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### **Research Article**

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# Purification of a Human Liver Cytochrome P-450 Immunochemically Related to Several Cytochromes P-450 Purified from Untreated Rats

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## Abstract

Among characterized forms of liver microsomal cytochromes P-450 in rats are four related isozymes (P-450f-i) notable for their lack of inducibility. Immunoblot analyses demonstrated that human liver microsomes contained several proteins related to these rat P-450s. A human liver P-450, termed HLx, was purified and found by immunochemical assays to resemble rat P-450g. Analysis of the NH<sub>2</sub>-terminal amino acid sequence of HLx indicates that it is related to rat P-450s f-i and human liver P-450MP. A monoclonal antibody was used to measure the amounts of HLx in 21 human liver specimens. No correlation between the levels of HLx protein in these specimens and the patients' environmental histories was observed. However, statistical analysis of the data suggests that the distribution of HLx is at least bimodal. We conclude that HLx is a member of a family of human liver P-450s that resembles in its structure, and possibly in its distribution, several liver P-450s found in other animals.

## Introduction

The cytochromes P-450 in the endoplasmic reticulum of the hepatocyte represent an important biochemical locus for the biotransformation of chemicals. This superfamily of hemoproteins catalyzes the oxidative metabolism of an array of endogenous and exogenous lipophilic compounds (1, 2). The rates at which these substrates are oxidized and the nature of the metabolites produced depend at least to some extent on the concentrations of individual isozymes of cytochromes P-450. These enzyme concentrations are controlled by genetic factors (3, 4) and also by drugs, diet, and environmental agents (1, 2, 5) that may increase or decrease specific cytochrome P-450 isozymes selectively.

Until recently, the types and amounts of cytochrome P-450 isozymes in the liver could be assessed only indirectly from measurements of the metabolism of prototype substrates

in vivo or by liver microsomes. However, advances in protein purification techniques, coupled with immunochemical methods including preparation of monospecific antibodies, has made it possible to purify more than 15 separate polypeptide forms of rat liver cytochrome P-450 (6) and to characterize their structure and regulation (6-8). This technology also offers an opportunity to critically assess the question of how relevant the abundant information about animal cytochromes P-450 is to man. For example, we recently demonstrated that human liver contains HLP (9), a cytochrome P-450 that resembles rat liver cytochrome P-450p (10) in its immunochemical characteristics, its NH<sub>2</sub>-terminal amino acid sequence, its catalytic activities, and its apparent inducibility in man by glucocorticoids or macrolide antibiotics (9). We have also purified from human liver two other novel cytochromes P-450: HLD, a cytochrome orthologous to the 3-methylcholanthrene-inducible P-450d in the rat (11), and HLJ, a cytochrome P-450 orthologous to rat cytochrome P-450j in being the major *N*-nitrosodimethylamine demethylase induced in humans drinking ethanol to excess or receiving isoniazid treatment (12).

In addition to such inducible forms of liver cytochrome P-450, there is a group of immunochemically related cytochromes in rats (13-16) and rabbits (17, 18) that comprise the bulk of cytochrome P-450 in untreated animals. It has been suggested that these cytochromes P-450, which are relatively refractory to induction by many xenobiotics, may be involved in pathways for metabolism of endogenous substrates at steps where modulation by environmental agents would be undesirable (19). To investigate the human representatives of this family, we purified a human liver cytochrome P-450 termed HLx. Through the use of immunochemical characterizations, we demonstrate that HLx is related to rat cytochromes P-450f-i.<sup>1</sup> Finally, we show that, as with cytochrome P-450g in outbred Long Evans rats (21) and in Sprague-Dawley rats (22), HLx does not appear to be inducible in man but rather exhibits a polymorphism, presumably under genetic control, in the level of its spontaneous expression.

