Identification of Residues 99, 220, and 221 of Human Cytochrome P450 2C19 as Key Determinants of Omeprazole Hydroxylase Activity*

(Received for publication, January 4, 1996, and in revised form, February 19, 1996)

Gordon C. Ibeanu, Burhan I. Ghanayem, Patricia Linko, Leiping Li, Lee G. Pedersen, and J Joyce A. Goldstein†

From the NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709

Human P450 2C19 is selective for 4'-hydroxylation of S-mephenytoin and 5-hydroxylation of omeprazole, while the structurally homologous P450 2C9 has low activity toward these substrates. To identify the critical amino acids that determine the specificity of human P450 2C19, we constructed chimeras of P450 2C9 replacing various proposed substrate binding sites (SRS) with those of P450 2C19 and then replaced individual residues of P450 2C9 by site-directed mutagenesis. The 339 NH2-terminal amino acid residues (SRS-1–SRS-4) and amino acids 160–383 (SRS-2–SRS-5) of P450 2C19 conferred omeprazole 5-hydroxylase activity to P450 2C9. In contrast, the COOH terminus of P450 2C19 (residues 340–490 including SRS-5 and SRS-6), residues 228–339 (SRS-3 and SRS-4) and residues 292–383 (part of SRS-4 and SRS-5) conferred only modest increases in activity. A single mutation Ile99 → His increased omeprazole 5-hydroxylase to ~51% of that of P450 2C19. A chimera spanning residues 160–227 of P450 2C19 also exhibited omeprazole 5-hydroxylase activity which was dramatically enhanced by the mutation Ile99 → His. A combination of two mutations, Ile99 → His and Ser200 → Pro, converted P450 2C9 to an enzyme with a turnover number for omeprazole 5-hydroxylation, which resembled that of P450 2C19. Mutation of Pro221 → Thr enhanced this activity. Residue 99 is within SRS-1, but amino acids 220 and 221 are in the F-G loop and outside any known SRS. Mutation of these three amino acids did not confer significant S-mephenytoin 4'-hydroxylase activity to P450 2C9, although chimeras containing SRS-1–SRS-4 and SRS-2–SRS-5 of P450 2C19 exhibited activity toward this substrate. Our results thus indicate that amino acids 99, 220, and 221 are key residues that determine the specificity of P450 2C19 for omeprazole.

The P450s cytochromes represent a ubiquitous superfamily of monooxygenases, which metabolize a vast array of endogenous and exogenous substrates (1–3). Multiple P450 enzymes appear to have arisen from a single ancestral gene by duplication and diverged by mutation and gene conversion to produce families of structurally related enzymes with overlapping but often distinct substrate specificities. The regio- and stereoselectivity of specific enzymes for particular substrates appears to be encoded in certain defined regions of the primary sequence. Considerable progress has been made in recent years in elucidating the structural determinants of substrate specificity (4). In some cases, substrate specificity between highly related members of the same subfamily has been shown to be defined by a few critical residues or even a single amino acid (5).

P450 2C19 is a member of the human CYP2C subfamily, which includes four structurally related enzymes (6). P450 2C9 and 2C19 are the most highly conserved of these forms, showing 91% structural identity, but have very distinctive substrate specificities. For example P450 2C19, which is polymorphic in man, is the principal enzyme responsible for the stereoselective 4'-hydroxylation of S-mephenytoin (7) and is highly selective for the 5-hydroxylation of the popular anti-ulcer drug omeprazole (46). The structurally related P450 2C9 exhibits little activity toward either of these substrates, but exhibits a high turnover number for hydroxylation of tolbutamide and phenytoin and specifically 7-hydroxylates S-warfarin (6). The present studies were designed to elucidate the key amino acids that determine the marked specificity of P450 2C19 for omeprazole and S-mephenytoin.

Since none of the mammalian P450 enzymes have been crystallized, the experimentally derived three-dimensional crystal structures of the bacterial enzymes, P450cam, P450BM-3, and P450terp, have served as models for predicting their structure and function. The current paradigm for such predictions has focused on alignment-based sequence similarities, α- and β-secondary structure, hydropathy indices, computerized modeling based on the crystal structures of the bacterial P450 enzymes and protein engineering using chimeric constructs and site-directed mutagenesis (8–12). Although the amino acid sequences of the soluble bacterial P450 differ considerably from those of the membrane-bound mammalian P450 enzymes, these methods have proven resourceful in identifying regions that determine the substrate specificities of the mammalian proteins.

