The turnover of cytochrome P450b

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The turnover of the heme and apocytochrome moieties of phenobarbital-inducible microsomal cytochrome P450 (P450b) was investigated. Adult male Sprague–Dawley rats were treated with phenobarbital for 5 days and injected with [^{35}S]methionine and the heme precursor δ-[^{3}H]-aminolevulinic acid. P450b was isolated by immunoprecipitation and quantitated by rocket immunoelectrophoresis. The isotope disappearance curves revealed a mean half-life ($T_{1/2}$) of 12.4 h for the heme moiety and a $T_{1/2}$ of 19.1 h for the apoprotein moiety of P450b. The apparently slower catabolic rate of the apoprotein may be due to reutilization of [^{35}S]methionine and does not exclude synchronous turnover of the two moieties. Our data are consistent with the kinetics of the drug-mediated induction of cytochrome P450b.

Cytochrome P450 *Heme* *Apocytochrome P450* *Phenobarbital* *Catabolic rate*

1. INTRODUCTION

Cytochrome P450 is the collective term for a family of hemoproteins which function as terminal oxidases in the metabolism of endogenous and exogenous substrates including steroids and fatty acids, drugs, insecticides, environmental pollutants and chemical carcinogens [1]. Cytochrome P450 isozymes determine the substrate specifity of microsomal oxidation reactions. The adaptative increase of these isozymes in response to various substrates alters the metabolic capacity, i.e., the rate of formation and the pattern of metabolites from a specific substrate [2]. Knowledge of the turnover rate of cytochrome P450 is a prerequisite for the interpretation of regulatory changes in its concentration. Previous indirect estimations of the catabolic rate of cytochrome P450 have been based on the rate of degradation of the heme moiety of cytochrome P450 of microsomes after removal of cytochrome b_5 by protease digestion [3,4]. No data are available on the turnover rate of the apocytochrome P450. We therefore have estimated the turnover rate of phenobarbital-inducible cytochrome P450 (cytochrome P450b) by following the disappearance of radioactivity from its simultaneous labeled heme and apoprotein moieties after isolation and quantitation of cytochrome P450b by immunological methods.

2. MATERIALS AND METHODS

Mature Sprague–Dawley rats (SD-rats), inbred at the Süddeutsches Tierzuchtinstitut (100–150 g body wt) had free access to commercial chow and water. Sodium phenobarbital (PB), dissolved in 0.9% NaCl solution was administered to rats by intraperitoneal injection (80 mg/kg) daily for 5 days. L-[^{35}S]methionine ([^{35}S]Met, 970–1227 Ci/mmol) and δ-[3,5-^{3}H(*N*)]aminolevulinic acid ([^{3}H]ALA, 1 Ci/mmol) were from New England Nuclear Corp. (Boston MA). The last dose of phenobarbital was given 1 h prior to injection of radioactive precursors. 250 μCi [^{3}H]ALA and 200 μCi [^{35}S]Met were injected intravenously and intraperitoneally, respectively, into each rat. After 15 min unlabeled methionine (0.2 μmol) was injected intraperitoneally. The rats were killed at various time periods thereafter and the livers perfused in situ through the portal vein with ice-cold 0.9% NaCl solution and homogenized in 0.25 M sucrose. Microsomes were isolated from the post-

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mitochondrial supernatant (10 000 × g for 20 min) by centrifugation at 105 000 × g for 1 h and were resuspended in 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4).

Aliquots of microsomes containing 1 nmol spectrally determined total cytochrome P450 were solubilized with 0.1 M $NaPO_4$ buffer (pH 7.4) containing 10% glycerol, 1% sodium cholate and 0.2% Emulgen 911 and immunoprecipitated with an antibody form specific for rat liver cytochrome P450b. Radioactivity was determined in one half of the immunoprecipitate and heme [5] and cytochrome P450 (spectrally) in the other half [6].