## Methods

*Human liver specimens.* Specimens were obtained at surgery in accordance with protocols approved by the Committee for the Conduct of Human Research at the Medical College of Virginia. All patients had normal transaminase and bilirubin levels and received atropine before

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1. Cytochrome P-450k (PB-1[20]) is a fifth member of this family of immunochemically related proteins in the rat (21). This protein, unlike cytochromes P-450f-i, is inducible by phenobarbital and shares 75% total sequence homology with cytochrome P-450f (20).

general anesthesia, except patient 17, who also received dexamethasone 5 h before surgery. Patient code numbers refer to individual liver samples. The patients' ages, genders, and medications are listed in Table I. Microsomes prepared by differential centrifugation (23) were stored as described previously under conditions in which the levels of cytochrome HLp (9) have remained stable for up to 2 yr. Protein concentrations of the microsomal samples were determined colorimetrically (24).

*Purification of human liver cytochrome P-450 HLx.* All steps of the purification were performed at 4°C unless otherwise noted. Liver microsomes (2 mg/ml of protein in storage buffer [25]; 1,650 mg of total protein, 880 nmol of cytochrome P-450) prepared from specimen 8 were solubilized by the dropwise addition of a 20% (wt/vol) solution of cholate (recrystallized two times) to a final concentration of 0.6%. The insoluble material was removed by centrifugation at 105,000 g for 1 h. The resulting supernatant (675 nmol of cytochrome P-450) was divided in half and each portion was applied to one of two identical

aminooctylamino-Sepharose 4B columns (2.6 × 37 cm) prepared as previously described (25) and equilibrated in 100 mM potassium phosphate buffer (pH 7.25) containing 1 mM EDTA, 20% glycerol, and 0.6% cholate. The columns were each washed with 5 column volumes of equilibration buffer containing 0.42% cholate. Hemoprotein was then eluted with equilibration buffer containing 0.33% cholate and 0.06% Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO).

The fractions from each column that contained hemoproteins comprised a narrow peak. The two peaks were combined and were concentrated to ~ 50 ml by ultrafiltration in an Amicon cell equipped with a PM30 filter. The concentrated hemoprotein solution was dialyzed against 1 liter of buffer A (5 mM potassium phosphate, pH 7.7, 0.1 mM EDTA, 20% glycerol, 0.1% Tergitol NP-10, and 0.2% cholate) for 72 h with five changes. The dialyzed material was stirred at room temperature for 30 min and then applied at a rate of 1 ml/min to a DEAE (Whatman Chemical Separations, Inc., Clifton, NJ) column series (DE51 [1.6 × 7 cm], DE52 [1.6 × 15 cm], and DE53 [1.6 × 25

Table I. Patient History and Immunoquantitation of Hepatic HLx, HLd, HLp, and HLj

Patient no.	HLx	HLd	HLp	HLj	Gender	Age	Source*	Medications
1	173	67	288	NA	M	—	D	Unknown
2	214	42	275	265	M	69	L	Erythromycin-base Neomycin
3	69	71	150	412	M	27	D	Ethanol (0.22%)
4	158	66	650	188	F	33	D	Dexamethasone Phenobarbital Diphenylhydantoin
6	59	84	200	165	M	26	D	Hydrocortisone Cimetidine
7	57	55	175	100	F	48	L	None
8	270	45	563	318	M	52	D	Dexamethasone Phenobarbital Diphenylhydantoin
9	86	85	313	352	M	36	D	Dexamethasone Diphenylhydantoin Amobarbital
10	108	98	225	235	F	36	L	None
11	149	77	525	353	F	38	D	Dexamethasone Diphenylhydantoin Furosemide Sulfamethoxazole Trimethoprim Gentamycin
12	151	33	100	158	M	46	L	None
13	69	11	763	BD	M	47	L	Triacetyloleandomycin
14	100	100	100	100	M	50	L	Flurazepam
16	69	42	179	159	F	45	D	None
17	105	6	116	565	M	56	D	Ethanol (0.30%) Allopurinol
18	108	105	233	247	M	26	D	None
19	69	152	106	118	M	69	L	None
20	133	23	309	160	F	51	L	Erythromycin base
21	70	15	163	182	F	41	D	Hydrochlorothiazide
22	87	14	167	588	F	43	L	Dexamethasone Isoniazid
23	104	64	113	194	M	47	L	None