Gotz et al. (13) predicted six potential substrate recognition sites (SRS) in the mammalian family 2 P450 enzymes based on an alignment with bacterial P450cam whose substrate-binding residues have been identified by x-ray crystallography and analysis of mutations that altered substrate specificity in experimental studies of the P450 2 subfamily. For example, site-directed mutagenesis studies have shown that the substrate and regiospecificity of the 2C subfamily of cytochrome P450 can be altered by substitution of critical amino acid residues at positions 112 to 115, 301, 359, 364 (14–19). Replacement of residues 117, 209, 365, and 481 in the 2A subfamily (5, 20–23) as well as residues 114, 206, 302, 363, 367, and 478 (24–28) in the 2B subfamily and residue 380 (29) in 2D1 have also been documented to alter substrate or regiospecificity.

To identify key amino acid residues responsible for the...
Sequence alignment and comparative analysis of amino acid variations system. Initial chimeras replaced regions of P450 2C9 with P450 2C9. Constructs were expressed in a yeast chimeras and mutant enzymes from the structurally related omeprazole and marked specificity of human P450 2C19 for two key substrates, cytosomes—transformation of yeast and preparation of recombinant microsomes—

### Table I

| Mutant | Amino Acid Change |
|--------|-------------------|
| 199H   | S220P/P221T       |
| Y243D  | S220P/P221T       |
| M325I  | S220P/P221T       |
| I99H   | S220P/P221T       |

Marked specificity of human P450 2C19 for two key substrates, omeprazole and S-mephenytoin, we constructed a total of 24 chimeras and mutant enzymes from the structurally related P450 2C9. Constructs were expressed in a yeast cDNA expression system. Initial chimeras replaced regions of P450 2C9 with similar segments of P450 2C19, using restriction enzymes sites located outside the six putative substrate binding domains proposed by Gotoh (13). Subsequently, we used primary sequence alignment and comparative analysis of amino acid variations within the appropriate segments of the human P450 2C proteins to identify residues that might play a role in the substrate specificity of P450 2C19. Site-directed mutagenesis was then used to identify key residues responsible for the substrate specificity of P450 2C19.

### Experimental Procedures

**Chemicals and Reagents**—Restriction and modification enzymes were purchased from New England Biolabs and sequencing kits and deoxyadenosine 5'-[35S]thiotriphosphate from Amersham Life Sciences. 32P-Labeled omeprazole (53 mCi/mmol), a gift from Glaxo Research and Development (Hertfordshire, United Kingdom [UK]), was unstable and therefore periodically purified to ~98% homogeneity by HPLC and stored at ~70°C under absolute ethanol. 32P-Labeled S-mephenytoin (20.7 mCi/mmol) was prepared as described previously (7). Purified P450 reductase was obtained from Human Biologics Inc.

**Transformation of Yeast and Preparation of Recombinant Microsomes**—The cloning of P450 2C9 and P450 2C19 into the yeast plasmids—

**Amino Acid Changes**—Amino acid changes were introduced in P450 2C9 and P450 2C19 by using synthetic oligonucleotides (Table I) containing the desired point mutations and a second primer, P450 2C9 and the 2C9/2C19 hybrids using synthetic oligonucleotides (13) with minor alterations (30). Microsomes were isolated from the pooled colonies was digested to linearize non-mutated repair-deficient bacteria strain, was transformed. Plasmid DNA isolated from the pooled colonies was digested to linearize non-mutated

**Restriction and Modification Enzymes**—Chemicals and Reagents—

**Enzymatic Assays**—S-Methylenzo 4-hydroxylation was assayed as described previously (7) except that cytochrome P450 was not added to the reactions. To assay omeprazole 5-hydroxylation, recombinant yeast microsomes were preincubated at 37°C for 5 min with purified P450 2C9 reductase (100 units/10 ml of P450) and dilauroylphosphatidylcholine (0.3 mg/ml of P450) and then placed on ice. 14C-Labeled omeprazole (400 μM, 22 mCi/mmol) was added to 50 ml HEPES buffer (pH 7.4) containing 0.1 mM EDTA and 1.5 mM MgCl2. The reaction was preincubated at 37°C with shaking for 5 min, initiated by addition of NADPH (2 mM), and terminated with equal volume of methanol after 15 min. After centrifugation, products were analyzed by reverse phase HPLC using an isocratic solvent phase consisting of 45% methanol and 55% water. Detection and quantitative analysis of radioactive peaks was accomplished through on-line radiochemical detector.