Cytochrome P450b was quantitated by rocket immunoelectrophoresis according to [7]. For the production of the form-specific antibody, cytochrome P450b was purified as in [8] to spec. act. 17 nmol/mg protein and antibodies raised in Swiss-domestic rabbits. Protein was determined as in [9]. Radioactive samples were dissolved in 10 ml Instagel (Packard) and counted in a Packard Tri Carb 460 CD liquid scintillation counter. Counting efficiencies were 60% for ^{35}S and 45% for ^{3}H. The degradation rate constant, k, was calculated from the relationship $A_t = A_0 e^{-kt}$, where A_t is the specific radioactivity at time t and A_0 is the initial radioactivity. In practice k is given by the slope of the line calculated from the linear regression of $\ln A_t$ against t and $T_{1/2} = \ln 2/k$.

3. RESULTS AND DISCUSSION

Our study represents the first simultaneous evaluation of the turnover of apoprotein and of prosthetic heme of an immunologically defined isozyme of cytochrome P450. Prolonged phenobarbital pretreatment was chosen to assure steady-state conditions of cytochrome P450b synthesis and degradation as a prerequisite for turnover studies. Indeed, the levels of total cytochrome P450 (not shown) as well as those of cytochrome P450b (fig.1) remained virtually unchanged during the

