Sequence Requirements for Cytochrome P-450 IID1 Catalytic Activity

A SINGLE AMINO ACID CHANGE (ILE\textsuperscript{380} → PHE) SPECIFICALLY DECREASES $V_{\text{max}}$ OF THE ENZYME FOR BUFURALOL BUT NOT DEBRISOQUINE HYDROXYLATION

Eiji Matsunaga, Tanya Zeugin, Ulrich M. Zanger, Toshifumi Aoyama, Urs A. Meyer, and Frank J. Gonzalez

From the Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 and the Department of Pharmacology, Biocenter, University of Basel, CH-4056 Basel, Switzerland

A cDNA coding for an allelic variant of rat IID1, designated IIDlv, was isolated that produced a P-450 having a 10-fold lower catalytic activity toward the substrate bufuralol when expressed in COS-1 cells (Matsunaga, E., Zanger, U. M., Hardwick, J. P., Gelhin, H. V., Meyer, U. A., and Gonzalez, F. J. (1989) Biochemistry, 28, 7349-7355). IID1 and IIDlv cDNA-deduced proteins differed in sequence by 4 amino acid residues. IID1 has Val, Phe, Arg, and Leu while IIDlv has Ile, Leu, Glu, and Phe at amino acid positions 123, 124, 173, and 380, respectively. Chimeric cDNAs between IID1 and IIDlv were constructed and expressed in hepatoma cells using vaccinia virus. A chimera having the Phe (IID1v) at amino acid 380, with the remaining 3 variant amino acid residues of IID1, was found to have a 10-fold decrease in $V_{\text{max}}$ and a 2 to 3-fold decrease in $K_m$ for (+)-bufuralol 1'-hydroxylation when compared to a converse chimera having Ile (IID1) in a background of IIDlv sequence. Although this enzyme lacked significant bufuralol metabolism, it was able to carry out debrisoquine 4-hydroxylation. In contrast, the chimera having Ile (IID1) at position 380 was lacking in debrisoquine 4-hydroxylation. Type I difference spectra analysis revealed that both forms could bind debrisoquine with similar spectral dissociation constants. These data demonstrate that the single amino acid substitution $\text{Ile}^{380} \rightarrow \text{Phe}$ differentially decreases the catalytic activity of IID1 toward bufuralol but not debrisoquine.

Heptic microsomal cytochrome P-450s\textsuperscript{1} are among the principal enzymes involved in metabolism of foreign compounds, including plant chemicals, drugs, and chemical carcinogens. Many forms of P-450 have been characterized from rodents and humans (Guenigerch, 1987). Although the precise number of different forms of P-450 in these organisms is not yet known, it is generally believed that between 60 and 200 are expressed in a given animal (Nebert and Gonzalez, 1987; Nebert et al., 1989). Moreover, P-450s are capable of metabolizing numerous structurally diverse compounds. A single chemical can also be metabolized by several different P-450s, albeit at different rates. These broad and overlapping substrate specificities are accompanied by rather low turnover numbers of usually less than 60 min\textsuperscript{-1}. In contrast, most enzymes of intermediary metabolism can have turnover numbers of up to several thousand min\textsuperscript{-1}.

Nine families of P-450s are expressed in all mammals (Nebert et al., 1989). Three of these, the CYP1, CYP2, and CYP3 families, are involved in the majority of foreign compound metabolism. A large number of P-450s that share high sequence similarity are found within the eight subfamilies in the CYP2 family. In addition, allelic variants of certain P-450 forms have been identified (Gonzalez, 1988). Many of these enzymes with high sequence similarity have distinct catalytic activities. These can be used to delineate those amino acid residues that are involved in substrate-binding and hydroxylation site specificities.

Recently, we isolated cDNAs for two allelic forms of rat P-450IID1, designated IID1 and IIDlv (Matsunaga et al., 1989). cDNA-expression in COS cells revealed that IID1 had a 10-fold higher rate of bufuralol metabolism than IIDlv, even though these enzymes exhibited only 4 amino acid differences. In this report, we have determined through construction and expression of a series of chimeric cDNAs, that a Phe residue at amino acid 380 is responsible for the deficient bufuralol metabolism of IIDlv. We also demonstrate that debrisoquine 4-hydroxylation is not significantly affected by this amino acid change. These studies establish that a single amino acid alteration can substantially decrease activity toward one substrate but not toward another.

