g) P-450 and comparison with the (+g) P-450 nucleotide sequence (line below) and the amino acid sequence (line above). Only the differences (boxed) from (−g) P-450 are indicated in the (+g) P-450 sequences. Initiation and termination codons are marked by asterisks. Numbers on the left indicate nucleotide position, starting with the initiation codon. The numbers on the right indicate amino acid positions. The three oligoprobes selected are underlined with a dashed line (g2 at bases 498–517 and +g and −g at bases 421–440). The polyadenylation signal is also underlined by a dotted line. The highly conserved heme binding region is outlined by a dotted box. Solid lines indicate 37 amino acids which are highly conserved among 14 known P-450s (Matsunaga et al., 1988). The arrows denote 2 amino acids which are conserved among these 14 amino acids, but differ for both P-450 (+g) and P-450 (−g). An asterick denotes that P-450g contains a threonine instead of a serine at base 127. This serine is the phosphorylation site of cAMP-dependent protein kinase for P-450 IIB1 and presumably other P-450s (Muller et al., 1985).

simultaneously through these variable regions using specific oligonucleotide primers. The differences between c5126 and g353 were verified at all nine nucleotides. In addition, two clones, F133 and F125, were isolated from a cDNA library constructed from hepatic mRNA from a male Fischer rat (an inbred −g strain). These clones were sequenced at the 3′ and

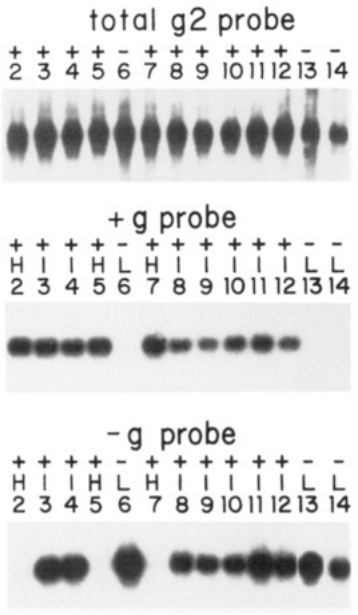

FIGURE 2: Northern blot analysis of hepatic mRNA isolated from male CD rats which are phenotypically variable in expression of P-450g showing hybridization with an oligoprobe complementary to a region present in both (+g) and (−g) cDNAs (g2) (top) versus oligoprobes specific for the (+g) cDNA (middle) and (−g) cDNA (bottom). Poly(A+) RNA (10 μg) was electrophoresed in 1% agarose–2.2 M formaldehyde gels and transferred to a Nytran membrane. The filters were then hybridized with oligoprobes g2 (bases 498–517)(total P-450g mRNA) and oligoprobes complementary to P-450 (+g) and P-450 (−g) cDNAs (bases 701–720) (see Figure 1) as described under Materials and Methods. The numbered lanes represent hepatic mRNA from 13 male CD rats. The P-450g phenotypes were determined by RIA and Western blot analysis of liver microsomes for P-450g. The two major phenotypes of male rats (high and low expression of P-450g) are represented by (+) or (−) above each sample. The high phenotype has been further subdivided by RIA analysis into those with the highest P-450g levels, H = high (>100 pmol of P-450g/mg of microsomal protein), and intermediate (I) P-450g levels (49–90 pmol/mg). The P-450g content of the low phenotype (L) was <10 pmol/mg.

5′ ends and in all of the nine regions which varied between g353 and c5126. Both F133 and F125 contained the 5′ end of P-450 (−g) and the polyadenylation signal but lacked the poly(A+) tail. Both clones contained all nine base changes seen in g353. Matsunaga et al. (1988) found that 39 amino acids were conserved in the amino acid sequences of 14 rat P-450s in the P-450I, -II, -III, and -IV families. We have reported that 37 of these 39 amino acids are also conserved in P-450g (McClellan-Green et al., 1989). These amino acid residues are underlined in Figure 1. Somewhat surprisingly, none of these conserved amino acids were altered in P-450 (−g). Moreover, we found no amino acid changes in the conserved heme binding region of P-450 (−g).