Immunoquantitation of the human liver cytochromes P-450 were performed as described in Methods with the densitometric value obtained for specimen 14 arbitrarily set as 100%. \* Sources of liver specimens were patients undergoing hepatic lobectomy (L) or brain-dead renal transplant donors (D). BD, below limit of detection. NA, not available for study.

cm]) equilibrated at room temperature with 1 liter of buffer A. The columns were washed with 300 ml of buffer A followed by 500 ml of buffer B (5 mM potassium phosphate, pH 7.7, 0.1 mM EDTA, 20% glycerol, 0.2% Tergitol NP-10, and 0.5% cholate). Then a 600-ml 0-to-250-mM NaCl gradient in buffer B was applied to the column and the first peak fractions containing 417 nm absorbing material were combined.

The pooled fractions were dialyzed against 1 liter of buffer C (5 mM potassium phosphate, pH 6.5, 20% glycerol, 0.1 mM EDTA, and 0.2% Tergitol NP-10) with three changes over 48 h and then applied at a rate of 1 ml/min to a CM52 (Whatman Chemical Separations, Inc.) column (1.6 × 30 cm) equilibrated in buffer C. Bound hemoprotein was eluted by application of a 500-ml 0-to-150-mM NaCl gradient in buffer C. Two peaks of 417 nm absorbing material were eluted. Those fractions in the second peak that contained only one protein, termed HLx, (as visualized by silver stained sodium dodecyl sulfate [SDS]-polyacrylamide gels [10%] [26]) were combined. Cytochrome P-450 HLx was concentrated and detergent was removed through the use of a small hydroxylapatite (Bio-Rad Laboratories, Richmond, CA) column (10).

**Preparation of murine monoclonal antibodies.** Five virus-free female Balb/c mice (15–18 g; Charles River Breeding Laboratories, Wilmington, MA) each received an intraperitoneal injection of 200 μl containing 50 μg of purified HLx in 50 mM potassium phosphate (pH 7.5) buffer containing 50 mM EDTA and 20% glycerol combined with an equal volume of Freund's complete adjuvant. After 21 d, a second injection of 20 μg of HLx was given. 6 d later serum samples from the mice were pooled and assayed for the presence of specific antibody by an enzyme-linked immunosorbent assay (ELISA). A final intravenous injection of 20 μg of HLx was given 3 d before surgery. Spleen cells were harvested from one immunized mouse and were fused with P3-X63-Ag multiple myeloma cells. The resulting hybridoma clones were expanded in vitro. Limiting dilutions were made to isolate individual clones. These were tested for specific antibody production by ELISA and, after the clones had expanded, the cells were injected into female Balb/c mice (5 × 10<sup>6</sup> cells per mouse) that had been primed with an intraperitoneal injection of pristane (0.5 ml) 2 wk previously. The ascitic fluid that appeared was collected 9–14 d later, clarified by centrifugation, and stored at –80°C.

**Immunochemical assays.** Immunoblot analyses were performed as previously described (9). Briefly, microsomal proteins separated by electrophoresis in 0.15 × 10 cm SDS-polyacrylamide gels (10%) (26) were transferred to nitrocellulose sheets electrophoretically. The sheets were incubated overnight in phosphate-buffered saline containing 3% bovine serum albumin and 10% calf serum and then were reacted sequentially with murine monoclonal anti-HLx, peroxidase-conjugated rabbit anti-mouse IgG (ICN ImmunoBiologicals, Lisle, IL), and, finally, 3,3'-diaminobenzidine tetrahydrochloride in hydrogen peroxide (0.006%). In the experiments where a rabbit IgG was used as the primary antibody, the second antibody was goat anti-rabbit IgG (ICN) and this was followed by peroxidase rabbit anti-peroxidase (ICN). The density and total area of the stained bands were determined using a scanning densitometer (Carl Zeiss, Inc., Thornwood, NY) (9). In preliminary analyses, dilutions of microsomes isolated from patient 14 produced integrated optical density values directly proportional to the amount of microsomal protein applied to the gel.