EXPERIMENTAL PROCEDURES

Materials - The cDNA clones for rat IID1 and IIDlv have been characterized previously (Matsunaga et al., 1989). Vaccinia virus strain WR and the vector pSC11 were obtained from Dr. Bernard Moss of the National Institutes of Health. H4-II-E-C3 rat hepatoma cells (ATCC CRL 1600) were purchased from the American Type Culture Collection. (-)-Bufuralol, (+)-bufuralol, 1'-hydroxybufuralol, and debrisoquine were gifts from Hoffman-La Roche, Basel.

Construction of Chimeric cDNAs and cDNA Expression -Chimeras were produced between IID1 and IIDlv cDNAs using restriction endonuclease sites common between the two DNAs (Fig. 1). Chimeras A and B were constructed using a common ApaI site that cleaves at nucleotides 1015 and 1016 in IIDlv and 969 and 970 in IID1. The chimeric cDNAs in pSC11 were inserted into vaccinia virus using the procedures outlined previously (Mackett et al., 1984; Chakrabarti et al., 1985; Aoyama et al., 1989). Recombinant vaccinia viruses were used to infect either rat

\textsuperscript{1}The cost of publication of this article was defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{2}The nomenclature used in this report is that described by Nebert et al. (1989). IID1 is encoded by the CYP2D1 gene. IIDlv is believed to be an allelic variant of IID1 (Matsunaga et al., 1989).
hepatoma cells H4-II-E-C3 (H4) or human hepatoma cells HepG2.

Analysis of cDNA-expressed P-450—Cells transfected with pCMV4 plasmids and cells infected with recombinant vaccinia were harvested after 48 h and washed with phosphate-buffered saline (Biofluids Inc., Rockville, MD). Cells were suspended in phosphate-buffered saline and lysed by brief sonication. Western immunoblots were prepared as described earlier (Matsunaga et al., 1989) using the human autoantibody anti-LKM1 (Zanger et al., 1988a) and peroxidase-labeled goat anti-human IgG. The immune complexes were visualized using dia-minoazobenzoic acid. Type I substrate binding difference spectra were carried out on lysate from vaccinia virus-infected cells dissolved in phosphate-buffered saline containing 20% (w/v) glycerol as described by Dekkoit (1978). Briefly, the samples were divided into two cuvettes and debrisoquine, dissolved in H2O, was added to the sample cuvette. An equal volume of H2O was added to the reference cuvette and difference spectra were recorded using an Amino DW-2000 spectrophotometer. The difference between the peak at 390 nm and trough at 428 nm was determined as a function of debrisoquine concentration. Spectral dissociation constant (Kd) values were then calculated. CO-reduced difference spectra were determined on total cell lysate dissolved in phosphate-buffered saline, 20% (w/v) glycerol, and 0.3% Emulgen 913 as described by Omura and Sato (1964). Bufuralol 1'-hydroxylase activity was determined with (+)- and (-)-bufuralol in whole cell extracts as described by Zanger et al. (1988b) and modified as in Matsunaga et al. (1989). In some cases, (+)-bufuralol was added directly to the media of transfected cells and the levels of 1'-hydroxy metabolites were quantified after 6 h. Debrisoquine 4'-hydroxylase activity was measured as described by Kronbach et al. (1987).