To verify the presence of the P-450 (−g) sequence in the mRNA of the (−g) phenotype, we hybridized hepatic mRNA from 13 male CD rats representing the high and low phenotypes with oligonucleotide probes complementary to the region 701–720 which contained 3 base changes between the sequences for P-450 (+g) and P-450 (−g) (Figure 1). We also examined the amount of total P-450g mRNA using the oligoprobe g2 (bases 498–517) which is specific for P-450g mRNA (McClellan-Green et al., 1989). The livers of these rats varied dramatically (20-fold) in P-450g content as previously analyzed by Western blot analysis and RIA analysis (McClellan-Green et al., 1989). As we previously reported, the amount of hepatic mRNA hybridizing with the oligoprobe g2 did not vary between the (+g) and (−g) rats, nor did the

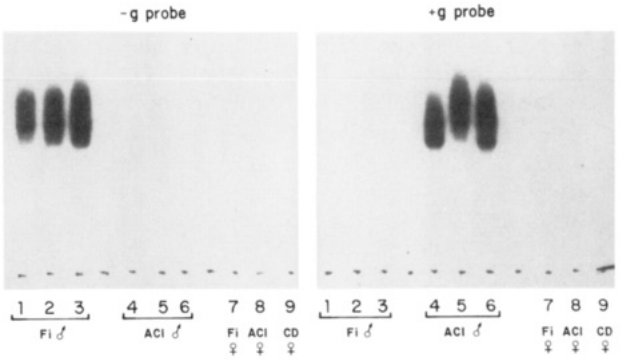

FIGURE 3: Northern blot analysis of hepatic mRNA isolated from individual male rats of inbred strains representing the high (ACI) and low (Fischer) phenotypes showing differential hybridization with oligoprobes complementary to (+g) and (−g) cDNAs. Each lane represents hepatic mRNA from an individual male Fischer (inbred −g) (lanes 1–3), from ACI rats (inbred +g) (lanes 4–6), and from female Fischer, ACI, and CD rats (lanes 7, 8, and 9, respectively). Northern blots were performed as indicated in Figure 2.

size of the hybridizable mRNA differ in the two phenotypes (~1800 bp) (Figure 2, top).

However, hybridization of Northern blots of these same 13 mRNAs with the specific oligoprobes (701–720) for (+g) and (−g) (labeled to similar specific activity) verified the presence of a mutated mRNA for P-450g in the (−g) phenotype (Figure 2). Hepatic mRNAs from outbred male CD rats 2, 3, 4, 5, 7, 8, 9, 10, 11, and 12, which expressed high levels of P-450g (+) (40–230 pmol/mg of P-450g as quantitated by RIA), were recognized by the probe for P-450 (+g). Likewise, hepatic mRNAs from rats 6, 13, and 14, whose livers contained little or no P-450g (the low phenotype) (L) (2–6 pmol/mg of P-450g by RIA), were only recognized by the probe unique for (−g). Interestingly, the (+g) male rats could be divided into two populations. Messenger RNA from livers of rats with very high hepatic levels of P-450g (2, 5, and 7) (indicated by an H) (100–230 pmol/mg) were recognized only by the probe for P-450 (+g). However, hepatic mRNA from rats 3, 4, 8, 9, 10, 11, and 12, whose livers expressed intermediate levels of P-450g (indicated by an I) (40–90 pmol/mg of P-450g), were recognized by both the (+g) and (−g) probes, indicating that these rats express both mRNAs and may be heterozygous for expression of P-450 (+g) and P-450 (−g) mRNAs.