Noncompetitive ELISA was performed as described (21) with all proteins coated on 96-well microtiter plates at 0.2 μg/ml. All of the primary antibodies were purified IgG fractions of hyperimmune sera from rabbits except anti-P-450a, which was raised in sheep. The polyclonal antibodies were made monospecific for the antigens of immunization by previously reported procedures (21, 27, 28).

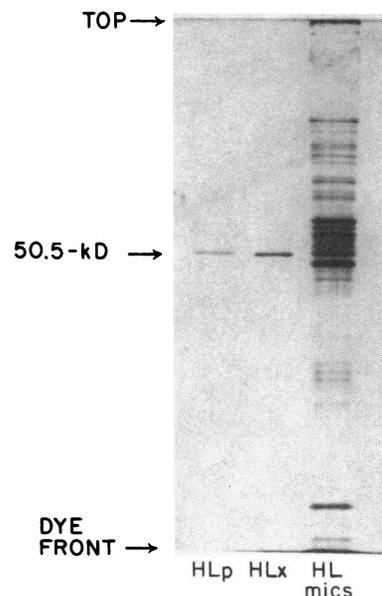
## Results and Discussion

We used the approach of sequential column chromatography of cholate-solubilized human liver microsomes on amino-

octylamino-Sepharose 4B, DEAE-cellulose, and CM-cellulose to purify a prominent isozyme of cytochrome P-450 from a single human liver. The purified protein, termed HLx, appeared homogeneous as judged by electrophoresis in SDS-polyacrylamide gels stained with silver (Fig. 1). The specific heme content of the HLx preparation was 9.5 nmol of CO-binding hemoprotein per mg of protein, representing a final yield of 1.9% of the starting material. The apparent molecular weight of HLx was 50.5 kD, a value slightly less than that determined for human liver cytochrome HLP (Fig. 1) (9). Absolute and CO-reduced spectral analyses disclosed, respectively, that HLx exists predominantly in the low-spin state and has a Soret wavelength maximum at 452 nm (not shown).

Amino-terminal amino acid sequence analysis is useful for characterizing even closely related forms of cytochrome P-450 (6, 14), so we submitted HLx for sequence analysis by automated Edman degradation (Table II). The 19-amino acid sequence we obtained for HLx revealed no significant homology with that previously determined for several other human cytochromes P-450 including HLP (9), HLd (11), and HLj (12) (Table II). However, the NH<sub>2</sub>-terminal amino acid sequence of HLx was homologous in 13 of the identified 18 residues with that reported for an isozyme of human cytochrome P-450 identified by its ability to catalyze the metabolism of mephenytoin (P-450MP) (29) (Table II). Moreover, when compared with rat cytochromes P-450 (6, 14), HLx shares high homology with the immunochemically related cytochromes P-450f, P-450g, P-450h, and P-450i (14), all found in untreated animals (Table II). Consistent with this similarity is the homology at 12 of the 19 identified amino acids between HLx and rabbit P-450 LM1 (Table II), a cytochrome that appears to be related to rat cytochrome P-450g (30). Finally, this NH<sub>2</sub>-terminal amino acid sequence for HLx was found to be much less similar to that reported (6) for rat cytochromes P-450a (5/19), P-450b (8/19), P-450c (3/19), P-450d (2/19), P-450e (8/19), P-450k (6/19), and P-450j (2/19).

Upon finding HLx to be most related to the family of rat cytochromes P-450f–i, we performed detailed comparisons of the immunoreactivity of purified HLx with specific anti-rat



**Figure 1.** Electrophoretic profile of human liver microsomes and purified cytochromes HLx and HLP. Liver microsomes (5 μg) and purified proteins (1 μg each) were prepared, subjected to electrophoresis in a SDS-polyacrylamide gel (10%), and visualized by silver staining, as described in Methods.

