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The role of phenylalanine 483 in cytochrome P450 2D6 is strongly substrate dependent

Barbara M.A. Lussenburg [a], Peter H.J. Keizers [a], Chris de Graaf [a], Mats Hidestrand [b], M. Ingelman-Sundberg [b], Nico P.E. Vermeulen [a], Jan N.M. Commandeur [a,*]

[a] *LACDR/Division of Molecular Toxicology, Department of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

[b] *Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden*

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Abstract

The polymorphic cytochrome P450 2D6 (CYP2D6) is involved in the metabolism of 30% of the drugs currently prescribed, and is thus clinically relevant. Typical CYP2D6 substrates generally contain a basic nitrogen atom and an aromatic moiety adjacent to the site of metabolism. Recently, we demonstrated the importance of active site residue F120 in substrate binding and catalysis in CYP2D6. On the basis of protein homology models, it is claimed that another active site phenylalanine, F483, may also play an important role in the interaction with the aromatic moiety of CYP2D6 substrates. Experimental data to support this hypothesis, however, is not yet available. In fact, in the only study performed, mutation of F483 to isoleucine or tryptophan did not affect the 1′-hydroxylation of bufuralol at all [Smith G, Modi S, Pillai I, Lian LY, Sutcliffe MJ, Pritchard MP, et al., Determinants of the substrate specificity of human cytochrome P-450 CYP2D6: design and construction of a mutant with testosterone hydroxylase activity. Biochem J 1998;331:783–92]. In the present study, the role of F483 in ligand binding and metabolism by CYP2D6 was examined experimentally using site-directed mutagenesis. Replacement of F483 by alanine resulted in a 30-fold lower V_{max} for bufuralol 1′-hydroxylation, while the K_m was hardly affected. The V_{max} for 3,4-methylenedioxy-methylamphetamine *O*-demethylenation on the other hand decreased only two-fold, whereas the effect on the K_m was much larger. For dextromethorphan, in addition to dextrorphan (*O*-demethylation) and 3-methoxymorphinan (*N*-demethylation), two other metabolites were formed that could not be detected for the wild-type. The substrate 7-methoxy-4-(aminomethyl)-coumarin was not metabolised at all by CYP2D6[F483A], a phenomenon that was reported also for CYP2D6[F120A]. The presented data show that next to F120, residue F483 plays a very important role in the metabolism of typical CYP2D6 substrates. The influence of F483 on metabolism was found to be strongly substrate-dependent.

Keywords: Cytochrome P450 2D6; Active site; Site-directed mutagenesis; Phenylalanine 483; Substrate selectivity

1. Introduction

The cytochrome P450 superfamily constitutes a large group of oxido-reductases that are responsible for the oxidation and reduction of many endogenous compounds as well as a wide variety of xenobiotics [1–3]. In humans, cytochrome P450 2D6 (CYP2D6) is one of the most important enzymes of this family [4]. Despite its low abundance – CYP2D6 represents only 4–8% of the total cytochrome P450 in human liver – it metabolises ~30% of the drugs currently on the market [5,6]. Its clinical relevance is even increased by the fact that CYP2D6 is highly polymorphic; 6% of the European population is classified as a 'poor metaboliser', while another 3% has the 'ultra-rapid metaboliser' phenotype [6–9], thus contributing to large interindividual differences in drug metabolism.

The development of accurate models of the active site of CYP2D6 is very useful to identify potential drug candidates that interact with the enzyme. Because no crystal structure is

Abbreviations: CYP, cytochrome P450; CPR, cytochrome P450 reductase; FU, fluorescence units; HAMC, 7-hydroxy-4-(aminomethyl)-coumarin; MAMC, 7-methoxy-4-(aminomethyl)-coumarin; MDMA, 3,4-methylenedioxy-methylamphetamine; MDA, 3,4-methylenedioxy-amphetamine; 3,4-OH-MA, 3,4-dihydroxy-methylamphetamine

* Corresponding author. Tel.: +31 20 5987595; fax: +31 20 5987610.
E-mail address: jnm.commandeur@few.vu.nl (Jan N.M. Commandeur).

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yet available for CYP2D6, the structural information required for such models has to be obtained from homology modeling [10,11] and experimental mutagenesis studies.

