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RAPID COMMUNICATION

CATALYTIC ACTIVITIES OF HUMAN DEBRISOQUINE 4-HYDROXYLASE CYTOCHROME P450 (CYP2D6) EXPRESSED IN YEAST

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Abstract-A 1.57kb *Bam*H1 fragment containing a full-length human debrisoquine 4-hydroxylase cytochrome P450 (*CYP2D6*) cDNA was inserted into the *Bgl*II site of the yeast expression plasmid pMA91 and the resulting recombinant plasmid, pELT1, introduced into *Saccharomyces cerevisiae* strain AH22. Microsomes prepared from AH22/pELT1 cells gave an absorption maximum at 448 nm and a P450 content of 67 ± 31 pmol/mg of microsomal protein. No P450 was detectable in microsomes prepared from AH22/pMA91 control cells. A western blot of microsomes prepared from yeast transformed with pELT1 were probed with a monoclonal antibody to CYP2D6 and revealed a strong band with a molecular mass consistent with that of CYP2D6 from human liver microsomes. No corresponding band was observed with microsomes from control yeast transformed with pMA91 alone. Microsomes from AH22/pELT cells showed catalytic activity towards metoprolol (α-hydroxylation and O-demethylation, 0.17 and 0.78 nmol/mg protein/h, respectively); and towards sparteine (2- and 5-dehydrogenation, 1.82 and 0.59 nmol/mg protein/h, respectively). The inhibition of metoprolol metabolism by quinidine (Qd) was 200 times more potent than that of quinine (Qn), both for α-hydroxylation (Qd IC_{50} = 0.05μM; Qn IC_{50} = 4μM) and O-demethylation (Qd IC_{50} = 0.05μM; Qn IC_{50} = 4μM). Negligible metabolism of tolbutamide and S-mephenytoin, substrates of the 2C sub-family, and of p-nitrophenol, a substrate of CYP2E1, was detected, although a trace of the N-deethylated metabolite of lignocaine, thought to be metabolised by CYP3A4, was detected with microsomes from *CYP2D6*-expressing yeast cells. The results indicate that yeast cells containing human *CYP2D6* cDNA express a functionally active form of the enzyme, the immunochemical and catalytic properties of which are consistent with those of human liver.

Debrisoquine 4-hydroxylase (CYP2D6) is a member of the cytochrome P450 super-gene family. It is the source of the debrisoquine/sparteine polymorphism, one of the most extensively studied genetic defects of oxidative drug metabolism in humans [1]. CYP2D6 plays a critical role in the metabolism of over 25 drugs, some of wide therapeutic use including anti-depressants, β-adrenergic antagonists and antiarrhythmics [2]. Associations have also been sought between the debrisoquine/sparteine polymorphism and diseases such cancer [3] and Parkinson's disease [4].

Heterologous expression is a powerful tool to study the substrate specificity and catalytic activity of individual human cytochromes P450. Also, in conjunction with in-vitro mutagenesis techniques and molecular modelling, it provides a systematic approach to the elucidation of structure-function relationships. Although a number of mammalian cytochromes P450 have been expressed in a variety of heterologous systems [for review, see 5], this report is the first to describe the expression of human *CYP2D6* in the yeast *Saccharomyces cerevisiae*. Validation of the substrate specificity and catalytic activity of the recombinant protein is also described.

MATERIALS AND METHODS

Molecular biology techniques, the expression plasmid (pMA91) containing the phosphoglycerate kinase (PGK) promoter and terminator, microbial strains (*Escherichia coli* DH5α, *S. cerevisiae* AH22), culture conditions and reagents were similar to those described previously for the expression of *CYP1A1* in yeast [6]. Plasmid pMP201 containing the full-length *CYP2D6* cDNA gene was obtained from Prof. C. R. Wolf (Imperial Cancer Research Fund Laboratories, University of Edinburgh). The *Eco*R1 fragment containing the *CYP2D6* gene was excised and modified with *Bam*H1 linkers [7]. Plasmid pELT1, which directed the synthesis of *CYP2D6* in yeast strain AH22, was constructed by ligating the *Bam*H1-modified *CYP2D6* gene into pMA91 [6]. Transformation of yeast cells with plasmids pMA91 and pELT1 was by electroporation [8]. Human liver [9] and yeast [6] microsomes were prepared as described previously. Western and immunoblotting techniques were carried out using standard methods [10]. Assays for the metabolism of sparteine [11], metoprolol [9], lignocaine [12], tolbutamide [13], S-mephenytoin [14] and p-nitrophenol [15] used published methods, as did cytochrome P450 [16] and protein [17] determinations.