**RESULTS AND DISCUSSION**

Chimeras—Twenty-four chimeric 2C9/2C19 and mutant P450 2C9 proteins were analyzed for their ability to hydroxylate omeprazole and S-mephenytoin. The structures of the chimeras and their relationships to the SRS proposed by Gotoh (13) are shown in Fig. 1A. The 2C9/2C19 chimera exhibited only a slight increase in omeprazole 5-hydroxylation activity over the wild-type P450 2C9, suggesting that the carbonyl terminus of P450 2C9 has relatively minor influence on its capacity to metabolize omeprazole (Fig. 2). Although this region encompasses SRS-5 and SRS-6, it contains some of the most highly conserved P450 domains including the heme-binding region. P450 cytochromes 2C9 and 2C19 differ by only 11 amino acids in this 152-residue domain, with a preponderance of conservative replacements. It is, therefore, not surprising that this region conferred only a small increase in omeprazole hydroxylase activity. In contrast, the chimeric 2C9/2C19 chimera contained 12 amino acid changes at the NH2-terminal end, which were not present in the 2C9/2C19 chimera, presumably accounting for the slightly higher activity of the former protein. Two other chimeric proteins containing only parts of the 160-383 fragment of 2C9, i.e. 2C9/2C19 and 2C9/2C19 chimera spanning SRS-2, SRS-5 of 2C19. The 2C9/2C19 chimera represented with residues 160-339, suggesting that this region may be required to substrate specificity. The 2C9/2C19 chimera contained 12 amino acid changes at the NH2-terminal end, which were not present in the 2C9/2C19 chimera, presumably accounting for the slightly higher activity of the former protein. Two other chimeric proteins containing only parts of the 160-383 fragment of 2C9, i.e. 2C9/2C19 and 2C9/2C19 chimera spanning SRS-2, SRS-5 and SRS-4, exhibited a smaller increase (3-7-fold) in omeprazole hydroxylase activity than the 2C9/2C19 chimera, suggesting that the region spanning residues 160-227 of P450 2C19 could be involved in substrate specificity.

**Mutants**—We initially generated five single amino acid mutants I99H (m1), Y243D (m2), M325I (m3), S451F (m4), and S451F (m2) and combinations of these mutations (m3, m4, m5, m3, m145, m1245, and m1345) in P450 2C9 (Fig. 1B). Each amino acid was mutated to the corresponding amino acid in P450 2C9. The amino acids were chosen as residues that might be involved in substrate recognition based on alignment with P450cam and mouse 2A4/5 and selection of amino acids in previously.
P450 2C19 that were unique compared to other members of the P450 2C subfamily. Ile99 → His was chosen because it is the only difference between P450 cytochromes 2C9 and 2C19 in SRS-1, and His99 is unique to P450 2C19. Ile257 is a unique amino acid in P450 2C19. Tyr243 → Asp is a unique nonsynonymous amino acid change near SRS-3. Ile362 corresponds to contact residue Val296 in P450cam and is the only unique amino acid in SRS-5. Ser451 → Phe is a unique nonsynonymous change located between SRS-5 and SRS-6. Of the single amino acid mutants, only I99H (Ile99 → His) in SRS-1 increased omeprazole 5-hydroxylase activity markedly (Fig. 3). The other mutations had essentially no effect. Multiple mutants containing the Ile99 → His mutation had comparable activity to that of I99H. In contrast, multiple mutants m34 and m45, which did not contain the Ile99 → His mutation, exhibited no increase in omeprazole 5'-hydroxylase activity (data not shown). Residue 99 maps to the interhelical region of B and B' helices, three residues from the NH₂ terminus of B', which comprises part of the substrate-contact loop in the structure of P450cam (36–38). Many mutations that alter the substrate specificity of members of the 2A, 2B, and 2C subfamilies of cytochrome P450 map to the B'-C interhelical region near the carboxyl terminus of SRS-1 (5, 39–43). Three-dimensional analysis of P450cam, P450terp, and P450BM-3 suggests that this substrate-contact loop, the largest in P450, extends from proximal end of the β1–5 sheet through the distal end of the B'-C coil (8). His99 of P450 2C19 corresponds to Pro87 of P450cam in the alignment of Gotoh (13), which is one of three amino acids in SRS-1 that contact camphor. Moreover, it represents the only amino acid change between P450 2C9 and P450 2C19 in SRS-1 and is the only residue in SRS-1 that is unique to P450 2C19. This change involves the replacement of a nonpolar, hydrophobic isoleucine with a polar and possibly charged histidine residue. This polar histidine has the capacity for hydrogen bonding. It is possible that the residue is involved in substrate binding or alternatively changes the configuration of the active site. Comparison of P450 2C19 with a three-dimensional model of P450 2B1 (19) predicts that this amino acid would be approximately 15 Å from the center of the substrate binding pocket. Alternatively, when omeprazole is docked into binding pocket in the three-dimensional model of P450cam, we find that Pro87 (which aligns with His99 in 2C19) is near the docked substrate (approximately 6 Å) as shown in Fig. 4. Moreover, the adjacent Phe88 of P450cam is even closer to the substrate (≈2 Å). Thus, the effect of the Ile99 → His mutation is consistent with this model.