RESULTS

In an earlier study we isolated and characterized two cDNAs that differ at only five nucleotides resulting in 4 amino acid substitutions at position 123, 124, 173, and 380 (Gonzalez et al., 1987; Matsunaga et al., 1989). IID1 has Val, Phe, Arg, and lle, respectively, at these positions while IIDlv has lle, Leu, Gin, and Phe, respectively. Despite these few amino acid differences, IIDlv expressed in COS cells had 10-fold less bufuralol 1'-hydroxylase activity than IID1 (Matsunaga et al., 1989). To determine which residue caused deficient bufuralol metabolism in IIDlv, chimeric cDNAs were constructed that contained various combinations of those 4 amino acids as shown in Fig. 1. Preliminary experiments were carried out to determine which chimeric P-450s possess (+)-bufuralol 1'-hydroxylase activity. At first, chimeric cDNAs were inserted into the COS cell vector pCMV4 as described (Matsunaga et al., 1989) and the constructs transfected onto COS-1 cells. Cells transfected with each plasmid were then lysed and assayed for (+)-bufuralol 1'-hydroxylase activities. Chimeric cDNAs A and C expressed P-450s having about 10-fold higher activities than those produced by chimeras B and D (Table I). These results were found when lysates were assayed in the presence of NADPH and O2 or with cumene hydroperoxide (CuOOH) (Zanger et al., 1988b). In addition, when cells were incubated with (+)-bufuralol, only those expressing the A and C chimeras P-450s were able to metabolize the drug in situ, as assessed by accumulation of 1'-hydroxybufuralol in the medium (Table I). These results revealed that P-450 chimeras A and C had catalytic activities similar to that of IID1, whereas P-450 chimeras B and D were more similar to IIDlv. Chimera A-P-450 differed from IID1 by only a single amino acid at position 380, by having an lle instead of Phe (Fig. 1). From these experiments it appeared that the amino acid at position 380 determined the extent of catalytic activity of IID1 toward bufuralol. We therefore focused further experimentation on P-450 chimeras A and B that represent IID1- and IIDlv-like P-450s, with respect to bufuralol 1'-hydroxylase activity.

In order to increase the yield of expressed protein we inserted the chimeras A and D cDNAs into vaccinia virus. Vaccinia virus is able to produce substantially more P-450 than the COS cell expression system. This higher level of P-450 production allowed us to perform kinetic and spectral analysis on the expressed proteins.

Table I

| Chimaera | Cell Lysate (+)-bufuralol 1'-hydroxylase | 1'-hydroxybufuralol δ | Buffuralol 1'-hydroxyrate δ | Debrisoquine 4'-hydroxyrate δ |
|----------|----------------------------------------|------------------------|-----------------------------|-------------------------------|
| IID1     | 510 pmol/min/mg protein                | 850 ng/ml/h             | 19.0                        | 116                           |
| IIDlv    | 37 pmol/min/mg protein                 | 1293 ng/ml/h            | 1.60                        | 8.50                          |

* COS-1 cells were transfected with recombinant pCMV4 plasmid DNA, harvested 48 h later, lysed by sonication, and assayed for (+)-bufuralol 1'-hydroxylase activity. Results are the average of duplicate determinations.

** Cells were transfected and 200 μM (+)-bufuralol was added 48 h later. The media was removed after 6 h and 1'-hydroxybufuralol quantitated.

These values are higher than those above or in Matsunaga et al. (1989). This may be due to a higher efficiency of transfection of the DNA into the COS cells. The single ratios of bufuralol metabolism between IID1 and IIDlv within a single transfection experiment are very reproducible.

To determine the P-450 content of cells infected with vaccinia viruses containing the chimeric A and B cDNAs, CO-reduced difference spectra were measured. Both chimeric P-450s yielded Soret bands with λmax of 450 nm (Fig. 2). Cells expressing chimaera A also had a peak with λmax at 420 nm. This may represent denatured cytochrome. Cells infected with wild type virus contained little detectable P-450. In the experiment shown in Fig. 2, the P-450 chimeras A and B were produced at a level of 18 and 23 pmol/mg solubilized cell lysate, respectively. The levels of expression varied less than 20% between experiments. These data demonstrate that the degrees of expression of each chimaera were similar.

Western immunoblot analysis using the anti-LKM1 antibody specific for human and rat IID proteins was performed on lysate from cells infected with vaccinia viruses containing the chimeric cDNAs. Cells infected with chimaera A and chimaera B cDNAs contained an immunoreactive protein of similar mobility to IID1 expressed in rat liver (Fig. 3). No protein was detected in cells infected with wild type vaccinia virus.

Fig. 1. Structure of the IID1, IIDlv, and various chimeric cDNAs. The circled amino acids, indicated by the single letter code, are those that differ between cDNA-deduced proteins. The enzymes Nael and Apal were used to construct the chimeras. The solid and hatched bars are coding sequences for IID1 and IIDlv, respectively, and the open bars are noncoding regions of the cDNAs.
The level of expression of both chimeric P-450s was comparable.

