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SHORT COMMUNICATIONS

Purification of a desmethylimipramine and debrisoquine hydroxylating cytochrome P-450 from human liver

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After administration of standard doses the plasma concentrations of many drugs vary markedly among subjects. Genetic polymorphism can contribute to this. Five to ten per cent of Caucasian subjects are poor oxidizers of debrisoquine [1], which is inherited as an autosomal recessive trait [2]. Poor metabolizers of debrisoquine also hydroxylate desmethylimipramine (DMI)* slowly, [3], suggesting a common regulation. Human liver microsomes from poor metabolizers hydroxylate both DMI and debrisoquine slowly [3, 4]. To elucidate the molecular mechanisms behind this, we have studied a DMI hydroxylating cytochrome P-450 isozyme from human liver.

Materials and methods

Preparation of microsomes and purification of enzymes. Human liver samples were obtained from kidney transplant donors [5], microsomes were prepared, solubilized and subjected to chromatography on lauric acid AH Sepharose 4 B as described [6]. The cytochrome P-450 fraction, containing about 170 nmol cytochrome P-450 eluted by 1% Emulgen 913, was applied to a CM Sepharose CL-6B column. This was eluted with a stepwise gradient of 40, 90 and 200 mM potassium phosphate. The 40 mM eluate contained a cytochrome P-450 fraction showing a single major band on SDS polyacrylamide gel electrophoresis. NADPH cytochrome P-450 reductase was isolated from liver microsomes of male rats [7].

Characterization of enzymes, and assays of metabolism. Polyacrylamide slab gel electrophoresis was performed in the presence of sodium dodecyl sulphate [8]. Gels were stained with Coomassie Brilliant Blue R 250. Catalytic activities of the purified cytochrome P-450 isozyme were determined in a standard reconstituted system [9]. DMI hydroxylation and imipramine demethylation were quantitated [3].

Structural analysis. Amino acid sequence degradations were performed in a liquid-phase sequencer [10], and phenylthiohydantoin derivatives were identified by reverse phase high performance liquid chromatography [11].

Results and discussion

We have prepared a purified DMI hydroxylating cytochrome P-450 by chromatography in two steps as shown in Table 1. This cytochrome P-450 preparation gave one major band on SDS-polyacrylamide gel electrophoresis with an apparent molecular weight of 51,500 (Fig. 1). It contained 8.5 nmol cytochrome P-450/mg protein and the yield was 3.4%. No cytochrome P-420 was detected. The specific cytochrome P-450 content of the preparation is less than theoretically possible. This may be due to loss of heme during purification, natural occurrence of the apoprotein of cytochrome P-450, or impurities. N-terminal amino acid

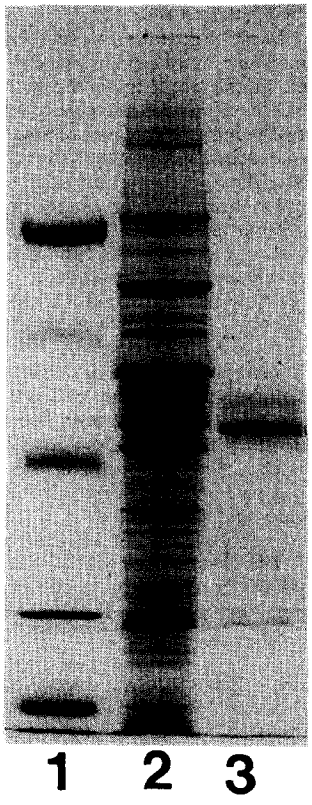

Fig. 1. SDS–polyacrylamide (9%) gel electrophoresis of human liver microsomes and purified cytochrome P-450. Well 1 contained molecular weight markers (94,000; 68,000; 43,000; 30,000, 21,000; and 14,300), well 2 microsomes from human liver, and well 3 purified cytochrome P-450.

* Abbreviations used: DMI, desmethylimipramine.

Table 1. Purification of cytochrome P-450 from human liver

Step	Total protein (mg)	Total P-450 (nmol)	Specific content of P-450 (nmol/mg)	Yield % (calculated P-450 content)	Purification factor
Microsomes	3047	1257	0.41	100	1
Solubilized microsomes	3044	1161	0.38	76	0.92
Lauric acid column	246	167	0.67	11	1.6
CM Sepharose CL-6B	5	42.5	8.5	3.4	21

Table 2. Catalytic activities of purified human liver enzyme compared to the activity of the microsomes

Substrate	Substrate concentration (μM)	Microsomes (nmol/min · nmol P-450)	Purified P-450 (nmol/min · nmol P-450)
Desmethylimipramine	50	0.11	0.95
Debrisoquine	500	0.19	0.45
Imipramine	50	—	0.27

sequence analysis revealed that the preparation was not chemically homogeneous. The purified fraction could be assigned into one major and one minor component, with likely N-terminal amino acid sequences AlaLeuIle- and MetLysGlu-, respectively. In two different preparations, the major component was estimated to be present in an amount of about 50–70%. It is possible that one of the components in our preparation is identical with a non-heme protein contaminating some debrisoquine hydroxylase fractions prepared by Distlerath *et al.* [12].

The purified enzyme preparation hydroxylated DMI at a rate of 0.95 nmol/min · nmol cytochrome P-450, which is nine times faster than the rate found in the microsomes (on a cytochrome P-450 basis). It was also able to 4-hydroxylate debrisoquine and N-demethylate imipramine (Table 2). Five hundred μM debrisoquine inhibited the hydroxylation of DMI by 82%. These data indicate that our purified enzyme is identical to the major debrisoquine hydroxylating cytochrome P-450 in man. The rate of debrisoquine hydroxylation with our protein was similar to that of a recently purified human liver debrisoquine hydroxylase [12], further supporting this suggestion. Our protein might also be the one present in a human liver cytochrome P-450 preparation hydroxylating bufuralol [13].

Summary, we have purified a human liver cytochrome P-450 by chromatography on lauric acid AH Sepharose 4B and CM Sepharose CL-6B columns. The preparation exhibits a single major band on SDS-polyacrylamide gel electrophoresis (M_r 51,500) and contains 8.5 nmol cytochrome P-450/mg protein. It oxidizes desmethylimipramine, debrisoquine and imipramine in a reconstituted system, and 500 μM debrisoquine inhibits the hydroxylation of DMI by 82%. The data indicate that we have purified a DMI hydroxylase which is identical to the major human liver debrisoquine hydroxylase. It also demethylates imipramine.

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The influence of pretreatment on the urinary metabolite profile of pseudoracemic hexobarbital

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Hexobarbital (HB; Fig. 1) is a well-known model substrate for the assessment of changes in the activity of hepatic cytochrome P-450 (P-450 for short) in animals and man [1, 2]. Sleeping time following HB-administration [3], and pharmacokinetic parameters of HB, like half-life [4] and clearance [5–7] have been frequently used to characterize such changes. A complicating factor in the interpretation of these parameters is the fact that HB is a chiral compound. The two enantiomers, S(+)HB and R(−)HB, differ considerably in pharmacokinetics and in urinary metabolic pattern [8, 9]. Yet HB has been used as a model substrate nearly exclusively in the racemic form.

For an unambiguous interpretation of pharmacokinetic data of racemic HB as a reflection of changes in hepatic drug