Analysis of Residues 160–227—Analysis of the chimeric data indicated that amino acids 160–227 may also play a crucial role in the specificity of P450 2C19 for omeprazole. We therefore constructed a 2C9/2C19160–227 chimera and an identical construct containing an Ile99 → His mutation. The 2C9/2C19160–227 chimera exhibited a 7-fold increase in omeprazole 5-hydroxylase activity compared to P450 2C9 (Fig. 5). More-


over, when combined with the an Ile<sup>99</sup> → His mutation, this chimera exhibited a dramatic 38-fold increase in activity. The activity of this mutated chimera was even greater than that of native P450 2C19.

We next sequentially modified amino acids in this region to those of the corresponding residues in P450 2C19 as shown in Fig. 1B. Six amino acids of P450 2C19 (three conservative and three nonconservative changes) differ from those of P450 2C9 between amino acids 160 and 227, with five of these mapping within 20 tandem amino acid residues of the carboxyl end of SRS-2. Mutations Lys<sup>206</sup> → Arg (K206R), Leu<sup>208</sup> → Val (L208V), Pro<sup>221</sup> → Thr (P221T), and Ser<sup>220</sup> → Thr in combination with Ile<sup>99</sup> → His did not increase omeprazole 5'-hydroxylase activity over that of I99H alone (Fig. 5), indicating that these residues are not the critical amino acids in this region. However, mutation of the polar serine at position 220 to a nonpolar hydrophobic proline residue (Ser<sup>220</sup> → Pro) increased activity to ~90% of that of P450 2C19. When a second nonconservative mutation was made in the tandem residue at position 221 (P221T) in conjunction with S220P and I99H, the omeprazole hydroxylase activity of the triple mutant (I99H/S220P/P221T) was equivalent to that of P450 2C19. Sequence alignment indicates that residues 220 and 221 reside midway between the COOH-terminal end of the F-helix and the NH<sub>2</sub>-terminal end of G-helix in the interhelical loop, outside of any known substrate binding sites. This region corresponds to the

FIG. 2. Omeprazole 5-hydroxylase activity of chimeras of P450 2C9 and P450 2C19 expressed in yeast. Construction of chimeras is shown in Fig. 1. The omeprazole hydroxylase activity was measured as described under “Experimental Procedures.”

FIG. 3. Omeprazole 5-hydroxylase activities of site-directed mutants of P450 2C9. Omeprazole hydroxylase activity is given as the means ± S.E. The single mutations are numbered m1–m5, with amino acid substitutions indicated in parentheses, and m134, m145, m1345, and m1245 represent the corresponding multiple mutants. Three other mutant enzymes, m2 (S451F), m34, and m45 (not shown), did not affect omeprazole hydroxylase activity.

FIG. 4. Model showing omeprazole docked in the substrate-binding pocket of P450 cam. Thr<sup>253</sup> is the highly conserved Thr in the I helix. The heme is shown in red, amino acid residues in blue, and substrate in black.