Table II. NH<sub>2</sub>-Terminal Amino Acid Sequences of HLx and Other Cytochromes P-450

Species	Isozyme	Residues identified																			Comparisons to HLx	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
Human	HLx	M	E	P	F	V	V	L	V	L	?	L	S	F	M	L	L	F	S	L	W	19/19
	HLp	(M)	A	L	I	P	D	<u>L</u>	A	M	Q	T	W	L	L	<u>L</u>	A	V	<u>S</u>	<u>L</u>	V	5/19
	HLj	—	A	A	L	G	<u>V</u>	T	<u>V</u>	A	L	<u>L</u>	V	W	A	A	F	L	L	<u>L</u>	—	4/17
	HLd	—	A	L	S	Q	<u>S</u>	V	P	F	S	A	T	E	L	<u>L</u>	<u>L</u>	A	<u>S</u>	A	—	3/17
	MP	<u>M</u>	D	S	L	<u>V</u>	<u>V</u>	<u>L</u>	<u>V</u>	<u>L</u>	?	<u>L</u>	<u>S</u>	?	L	<u>L</u>	<u>L</u>	L	<u>S</u>	<u>L</u>	<u>W</u>	13/18
Rat	P-450f	<u>M</u>	D	L	V	T	F	<u>L</u>	<u>V</u>	<u>L</u>	T	<u>L</u>	<u>S</u>	S	L	I	<u>L</u>	L	<u>S</u>	<u>L</u>	<u>W</u>	10/19
	P-450g	<u>M</u>	D	P	V	V	<u>V</u>	<u>L</u>	L	<u>L</u>	S	<u>L</u>	F	F	L	<u>L</u>	—	—	—	—	—	9/14
	P-450h	<u>M</u>	D	P	V	L	<u>V</u>	<u>L</u>	<u>V</u>	<u>L</u>	T	<u>L</u>	S	S	L	L	<u>L</u>	L	<u>S</u>	<u>L</u>	<u>W</u>	13/19
	P-450i	<u>M</u>	D	P	F	V	<u>V</u>	<u>L</u>	<u>V</u>	<u>L</u>	S	<u>L</u>	<u>S</u>	F	L	<u>L</u>	<u>L</u>	L	Y	?	<u>W</u>	14/18
Rabbit	LM1	<u>M</u>	D	P	V	<u>V</u>	<u>V</u>	<u>L</u>	<u>V</u>	<u>L</u>	G	<u>L</u>	C	C	L	<u>L</u>	<u>L</u>	L	<u>S</u>	I	<u>W</u>	12/19

The NH<sub>2</sub>-terminal amino acid sequence of HLx was determined by automated Edman degradation using a gas-phase sequencer (9). Yield on the NH<sub>2</sub>-terminal Met was 76 pmol (15% yield).

cytochrome P-450 antibodies as determined by ELISA and immunoblot analyses. Cytochrome HLx gave a significant positive ELISA reaction with a monospecific polyclonal anti-P-450g IgG that does not react with other rat cytochrome P-450 isozymes (21) (Table III). In contrast HLx was not recognized by monospecific antibodies to P-450f, P-450a, P-450b, P-450c, P-450d, or P-450i (Table III). In addition, none of five monoclonal antibodies specific for cytochrome P-450h (31) gave a significant reaction with HLx in the ELISA (data not shown). Moreover, immunoblots of purified HLx developed with antibody preparations monospecific for cytochrome P-450f, P-450i, or P-450g demonstrated that only the last antibody, anti-P-450g, recognized HLx (Fig. 2 A). However, immunoblots of HLx developed with polyclonal antibodies directed against cytochrome P-450f, P-450g, or P-450i that had not been absorbed to render them monospecific each reacted strongly with HLx (Fig. 2 B). These results indicate that HLx is related not only to P-450g but also to P-450f and P-450i. Thus, the results from these immunochemical comparisons indicate that HLx is immunochemically most closely related to P-450g.