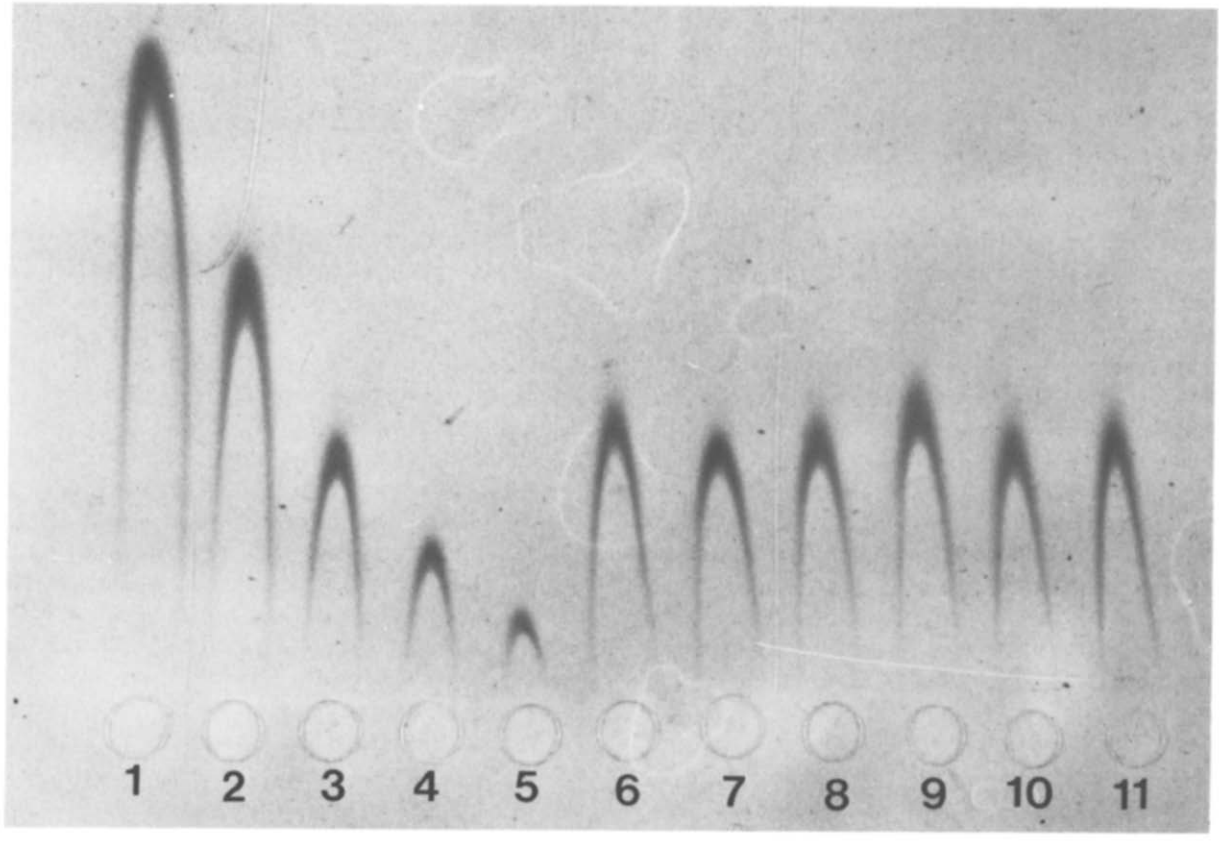

Fig.1

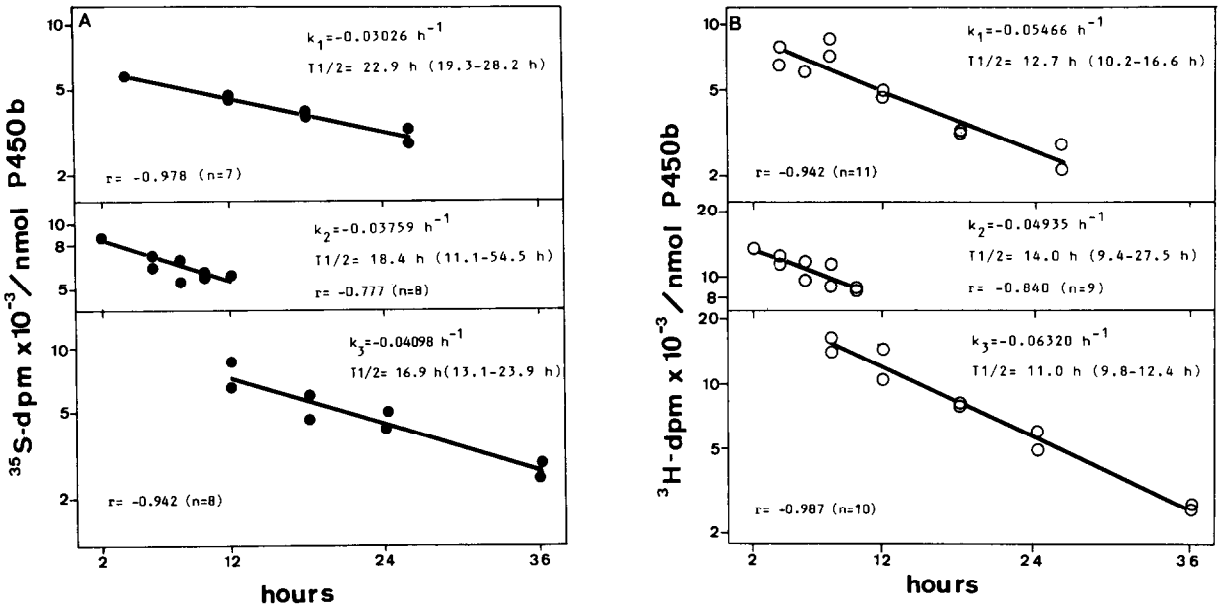

Fig.2. Disappearance of radioactivity from immunoprecipitated cytochrome P450b quantified by rocket immunoelectrophoresis. Phenobarbital-induced (80 mg/kg for 5 days) adult male rats were injected with 200 μCi [^{35}S]Met (intraperitoneally) and 250 μCi [^{3}H]ALA (intravenously) 1 h after the last dose of phenobarbital and killed at various times thereafter. Microsomes were prepared from liver homogenate, solubilized, and the radioactivity found in the immunoprecipitate from 1 nmol total cytochrome P450 was determined. The values plotted are the observed specific activities. The lines were calculated by linear regression. The mean rate constant of degradation, $\bar{k}$, of 3 experiments was calculated from $\bar{k} = 1/3(k_1 + k_2 + k_3)$ and the mean standard deviation of $\bar{k}$ from:

$$S_{\bar{k}} = \sqrt{\frac{(n_1 - 2)S_1^2 + (n_2 - 2)S_2^2 + (n_3 - 2)S_3^2}{n_1 + n_2 + n_3 - 6}}$$