FIG. 5. Analysis of the contribution of His<sup>99</sup> and region 160–227 of 2C19 to omeprazole 5-hydroxylase activity using chimeras and site-directed mutants. The construction of the 2C9/2C19<sub>160–227</sub> chimera is shown in Fig. 1. A second 2C9/2C19<sub>160–227</sub> chimera contained the Ile<sup>99</sup> → His mutation. Individual amino acids in the 160–227 region were mutated in P450 2C9 in combination with the Ile<sup>99</sup> → His mutation as shown in Fig. 1B. Values represent means ± S.E.
could allow strong hydrogen bonding with His99. However, the branched structure of S-mephenytoin may require additional three-dimensional accommodation in the active site. These results suggest that specificity of P450 2C19 for S-mephenytoin may require a more complex enzyme configuration than that for omeprazole, possibly involving multiple substrate recognition domains acting in concert.

Conclusions—Chimeric proteins and amino acid mutations have been widely used to determine critical residues responsible for substrate specificity of closely related P450 proteins. We have utilized this approach to identify amino acids that are critical determinants of omeprazole 5-hydroxylase activity of human P450 2C19. We first identified regions important in substrate specificity by analyzing the catalytic activity of chimeras and then sequentially replaced individual residues in P450 2C9 by site-directed mutagenesis. We have identified three key amino acids at positions 99, 220, and 221 which are critical determinants of omeprazole 5-hydroxylase activity. These residues map to SRS-1 and the F-G loop.

Acknowledgments—We express our gratitude to Glaxo Research and Development (Hertfordshire, UK) for the generous gift of 14C-labeled omeprazole. We also thank Dr. James R. Halpert, University of Arizona, Tucson, for generously providing the coordinates for a structural model of cytochrome P450 2B1 and for a preprint of a manuscript in press (19). We thank Tamara McIntyre, Heath LeFevres, and Angela Stanley for HPLC analysis of omeprazole metabolites.

REFERENCES

1. Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyerisen, R., Gonzalez, F. J., Coon, M. J., Gunzilus, I. C., Gotoh, O., Okuda, K., and Nebert, D. (1993) DNA Cell Biol. 12, 1–51
2. Porter, T. D., and Coon, M. J. (1991) J. Biol. Chem. 266, 13469–13472
3. Guengerich, F. P. (1991) J. Biol. Chem. 266, 10019–10022
4. J ohnson, E. S. (1992) Trends Pharmacol. Sci. 13, 122–126
5. Lindberg, R., and Negishi, M. (1989) Nature 340, 632–634
6. Goldstein, J. A., and de Morais, S. M. F. (1994) Pharmacogenetics 4, 285–299
7. Goldstein, J. A., Faletto, M. B., Romkes-Sparks, M., Sullivan, T., Kitaeevan, S., Raucy, J. L., Lasker, J. M., and Ghanayem, B. I. (1994) Biochemistry 33, 1743–1752
8. Hasemann, C. A., Kurumbali, R. G., Boddupalli, S. S., Peterson, J. A., and Disenhoffer, J. (1995) Structure (Lond.) 3, 41–62
9. Laughton, C. A., Neidle, S., Zveibil, M. J., and Sternberg, J. M. (1990) Biochim. Biophys. Res. Commun. 171, 1160–1167
10. Laughton, C. A., Zveibil, M. J., and Neidle, S. (1993) J. Steroid Biochem. Mol. Biol. 44, 399–407
11. Lewis, D. F. V., and Moereels, H. (1992) Comput-Aided. Mol. Design 6, 235–252
12. Zveibil, M. J., Wolf, J. M., and Sternberg, M. J. E. (1993) Proteins 4, 271–292
13. Gotoh, O. (1992) J. Biol. Chem. 267, 83–90
14. Straub, P., Lloyd, M., Johnson, E. F., and Kemper, B. (1993) J. Biol. Chem. 268, 21997–22003
15. Straub, P., Johnson, E. F., and Kemper, B. (1993) Arch. Biochem. Biophys. 306, 521–527
16. Ishi, M., and Nakamura, M. (1989) Biochim. Biophys. Res. Commun. 158, 717–722
17. Sullivan, T. H., Ghanayem, B. I., Bell, D. A., Zhang, Y. Z., Kaminisky, L. S., Shenfield, G. M., Miners, J. O., Birkett, D. J., and Goldstein, J. A. (1996) Pharmacogenetics, in press
18. Richardson, T. H., and Johnson, E. F. (1994) J. Biol. Chem. 269, 23937–23943
19. Szklar, G. D., He Y.-a., and Halpert, J. R. (1995) Biochemistry, 34, 13132–13139
20. Juvonen, R., O., Iwasaki, M., and Negishi, M. (1991) J. Biol. Chem. 266, 16431–16435
21. Iwasaki, M., Lindberg, R. L. P., Juvonen, R. N., and Negishi, M. (1993) Biochem. J. 291, 569–573
22. Iwasaki, M., Darden, T. A., Pedersen, L. G., Davis, D. G., Juvonen, R. O., Sueyoshi, T., and Negishi, M. (1993) J. Biol. Chem. 268, 759–762
23. Iwasaki, M., Darden, T. A., Pedersen, L. G., and Negishi, M. (1994) Biochemistry 33, 5054–5059
24. Aoyama, T., Koriyama, K., Nagata, K., Adesnik, M., Reiss, A., Lapenson, D. P., Gillette, J., Noll, H. V., Waxman, D. J., and Gonzalez, F. J. (1989) J. Biol. Chem. 264, 21327–21333
25. Kedzie, K. M., Balfour, C. A., Escobar, G. Y., Grimm, S. W., He, Y., Peppinell, D. J., Regan, J. W., Stevens, J. C., and Halpert, J. R. (1991) J. Biol. Chem. 266, 22535–22521
26. He Y.-a., Luo, Z., Kletsova, P. A., Burnett, V. L., and Halpert, J. R. (1994) Biochemistry 33, 4419–4424
27. Halpert, J. R., and He Y.-a. (1993) J. Biol. Chem. 268, 4453–4457
28. Hasler, J. A., Harlow, G. R., Szklar, G. D., Oh, G. H., Kedzie, K. M., Burnett, V. L., Kaminsky, L. S., and Halpert, J. R. (1994) Mol. Pharmacol. 46, 338–343
29. Matsunaga, E., Zeugin, T., Zanger, U. M., Aoyama, T., M., and Y. U., A. and
Determinants of Substrate Specificity of P450 2C19