Most substrates of CYP2D6 contain a basic nitrogen at a distance of approximately 5–7 Å from the site of oxidation, and an adjacent aromatic moiety [12]. The negatively charged active site residues E216 and D301 have been shown to play a role in fixation of the basic nitrogen atom [13–15]. Pharmacophore and homology models suggest a role for aromatic residues in the active site to undergo VanderWaals interactions with aromatic moieties of the ligands [16]. Three aromatic phenylalanine residues have been proposed as active-site residues, F120, F481 and F483. Recently, it was experimentally underlined that F120 is indeed one of the aromatic active site residues that plays an important role in substrate binding and metabolism [17,18]. In earlier CYP2D6 homology models based on bacterial cytochrome P450 crystal structure templates it was suggested that another aromatic residue associated with ligand binding is F481 [16,19,20]. Substitution of F481 by non-aromatic residues reduced the affinity of several typical CYP2D6 substrates [16]. In more recent homology models based on rabbit CYP2C5, however, F481 is positioned outside the binding pocket, but in close contact with active site residue F483 [14,21–23]. Our model (Fig. 1) [21] and several others [14,22–24] suggest that this aromatic active site residue, i.e. F483, is also a potential ligand-contact residue (Fig. 1). In a modeling study by Kemp et al. phenylalanines 120 and 483 are referred to as very important residues in the active site of CYP2D6, which are involved in the binding of various NCI compounds [24]. However, experimental data supporting a role for F483 in binding known CYP2D6 substrates is not yet available. In fact, in the only experimental study on this residue, it was shown that substitution of F483 by isoleucine or tryptophan did not affect the 1′-hydroxylation of the typical CYP2D6 substrate bufuralol [23]. Interestingly, the F483I mutant was able to catalyse the 15α-hydroxylation of testosterone, which is not a substrate for wild-type CYP2D6 [23]. Therefore, it is not yet clear whether this residue plays a role in the binding of typical CYP2D6 substrates. Recently, we demonstrated that the role of another active-site phenylalanine-residue, F120, is very substrate dependent [17,18]. The F120A mutant completely lost the ability to metabolise 7-methoxy-4-(aminomethyl)coumarin

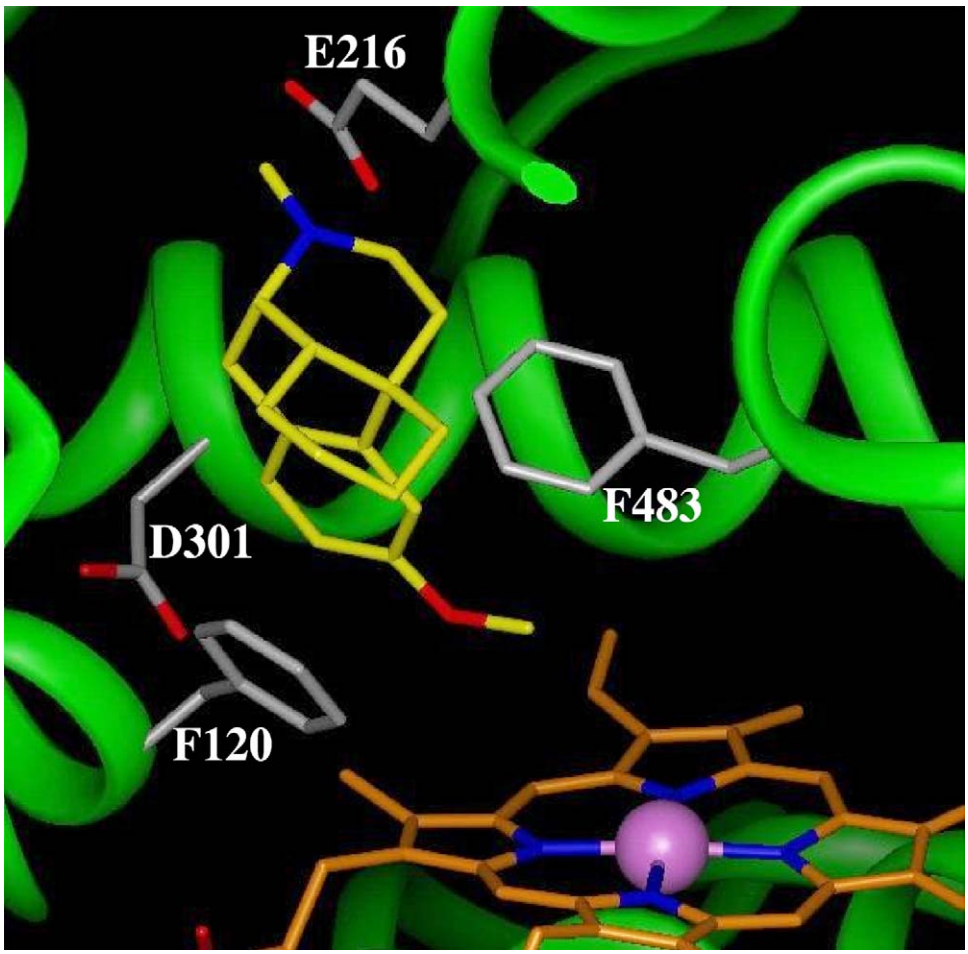

Fig. 1. The active site of the homology model of CYP2D6 [17], showing the active site residues F120, E216, D301 and F483. The pink ball at the bottom represents the heme iron atom. In yellow, the substrate dextromethorphan is depicted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)