In contrast, individual mRNAs from livers of three male inbred ACI (Harlan) rats which are homozygous for high levels of P-450g are recognized only by the (+g) oligoprobe on Northern blots, whereas mRNAs from three inbred homozygous male Fischer rats, which represent the (−g) phenotypes, are only recognized by the (−g) oligoprobe (Figure 3). Predictably, hepatic mRNA isolated from female CD, ACI, and Fischer rats is not recognized by either oligoprobe for this male-specific isozyme. The ability of specific oligoprobes to differentiate between mRNA from (+g) and (−g) phenotypes demonstrates that the base changes identified in this study constitute real differences between the mRNAs for the (−g) and (+g) phenotypes.

Discussion

In the present study, we used specific oligonucleotide probes complementary to P-450 (+g) to isolate one full-length clone, one nearly full-length clone (missing only the first two 5′-bases), and one partial clone (962 nucleotides) from a cDNA library constructed from liver of a phenotypically low (−g) male Sprague-Dawley rat. Sequencing of the P-450 (−g) cDNAs showed that they differed by only nine bases in the

coding region from their P-450 (+g) counterpart, c5126. Two of these base changes were conservative, but the remaining seven base changes would result in seven predicted amino acid substitutions for the P-450 (+g) polypeptide sequence. There were no changes in the 5′ noncoding or 3′ noncoding regions. Using a unit evolutionary period of 4.0 (millions of years needed for a 1% divergence in amino acid sequence) (Nebert & Gonzalez, 1987), one would estimate that the (−g) polymorphism arose approximately 2.4 million years ago. Therefore, it is not surprising that the same nine base changes were also found in two cDNA clones constructed from mRNA from an inbred (−g) Fischer rat.

The presence of mutations in the (−g) phenotype was verified in the present studies by hybridization of mRNA from both phenotypes with specific oligoprobes to a region containing three base changes between the (+g) and (−g) sequences. These oligoprobes successfully differentiated between the mRNA for (+g) and (−g), confirming that these base changes occur in vivo. The oligoprobe for P-450 (+g) recognized only the mRNA from the (+g) phenotype, and the (−g) phenotype was recognized only by the (−g) oligoprobe. Somewhat surprisingly, mRNAs from rats with intermediate levels of P-450g were recognized by both probes. However, these results provide a clear explanation of the results of breeding studies by Rampersaud et al. (1987), who found intermediate levels of P-450g in heterozygous male hybrid rats. They suggested the presence of cis-acting regulatory element. Our results clearly demonstrate that this is not the explanation for the intermediate phenotype. Instead, the presence of both the (+g) and (−g) mRNAs leads to intermediate levels of P-450g, since the (−g) mRNA is not expressed in either the intermediate or the high phenotype.

Polymorphisms of several forms of P-450 have been discovered in humans, including cytochromes involving in debrisoquine metabolism (P-450db), nifedipine metabolism ($P\text{-}450_{NF}$), mephenytoin metabolism ($P\text{-}450_{MP}$), and P-450HLx (Guengerich et al., 1987). These polymorphisms contribute to interindividual variations in drug metabolism in humans, and possible relationships to cancer and other diseases have been proposed. Cloning experiments have demonstrated the presence of at least three aberrantly spliced mRNAs for P-450db in the low phenotype (Gonzalez et al., 1988). These mRNAs either retained an intron or lost part of an exon as the result of abnormal splicing. A polymorphism in $P\text{-}450_{NF}$, which metabolizes nifedipine as well as a number of steroids, has been related to variations in the levels of $P\text{-}450_{NF}$ mRNA levels (Bork et al., 1989).

In contrast, the defect in the (−) P-450g phenotype represents only nine single base changes, indicating that single point mutations in the structural gene for P-450 (−g) are responsible for a defective protein. Such missense mutations have been reported in a study by Higashi et al. (1988) in adrenal P-450 (c21)-deficient patients, in which they characterized three clustered point mutations in the sixth exon on the P-450 (c21) gene which impaired expression of catalytic activity when the mutated DNAs were transfected into COS cells. The presence of point mutations may be a common mechanism for P-450 polymorphisms. However, to our knowledge, P-450g is the first example of a phenotypic difference in expression of a P-450 protein resulting from point mutations in the mRNA.