Kinetics of bufuralol metabolism were measured to determine whether the impaired metabolism by chimera B P-450 is due to an altered \( V_{\text{max}} \) or \( K_m \). We first established that the formation of 1'-hydroxybufuralol was proportional to protein concentration and linear with time. Indeed, lysates from HepG2 cells expressing both chimeras were linear in product formation with up to 700 \( \mu \)g of protein/assay. At a protein content of 300 \( \mu \)g/ml, the reactions were linear for 15 min. We therefore performed kinetic analyses using 300 \( \mu \)g/ml of cell lysate protein and 10-min incubation times. The results, displayed in Fig. 4, revealed that chimera A is considerably more active than chimera B for hydroxylation of (+)-bufuralol. Results of two experiments, using either NADPH/O\(_2\) or CuOOH as oxygen donors, are shown in Table II. Chimera B P-450, containing Phe\(_{280}\) actually had a \( K_m \) of about one-third that of chimera A regardless of the oxygen source. The low rates of (+)-bufuralol metabolism by chimera B were due to an almost 17-fold lower \( V_{\text{max}} \). Another interesting finding was that the chimera A P-450 metabolized (+)- and (-)-bufuralol equally well, whereas chimera B favored the (+)-stereoisomer. The general specificities of expressed chimera A and B toward these isomers were comparable to IID1 and IID1v, respectively. It must be emphasized that full kinetic analysis of the parental P-450s was not carried out due to the lower levels of expression in the COS cell system and the inability to accurately measure levels of expressed protein by direct spectral determination. Therefore, chimeric and parental enzymes cannot be directly compared.

We next determined the activities of chimeras A and B toward debrisoquine 4-hydroxylation. Chimera B P-450 displayed considerable activity for debrisoquine 4-hydroxylation even though it produced a little 1'-hydroxybufuralol (Fig. 5). The \( K_m \) and \( V_{\text{max}} \) were 160 \( \mu \)M and 16 min\(^{-1}\), respectively.
contrast, chimera A had a $V_{max}$ of only 0.3 min$^{-1}$. In fact, the activity was too low to accurately determine a $K_m$ value. Analysis of COS cell expressed IID1 and IID1v revealed that both enzymes are capable of hydroxylating debrisoquine, although the latter P-450 had a 50% higher activity. These data would suggest that one or more of the 3 amino acids located at positions 123, 124, and 173 participate with the residue at position 380 to confer extent of debrisoquine 4-hydroxylase activity. Again, direct comparison of debrisoquine metabolism kinetics between parental and chimeric enzymes awaits expression of the parental forms in vaccinia virus.

Type I substrate-binding spectral analysis was carried out with debrisoquine on lysates of cells infected with recombinant vaccinia viruses. Typical Type I spectra with peak and trough values of 390 and 428 nm, respectively, were observed between substrate binding, as measured by changes in iron associated with the catalytic activities of this type of hemo-protein and lipid in the total cell lysate. Surprisingly, both these values are much greater than those published earlier for rat liver microsomes (Kupper et al., 1982) but only about 25-50% higher than our $K_m$ value for debrisoquine hydroxylation. Differences between $K_a$ and $K_m$ could be due to partitioning of substrate into the large amount of excess protein and lipid in the total cell lysate. Surprisingly, both chimeric P-450s bind debrisoquine with similar affinities indicating that the low debrisoquine metabolism by chimerA was due to an alteration in the catalytic rather than the substrate-binding properties of the enzyme. It must be considered, however, that correlations frequently do not exist between substrate binding, as measured by changes in iron spin state, and catalytic activities (Guengerich, 1983). In this regard, bufuralol was not able to elicit a Type I spectral change in IID1 so we could not compare its binding affinity with that of debrisoquine. The reasons why some actively metabolized substrates do not elicit measurable changes in the iron spin state is not clear.