RESULTS AND DISCUSSION

Microsomes prepared from yeast AH22 cells transformed with pELT1 (AH22/pELT1) showed a Soret absorption maximum at 448nm and a P450 content of 67 ± 31 pmol/mg microsomal protein (range 140 - 44 pmol/mg, n = 10). No P450 was detectable in microsomes prepared from control AH22 cells transformed with pMA91 (AH22/pMA91) (Fig. 1). The level of expression of *CYP2D6* compares favourably with that of other human P450s expressed in yeast cells including $P450_{MP}$, 10 pmol/mg microsomal protein (mp) [18]; CYP1A1, 33 pmol/mg mp [6]; CYP2C10, 40 pmol/mg mp [19] and CYP3A4, 90 pmol/mg mp [20]; and is greater than human P450 expression, including CYP2D6, in mammalian COS 1 cells (5-40 pmol/mg mp) [21] and human lymphoblastoid cell-lines (20-40 pmol/mg mp) [22].

The western blot of AH22/pELT1 microsomes when probed with an antibody raised against human CYP2D6 revealed a strong band with a molecular mass consistent with that of CYP2D6 from human liver microsomes. No corresponding band was observed with microsomes from AH22 cells transformed with pMA91 (Fig. 2). The intensity of signal with AH22/pELT microsomes was greater than that obtained with human liver microsomes, when compared on a μg of microsomal protein basis, indicating an efficient expression of the *CYP2D6* gene in yeast.

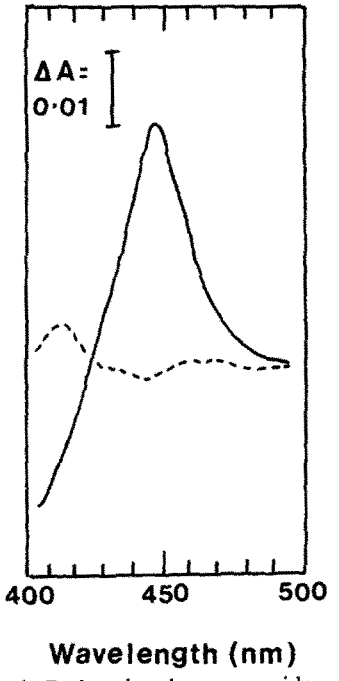

Fig. 1. Reduced carbon monoxide difference spectra of microsomes from AH22/pELT1 (—) and AH22/pMA91 control (- - -) yeast cells. Microsomal protein concentration, 9 mg/mL.

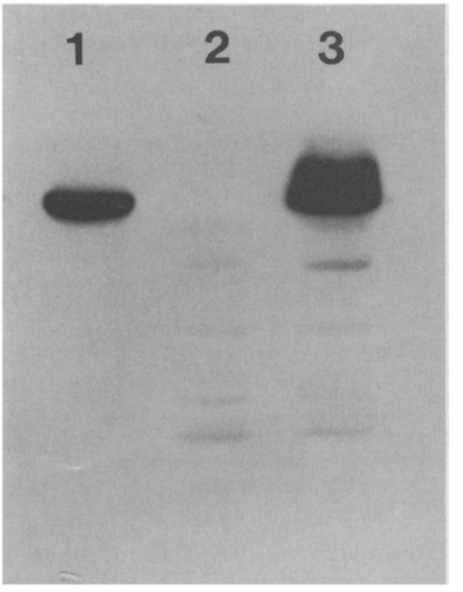

Fig. 2. Immunoblot analysis of microsomes probed with a monoclonal antibody raised against human CYP2D6. Lane 1, human liver (15 μg); lane 2, AH22/pMA91 control yeast (30 μg); lane 3, AH22/pELT1 yeast (30 μg).

The substrate specificity of CYP2D6 in microsomes prepared from yeast AH22/pELT1 is consistent with that anticipated from human liver microsomes. Sparteine and metoprolol, two classical substrates of CYP2D6, are metabolised by the recombinant enzyme, while tolbutamide and S-mephenytoin, substrates of the CYP2C sub-family [23], and p-nitrophenol, a substrate of CYP2E1 [24], are not metabolised (Table 1). However, a small amount of the N-deethylated metabolite of lignocaine, monoethylglycinexylidide (MEGX), amounting to 4% of that observed with human liver microsomes, was detected with microsomes from yeast cells expressing the recombinant protein. This reaction is reported to be catalysed by CYP3A4 [25]. Negligible metabolism of the substrates was observed with yeast control AH22/pMA91 microsomes, while all substrates were metabolised by human microsomes prepared from the liver of an extensive metaboliser (Table 1).