Gonzalez, F. J. (1990) J. Biol. Chem. 265, 17197–17201
30. Faletto, M. B., Linko, P., and Goldstein, J. A. (1992) J. Biol. Chem. 267, 2032–2037
31. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 200, 81–88
32. Rose, M. D., Winson, F., and Hieter, P. (1990) in Methods in Yeast Genetics (Rose, M. D., Winson, F., and Hieter, P., eds) pp. 180–187, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
33. Oeda, K., Sakaki, T., and Ohkawa, H. (1985) DNA (N. Y.) 4, 203–210
34. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
35. Omura, T., and Sato, R. (1964) J. Biol. Chem. 239, 2370–2378
36. Pollos, T. L., Finzel, B. C., and Howard, A. J. (1987) J. Mol. Biol. 195, 687–700
37. Atkins, W. M., and Sligar, S. G. (1988) J. Biol. Chem. 263, 18842–18849
38. Nelson, D. R., and Strobel, H. W. (1989) Biochemistry 28, 656–660
39. Lin, D., Black, S. M., Nagahama, Y., and Miller, W. M. (1993) Endocrinology 132, 2498–2506
40. Kronbach, T., Larabee, T. M., and Johnson, E. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8262–8265
41. Kronbach, T., Kemper, B., and Johnson, E. F. (1991) Biochemistry 30, 6097–6102
42. Uno, T., and Imai, Y. (1992) J. Biochem. (Tokyo) 112, 155–162
43. Johnson, E. F., Kronbach, T., and Hsu, M.-H. (1992) FASEB J. 6, 700–705
44. Uno, T., and Imai, Y. (1989) J. Biochem. (Tokyo) 106, 569–574
45. Luo, Z., He, Y.-a., and Halpert, J. R. (1994) Arch. Biochem. Biophys. 309, 52–57
46. Ghanayem, B. I., Karam, W. G., and Goldstein, J. A. (1994) Sixth North American International Society for the Study of Xenobiotics Meeting, October 23–27, 1994, Raleigh, NC, (abstr.), p. 50