Next, we examined interindividual differences among 21 human liver specimens for the amounts of HLx protein. We prepared a murine monoclonal antibody against purified HLx

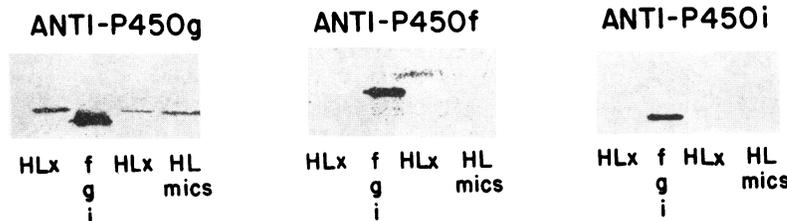
Table III. Reactivity of Purified Human Cytochrome HLx with Polyclonal Antibodies Made against Several Rat Cytochrome P-450 Isozymes in an ELISA

Antibody	Absorbance at 490 nm		HLx % reactivity
	Homologous rat protein	HLx	
Anti-P-450a	0.92	0.00	ND
Anti-P-450b	1.15	0.01	<2%
Anti-P-450c	1.88	0.00	ND
Anti-P-450d	0.74	0.00	ND
Anti-P-450f	1.62	0.00	ND
Anti-P-450g	1.31	0.25	19
Anti-P-450i	1.23	0.00	ND

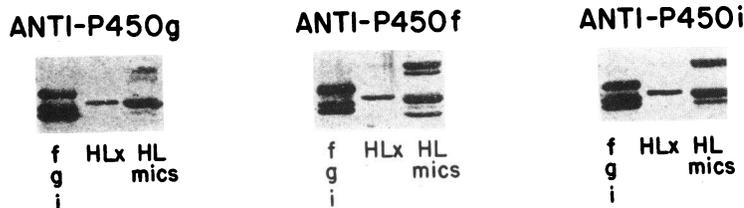
Conditions for the noncompetitive ELISA and the sources of the antibodies are given in Methods. Antibodies used are monospecific for the antigen of immunization, except anti-P-450b, which also recognizes cytochrome P-450e (32).

for immunoquantitation analyses (Fig. 3). This monoclonal antibody gave a negative reaction when tested by immunoblot analysis or ELISA with any of 12 purified rat cytochromes P-450 (P-450a-k and P-450p, data not shown). Analyses of 21 human liver microsomal samples immunoblots developed with monoclonal anti-HLx revealed, in each instance, a single well-defined band having the mobility of HLx (data not shown). We made quantitative measurements of HLx by scanning densitometry of immunoblots developed with the monoclonal anti-HLx IgG (Table I), the results being expressed relative to the value for patient 14 (arbitrarily set to 100%). There was a 4.7-fold range in the level of liver microsomal immunoreactive HLx (Table I) among the 21 samples. This range is less than that determined in the same samples for cytochromes HLj (5.9-fold), HLp (7.6-fold), or HLd (25-fold) (Table I). The values for the concentration of immunoreactive HLx gave no significant correlation with the age, gender, or history of medications for the patients (Table I). However, as demonstrated in previous studies, HLj levels were significantly elevated in patients receiving isoniazid or ethanol (12), and HLp levels were significantly elevated in patients receiving dexamethasone or triacetyloleandomycin (9). In addition, the widely varying HLd levels did not segregate with any of the parameters examined (11). Furthermore, the amounts of HLx immunoreactive protein were unrelated to that for three other human liver cytochromes P-450, HLd (11), HLj (12), and HLp (9) (Table I). On inspection of this HLx immunoquantitation data, it is evident that the seven highest values (> 133) represent a population distinct from the remaining 14 low values. Statistical analysis of this data indicated that the distribution of HLx in these patients is at least bimodal, with the break occurring as indicated above (Ward's minimum variance cluster analysis [33]). Although additional samples are needed to confirm the bimodal distribution of HLx, the present data suggest that there may be a polymorphism among humans for high and low expression of immunoreactive HLx protein. Whereas this conclusion is unprecedented for other human cytochromes P-450, the levels of P-450g in Long Evans rats (21) and LM1 in New Zealand White rabbits (34) are at least bimodally distributed. For example, among individual Long Evans rats there is a 50-fold difference in P-450g levels (21) and among New Zealand White rabbits there is a 10-fold difference in LM1 levels (34). However, our observations do not