Recent studies have shown, however, that minor point mutations in a P-450 mRNA can dramatically affect the substrate specificity of the P-450 protein. Lindberg and Negishi (1989) recently demonstrated that 2 mouse liver P-450s ($P\text{-}450_{15\alpha}$ and $P\text{-}450_{coh}$), which differed by only 11 amino acid residues, exhibited completely different substrate specificities when their cDNAs were expressed in Cos cells. $P\text{-}450_{15\alpha}$ hydroxylated testosterone at the 15α-position but did not hydroxylate coumarin. In contrast, $P\text{-}450_{coh}$ hydroxylated coumarin at a rate >100-fold higher than $P\text{-}450_{15\alpha}$. Site-directed mutagenesis studies demonstrated that the substitution of a single amino acid at position 209 (phenylalamine to leucine) in $P\text{-}450_{coh}$ resulted in a 100-fold increase in 15α-steroid hydroxylase activity. The substitution of three amino acids in $P\text{-}450_{coh}$ to the amino acids found in $P\text{-}450_{15\alpha}$ completely abolished coumarin hydroxylase activity. Therefore, it appears that a mutation of a single amino acid can completely alter the substrate specificity of a P-450.

One might predict that mutations in regions which are highly conserved among different P-450s would be more likely to deleterious than mutations in other areas. However, the highly conserved regions of cytochrome P-450s are the same in the (+g) and (−g) phenotypes. For example, the heme binding region between amino acids 428 and 448 containing the highly conserved cysteine at position 435 is not altered. The highly conserved threonine-301 and proline-404 (amino acid numbering of P-450g, Figure 1) (Gonzalez et al., 1988; Nelson & Strobel, 1988) are also present in both sequences. Matsunaga et al. (1988) reported that 39 amino acid residues are conserved among 14 rat amino acid sequences from the P-450I, -II, -III, and -IV gene families. Two of these residues are not present in P-450g (McClellan-Green et al., 1989). However, none of the remaining 37 conserved amino acids were altered by the 7 mutations found in the (−g) phenotype.

The cluster of mutations present in the sequence of the (−g) cDNA at residues 234, 237, and 240 occurs in an established area of hypervariability between P-450s (Nelson & Strobel, 1988) and may represent a substrate binding site for the cytochromes. Mutations in this area, such as those present in (−g), may conceivably produce an unstable protein that is rapidly degraded. It is also possible that the substitution of leucine for proline in the (−g) sequence at residue 22 could change the insertion pattern of the P-450 into the membrane. A recent paper by Sakaguchi et al. (1987) suggests that a short amino-terminal segment (<29 residues) of the cytochrome P-450 functions as both an insertion signal and as a stop–transfer sequence. On the basis of studies with plasmid constructs, they suggest that the important part of the signal is present in residues 21–29. In this event, the amino acid change from proline to a more hydrophobic leucine at residue 22 might cause incomplete or improper insertion of the cytochrome into the membrane, leading to its rapid degradation. Additional studies using site-specific mutagenesis are planned in our laboratory to elucidate the relative contributions of each of these changes in producing a defective polypeptide.

In summary, sequencing of the cDNA for P450 (−g) has revealed nine single base changes in the coding region from the sequence of the (+g) cDNA, which would result in seven amino acid substitutions in the (−g) phenotype. Using specific probes for (+g) and (−g) mRNA, we have shown that three of these base changes occur in vivo in (−g) rats, demonstrating unequivocally that phenotypic differences in hepatic levels of P-450g are due to the expression of a variant mRNA for P-450g in the low phenotype containing nine single base mutations. To our knowledge, this is the first report that a phenotypic difference in P-450 expression results from simple point mutations in the mRNA.

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Registry No. Cytochrome P-450, 9035-51-2; DNA (rat liver g phenotype cytochrome P-450g mRNA complementary), 124153-04-4; cytochrome P-450g (rat liver g phenotype protein moiety reduced), 124153-05-5.

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