DISCUSSION

Two regions of the P-450 molecule have been shown to be associated with the catalytic activities of this type of hemo-protein. The first region, sometimes called HR2 or proximal region, is located near to the carboxyl terminus of all P-450s and contains the Cys that is the fifth thiolate ligand to the heme iron at the active site. This was established first by sequence comparisons of mammalian and bacterial P-450s (Gotoh et al., 1983) followed by the crystal structure of the bacterial P-450$_{cam}$ (Poulos et al., 1985, 1986). Recent studies, using site-directed mutagenesis of rat P-450IA2, have further delineated the importance of conserved amino acids surrounding this residue (Shimizu et al., 1988). The second region has been termed the Thr region or the distal region and centers around the Thr residue at position 292 in P-450$_{cam}$ that is located at the heme surface (Poulos, 1985; Poulos et al., 1986). This residue is also conserved in many mammalian P-450s and when changed to a His via site-directed mutagenesis of two rabbit P-450s, caused a loss in their ability to hydroxylate or bind the substrates lauric acid and testosterone (Imai and Nakamura, 1988). Others found that the catalytic activity of P-450IA2 toward acetanilide hydroxylation was impaired when this and neighboring residues were changed by site-directed mutagenesis (Furuya et al., 1989). In addition, an alteration in regioselective hydroxylation was noted. The identification of the proximal and distal regions as being important for P-450 catalytic activities relied upon primary sequence comparisons and predictions based on P-450$_{cam}$ three-dimensional structure. It is uncertain, however, whether this structure can be used as a model for the mammalian P-450s given the chemical and physical difference between the membrane-bound mammalian and the soluble bacterial enzymes (Poulos, 1989).

The use of closely related P-450s with different substrate specificities is another approach that has been used to identify important amino acid residues in mammalian P-450s. Recently Lindberg and Negishi (1989) expressed two cDNAs that encoded enzymes having 11 amino acid differences and distinct substrate specificities; one hydroxylated testosterone at the 15α position, whereas the other was a coumarin 7-hydroxylase. By changing each amino acid in one P-450 to the corresponding amino acid in the other, it was determined that residue 209 played a major role in determining substrate specificity. When P-450$_{cam}$ is used as a model, this residue does not appear to be near to the active site or the putative substrate channel.

In the current report, we have used cDNAs encoding two allelic variants that differ by only 4 amino acids to decipher those residues required for substrate specificity of rat IID1 for bufuralol and debrisoquine. One residue, Ile$^{380}$ located just distal from the heme-binding Cys$^{446}$ appears to be associated with bufuralol 1'-hydroxylation. However, its spatial location within the enzyme is unknown due to the lack of a crystal structure of a mammalian P-450. It is noteworthy that position 380 in IID1 would appear to correspond to position 301 in P-450$_{cam}$ which lies on the surface of the molecule at the end of β-sheet 3, quite distant from the active site and substrate channel (Poulos et al., 1986). Again, it is still uncertain whether the structure of the bacterial enzyme in any way resembles that of a mammalian P-450. In any case, the single amino acid change Ile$^{380}$$\rightarrow$ Phe resulted in a markedly lower catalytic activity for bufuralol. This conclusion was deduced from comparing the two chimeric P-450s with COS cell-expressed parental P-450s. We also found that hydroxylation of the structurally unrelated substrate debrisoquine was high in the chimeric enzyme having low bufuralol 1'-hydroxylation activity and low in the chimeric enzyme having high bufuralol 1'-hydroxylase activity. Until the parental P-450s are analyzed for debrisoquine metabolism kinetics using the vaccinia virus expression system, it is impossible to tell whether the high and low level activities are due to the residue at amino acid 380, or perhaps more intriguingly, to two or more of the 4 variable residues analyzed in this study.

Our detailed kinetic and spectral analyses of the functional properties of the Ile$^{380}$ and Phe$^{380}$ enzymes allow some conclusions on the mechanistic basis for the observed change in substrate specificity. Two fundamentally different assay systems were applied using either NADPH/O$_2$ and NADPH P-450 oxidoreductase to test the monoxygenase function or the organic hydroperoxide CuOOH to assess the peroxygenase function of the enzymes. Substrate hydroxylation by the latter mechanism, which circumvents any electron transfer steps from NADPH via NADPH P-450 oxidoreductase to P-450, was equally affected by the amino acid change at position 380 as compared to the NADPH-dependent reaction. Therefore, an effect of the mutation on P-450 reduction by NADPH P-450 oxidoreductase seems unlikely. Furthermore, an effect on substrate-binding properties as cause for altered selectivity seems unlikely on the basis of similar spectral dissociation constants for debrisoquine of both enzyme forms and on the finding of even lower $K_m$ for bufuralol of the chimera with lower activity for this substrate. Taken together, these findings indicate that the observed change in substrate selectivity in this case may be predominantly caused by differences in
the rates of those catalytic events involving oxygen transfer from the enzyme active site to the various substrate molecules. We cannot, however, speculate on the structural basis for this change in catalytic activity in the absence of a valid model for the three-dimensional structure of mammalian P-450s. It is difficult to see, at this stage, how substitution of 1 nonpolar amino acid with another would have major effects on the conformation of the enzyme or would play important catalytic roles at the active site.