Values in parentheses denote 95% confidence limits. (A) Disappearance of [^{35}S]Met-labeled microsomal apocytochrome P450b. The mean half-life from 3 experiments was $T_{1/2} = 19.1$ h (95% confidence limits: 17.8–20.6 h; $\bar{k} = 0.0363\ h^{-1}$, $S_{\bar{k}} = 0.00132\ h^{-1}$). (B) Disappearance of ^{3}H-labeled heme of microsomal cytochrome P450b. The mean half-life was $T_{1/2} = 12.4$ h (9.7–17.4 h; $\bar{k} = 0.05574\ h^{-1}$, $S_{\bar{k}} = 0.00791\ h^{-1}$).

turnover experiments. A mean $T_{1/2}$ of 12.4 h for the heme moiety and a mean $T_{1/2}$ of 19.1 h for the apoprotein moiety of cytochrome P450b was found (fig.2). Since an unknown degree of reutilization of methionine has to be assumed, our studies cannot exclude that the heme and apoprotein moieties are turning over synchronously in vivo. Our data (table 1) suggest, that the radioactivity derived from [^{3}H]ALA most probably represents heme of holocytochrome P450b, since equal amounts of spectrally measured cytochrome P450, spectrofluorimetrically determined heme and immunologically measured apocytochrome were observed. The isotope disappearance curve for the heme moiety

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Fig.1. Electroimmunochemical precipitation profiles of cytochrome P450b. Rocket immunoelectrophoresis was performed with detergent-solubilized liver microsomes from phenobarbital-treated rats [7]. Each well contained 7 pmol spectrally measured cytochrome P450. The microsomes were analyzed 4 h (well 6), 6 h (well 7), 8 h (well 8), 12 h (well 9), 18 h (well 10) and 26 h (well 11) after the injection of [^{35}S]Met and [^{3}H]ALA (see also fig.2). Wells 1–5 contained various concentrations of cytochrome P450b as standard.

Table 1

Apocytochrome and heme of cytochrome P450b

	Protein or heme (pmol) $m \pm SD$ (n)
(A) Rocket immunoelectrophoresis of 1 nmol total cytochrome P450	388 ± 96 (11)
(B) CO-binding spectrum of cytochrome P450b in immunoprecipitate from 1 nmol total cytochrome P450	318 ± 36 (11)
(C) Heme concentration by the oxalic acid method in immunoprecipitate from 1 nmol total cytochrome P450	339 ± 53 (8)

appeared monophasic in contrast to a biphasic curve reported on the basis of studies in protease-digested microsomes containing multiple cytochrome P450 isozymes and small contents of cytochrome b_5 [3,4].

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REFERENCES

[1] White, R.E. and Coon, M.J. (1980) Annu. Rev. Biochem. 49, 315–365.
[2] Lu, A.Y.H. and West, S.B. (1980) Pharmacol. Rev. 31, 277–295.
[3] Meyer, U.A. and Marver, H.S. (1971) Science 171, 64–66.
[4] Levin, W. and Kuntzman, R. (1969) J. Biol. Chem. 244, 3671.
[5] Morrison, G.R. (1965) Anal. Chem. 37, 1124–1126.
[6] Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2370–2378.
[7] Pickett, C.B., Jeter, R.L., Morin, J. and Lu, A.Y.H. (1980) J. Biol. Chem. 256, 8815–8820.
[8] West, S.B., Huang, M.T., Miwa, G.T. and Lu, A.Y.H. (1979) Arch. Biochem. Biophys. 193, 42–50.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.