## A MONOSPECIFIC IgGs



## B POLYSPECIFIC IgGs



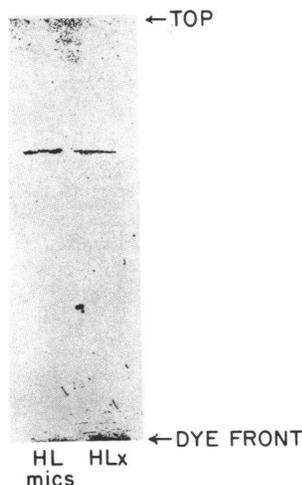
**Figure 2.** Immunoblots of human liver microsomes and cytochrome HLx and rat liver cytochromes P-450f, P-450g, and P-450i. Purified human liver cytochrome HLx (0.2  $\mu$ g, left; 0.05  $\mu$ g, right) and rat liver cytochromes P-450f, P-450g, and P-450i (0.2  $\mu$ g each) and human liver microsomes isolated from patient 8 (5  $\mu$ g) were immunoblotted as described in Methods with (A) antibodies rendered monospecific by immunoabsorption for rat liver cytochromes P-450g, P-450f, or P-450i or (B) antibodies against rat liver cytochromes P-450g, P-450f, and P-450i before immunoabsorption.

eliminate the possibility that the bimodal distribution of HLx levels reflect the influences of yet to be identified environmental factors.

It was possible that the function of HLx might resemble that of either rat cytochrome P-450g or rabbit cytochrome P-450 LM1. For example, LM1 catalyzes the 21-hydroxylation of progesterone in rabbits resulting in formation of deoxycorticosterone (17). However, human liver microsomes do not catalyze progesterone hydroxylation at the 21 position (35). In a reconstituted system consisting of purified HLx, optimal phospholipid, and excess purified rat NADPH-cytochrome P-450 reductase, no detectable metabolism of progesterone was observed, including hydroxylation at the 21 position. However, very low but detectable (nmol product/min per nmol HLx) metabolism by HLx was observed with benzo(a)pyrene (0.15), 7-ethoxycoumarin (0.6), and testosterone (0.3, 16 $\alpha$ -hydroxytestosterone). In addition, HLx actively metabolized benzphetamine (9.7). Therefore, although HLx is catalytically active, its metabolism of progesterone clearly differs from rabbit LM1 (17) and rat P-450g (36). Such a loss of correspondence in substrate specificity of structurally related cytochromes has been noted previously for other interspecies comparisons. For example, cytochrome P-450p metabolizes mephenytoin in the rat (37), whereas in humans this drug is oxidized not by its

ortholog HLP, but rather by P-450MP, a cytochrome structurally related to but clearly distinct from HLx.

Recently, a full-length complementary DNA (cDNA) clone to a human liver mRNA has been isolated with the use of an antibody probe to rabbit LM1 (38). The deduced NH<sub>2</sub>-terminal amino acid sequence of the protein encoded by this cDNA (38) and that described here for HLx protein (Table II) match each other exactly. Analyses of human genomic DNA with this probe suggest that the encoded protein, probably HLx or a highly related isozyme, is a representative of a large multigene family (Robert H. Tukey, personal communication). This interpretation is supported by our finding multiple protein bands on immunoblots of human liver microsomes developed with antibodies that recognize rat cytochromes P-450f-i (Fig. 2 B). Based on comparisons of NH<sub>2</sub>-terminal amino acid sequences (Table II) and complete cDNA sequences (39; Robert H. Tukey, personal communication), we believe that HLx and P-450MP, a human liver mephenytoin-metabolizing isozyme (29), are structurally related members of the same gene family. A better understanding of the function and genetic control of this family of "uninducible" isozymes may help to explain clinically important interindividual differences in the physiologic and toxicologic processes dependent on the cytochromes P-450.



**Figure 3.** Immunoblot of human liver microsomes and purified cytochrome HLx developed with monoclonal anti-HLx. Human liver microsomes (25  $\mu$ g) isolated from patient 8 and cytochrome HLx (0.5  $\mu$ g) were immunoblotted as described in Methods using a murine monoclonal anti-HLx IgG.

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