The metabolite ratios of 2-dehydrosparteine:5-dehydrosparteine and O-demethylmetoprolol: α-hydroxymetoprolol were slightly lower with microsomes from AH22/pELT1 yeast cells (3.1 and 4.6, respectively) compared with human liver microsomes (4.2 and 5.7, respectively). This is consistent with the involvement of an additional P450 in the 2-dehydrogenation of sparteine [26] and the O-demethylation of metoprolol in human liver microsomes [9].

The rate of metoprolol metabolism by microsomes from AH22/pELT1 cells, when expressed on a mg of microsomal protein basis, was 31-39% that of human liver microsomes, while the rate of sparteine metabolism approached that of human liver microsomes (73-100%) (Table 1). The reduced rate of metoprolol metabolism

Table 1. Catalytic activities of yeast and human liver microsomes

Substrate	Product	Microsomes		
		AH22/ pMA91	AH22/ pELT1	Human Liver
Sparteine	2DHS	<0.02	1.82	2.49
	5DHS	<0.01	0.59	0.59
Metoprolol	αHM	<0.01	0.17	0.44
	ODM	<0.01	0.78	2.51
Tolbutamide	OHTB	<0.01	<0.01	2.15
S-Mephenytoin	4HM	<0.01	<0.01	0.27
	NDM	<0.03	<0.03	0.55
p-Nitrophenol	4NC	<0.50	<0.50	52.8
Lignocaine	MEGX	<0.02	0.20	4.52

2DHS, 2-dehydrosparteine; 5DHS, 5-dehydrosparteine; αHM, α-hydroxymetoprolol; ODM, O-demethylmetoprolol; OHTB, hydroxytolbutamide; 4HM, 4'-hydroxymephenytoin; NDM, N-demethylmephenytoin; 4NC, 4-nitrocatechol; MEGX, monoethylglycinexylidide. Values are nmol product/mg protein/hour, mean of two replicates. P450 content of yeast AH22/pELT1 and human liver microsomes were 67 and 1314 pmol/mg protein, respectively.

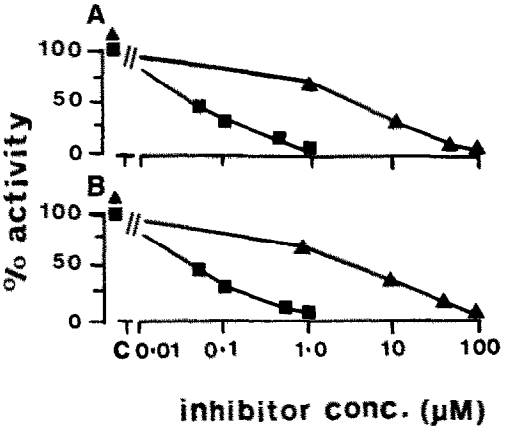

Fig. 3. Effect of quinidine (■) and quinine (▲) on the metabolism of metoprolol by microsomes from AH22/pELT1 yeast cells. A, inhibition of α-hydroxylation; B, inhibition of O-demethylation. Each point is a mean of duplicate analyses. C, control.

with recombinant CYP2D6 is at variance with the apparent greater level of expression in the yeast microsomes as indicated by the immunoblot (Fig. 2). However, the recombinant protein could have a lower turn-over number and/or a proportion of the enzyme could be inactive. When catalytic activity is expressed on a total P450 basis however, the rate of metoprolol metabolism with recombinant protein is greater than with human liver microsomes (40-fold and 20-fold for O-demethylation and α-hydroxylation, respectively).

The inhibition of metoprolol metabolism by quinidine (IC_{50}=0.05μM) was 200 times greater than inhibition by quinine (IC_{50}=4μM), both for α-hydroxylation and O-demethylation (Fig. 3). Similar stereoselective inhibition of metoprolol metabolism by quinidine and quinine using human liver microsomes has been established previously [9]. As anticipated, complete inhibition of O-demethylation of metoprolol by the yeast microsomes could be achieved with both quinidine and quinine (Fig. 3.), whereas in human microsomes only 80% of this activity can be inhibited [9], indicating the involvement of another enzyme, in addition to CYP2D6, in the O-demethylation of metoprolol.

The results indicate that the substrate specificity, catalytic activity and immunologic properties of human debrisoquine 4-hydroxylase expressed in yeast are comparable with those of CYP2D6 in human liver. The recombinant CYP2D6 should be useful in identifying drugs undergoing oxidative catalysis by this enzyme and for determining its structure-function relationships.

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