REFERENCES
Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1989) J. Biol. Chem. 264, 8222–8229
Aoyama, T., Yamano, S., Waxman, D. J., Lapenson, D. P., Meyer, U. A., Fisher, V., Tyndale, R., Inaba, T., Kalow, W., Gelboin, H. V., and Gonzalez, F. J. (1989) J. Biol. Chem. 264, 10388–10395
Chakrabarti, S., Brechling, K., and Moss, B. (1985) Mol. Cell. Biol. 5, 3403–3409
Furuya, H., Shimizu, T., Hatano, M., and Fujii-Kuriyama, Y. (1989) Biochem. Biophys. Res. Commun. 160, 669–676
Gonzalez, F. J. (1988) Pharmacol. Rev. 40, 243–288
Gonzalez, F. J., Matsunaga, T., Nagata, K., Meyer, U. A., Nebert, D. W., Pastewka, J., Kozak, C. A., Gillette, J., Gelboin, H. V., and Hardwick, J. P. (1987) DNA (N. Y.) 6, 149–161
Gotoh, O., Tagashira, Y., and Fujii-Kuriyama, Y. (1983) J. Biochem. (Tokyo) 93, 807–817
Guengerich, F. P. (1983) Biochemistry 22, 2811–2820
Guengerich, F. P. (1987) Mammalian Cytochromes P-450, Vols. I and II, CRC Press, Boca Raton, FL
Imai, Y., and Nakamura, M. (1988) FEBS Lett. 234, 313–315
Jefcoate, C. R. (1978) Methods Enzymol. 52, 258–279
Kronbach, T., Mathys, D., Gut, J., Catlin, T., and Meyer, U. (1987) Anal. Biochem. 162, 24–32
Kupfer, A., Al-Dabbagh, S. G., Richie, J. C., Idle, J. R., and Smith, R. L. (1982) Biochem. Pharmacol. 31, 3193–3199
Lindberg, R. L. P., and Negishi, M. (1989) Nature 339, 632–634
Mackett, M. Smith, G. L., and Moss, B. (1984) J. Virol. 49, 857–864
Matsunaga, E., Zanger, U. M., Hardwick, J. P., Gelboin, H. V., and Gonzalez, F. J. (1989) Biochemistry 28, 7349–7355
Nebert, D. W., and Consalve, F. J. (1987) Annu. Rev. Biochem. 56, 943–993
Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R., and Waterman, M. R. (1989) DNA (N. Y.) 8, 1–13
Omura, T., and Sato, R. (1960) J. Biol. Chem. 239, 2379–2385
Poulos, T. (1989) Nature 339, 580–581
Poulos, T. L., Finzel, B. C., Gunsalus, I. C., Wagner, G. C., and Kraut, J. (1985) J. Biol. Chem. 260, 16122–16130
Poulos, T. L., Finzel, B. C., and Howard, A. J. (1986) Biochemistry 25, 5314–5322
Shimizu, T., Hirano, K., Takahashi, M., Hatano, M., and Fujii-Kuriyama, Y. (1988) Biochemistry 27, 4138–4141
Zanger, U. M., Hauri, H.-P., Loep, J., Homberg, J. C., and Meyer, U. A. (1988a) Proc. Natl. Acad. Sci. U. S. A. 85, 8264–8269
Zanger, U. M., Vilbois, F., Hardwick, J. P., and Meyer, U. A. (1988b) Biochemistry 27, 5447–5454
Sequence requirements for cytochrome P-450IID1 catalytic activity. A single amino acid change (Ile380 Phe) specifically decreases Vmax of the enzyme for bufuralol but not debrisoquine hydroxylation.

E Matsunaga, T Zeugin, U M Zanger, T Aoyama, U A Meyer and F J Gonzalez

*J. Biol. Chem.* 1990, 265:17197-17201.

Access the most updated version of this article at [http://www.jbc.org/content/265/28/17197](http://www.jbc.org/content/265/28/17197)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/265/28/17197.full.html#ref-list-1](http://www.jbc.org/content/265/28/17197.full.html#ref-list-1)