

Effect of unbalanced diets on incorporation of δ -aminolevulinic acid into cytochrome P-450

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The *in vivo* syntheses of two liver microsomal cytochromes P-450 PB_{3a}, P-450 UT₅₀ [(1987) Eur. J. Biochem., submitted] (*M*_r 50 000, 52 000) have been estimated by measuring the specific activity 2 h after *i.p.* administration of δ -[³H]aminolevulinic acid to male Sprague Dawley rats. The animals were fed either a standard rat chow (5% lard, 22% casein) or unbalanced diets (high lipid, 30% lard or low protein, 6% casein) with or without 50 ppm Phenoclor DP6. The high-lipid diet supported a more rapid body weight gain but had little impact on cytochrome P-450 content, expressed either per whole liver or per mg microsomal protein, and on the incorporation of the precursor into cytochrome P-450. The latter was determined by measuring the radioactivity incorporated into the cytochrome P-450 fraction, partially purified by affinity chromatography, as well as into two cytochrome P-450 isozymes (*M*_r 50 000 or 52 000) purified by DEAE-52 cellulose ion-exchange chromatography. The low-protein diet, on the other hand, severely depressed body weight gain and cytochrome P-450 content as well as incorporation of radioactivity, the lower-*M*_r cytochrome (*M*_r 50 000) being particularly affected. However, when a potent inducer, Phenoclor DP6, was added to the low-protein diet, cytochrome synthesis was restored indicating that the effect was reversible.

Cytochrome P-450 synthesis; Dietary protein; Polychlorobiphenyl; Nutritional effect; δ -Aminolevulinic acid

1. INTRODUCTION

In rat liver several diverse cytochromes (cyt.) P-450 exist, many of which can be increased by treatment with different xenobiotics [1-3]. Moreover, nutritional disorders may cause changes in the levels of total cytochrome P-450 [4-7] and their characteristics [8-10]. Knowledge of the rate of synthesis of cyt. P-450 is needed for the interpretation of regulatory mechanisms in concentration changes.

Here, we have evaluated the synthesis rate of two cyt. P-450 isoenzymes from liver of rats fed

either standard or unbalanced diets with or without treatment with the inducer Phenoclor DP6. From N-terminal sequence analysis, these proteins cyt. P-450 PB_{3a} and cyt. P-450 UT₅₀ can be compared with cyt. P-450b and P-450_i described by Botelho et al. [2] and Levin et al. [11], respectively.

2. MATERIALS AND METHODS

Weanling Sprague Dawley male rats were fed a standard diet (St) containing 22% casein and 5% lard *ad libitum* for 1 week and then divided into 3 groups. Group 1 was fed the standard diet, group 2 a high-lipid diet (HL 30% lard), and group 3 a low-protein diet (L_P 6% casein). Experimental diets were fed for 6 weeks. Each group was further divided, half of the animals being fed the ex-

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perimental diet containing 50 ppm Phenoclor DP6 [a French polychlorobiphenyl (PCB) from Prodelec] for the last 4 weeks. δ -[^3H]Aminolevulinic acid (δ ALA, 510 mCi/mmol) from New England Nuclear dissolved in 0.9% NaCl solution was administered to rats by intraperitoneal injection (50 $\mu\text{Ci}/100$ g body wt) 2 h before killing. Microsomes were isolated from liver as in [12]. Cyt. P-450 was solubilized with sodium cholate and purified by affinity chromatography on ω -*n*-diaminooctyl-Sepharose 4B (AOS), prepared as described [13]. Purified cyt. P-450 was eluted with 0.08% non-ionic detergent (Emulgen 911) at a specific content of 17 nmol per mg protein with a recovery of approx. 45% of total microsomal cyt. P-450. Cyt. P-450 eluted from the affinity column was immediately pooled and dialyzed overnight against 10 mM phosphate buffer and applied on a Whatman DEAE-52 cellulose ion-exchange column previously equilibrated with 600 ml of 10 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, 0.1 mM EDTA and 0.2% sodium cholate. After sample application the column was washed with equilibration buffer followed by a linear gradient of NaCl (0–300 mM) in 500 ml of the same buffer during which cytochrome isoenzymes were sequentially eluted. Each showed a single protein staining band when analyzed by SDS-polyacrylamide gel electrophoresis and molecular masses of 50 and 52 kDa, respectively. The identification of cytochrome isoenzymes by means of antibody cross-reaction, amino acid analysis, catalytic activity and N-terminal analysis has been reported separately [14]. The specific contents were 14–19 nmol cyt. P-450/mg protein. Samples of total cyt. P-450 eluted from the aminooctyl-Sepharose column (AOS P-450) and the purified isoenzymes were dissolved in soluene 350 and toluene PPO-POPOP mixture. Radioactivity was determined using a Searle 300 liquid scintillation counter. Protein was assayed as described by Lowry et al. [15]. Total microsomal cyt. P-450 was measured as reported by Omura and Sato [16].

3. RESULTS AND DISCUSSION

The rate of biosynthesis of total cyt. P-450 and those of two purified cyt. P-450 isozymes were estimated in the liver of rats treated with standard

and unbalanced diets with and without Phenoclor DP6, a potent cyt. P-450 inducer.

Levels of translatable mRNA coding for cytochromes P-450 are increased by various inducers [17] indicating an increased rate of cyt. P-450 synthesis. Previous estimations of the turnover rate of cyt. P-450 have been based on the rate of degradation of the heme moiety of cyt. P-450 after removal of cyt. *b*₅ by protease digestion [18]. In 1981 we showed [19] by using simultaneously [^3H] δ ALA and [^{14}C]guanidinoarginine (low reutilizable precursor) that the turnover of the two moieties of cyt. P-450 was synchronous. This observation was confirmed in 1982 in purified cyt. P-450 [20]. Thus, the level of incorporation of [^3H] δ ALA into the cyt. P-450 heme can be related to the synthesis rate of the two cytochrome moieties.

Prolonged dietary and inducer treatments were chosen to ensure steady-state conditions for cyt. P-450 isozyme biosynthesis. Therefore, when the precursor was injected the precursor pool/cyt. P-450 pool ratio was quite different in the experimental groups. Thus, the specific values calculated in dpm/nmol cyt. P-450 must be corrected by a factor to account for these differences as first suggested by Kuriyama et al. [21], these factors being reported in table 1. For example, control rats were injected with 50 $\mu\text{Ci}/100$ g body wt, namely 148 μCi . The total content of liver cytochrome was 253 nmol. For the low-protein group 63 μCi δ ALA were injected, the cyt. P-450 liver content being 68 nmol. The precursor/cytochrome ratio was calculated as follows: body wt of the standard diet group $\times$ total liver cyt. P-450 of the treated group/body wt of treated group $\times$ total liver cyt. P-450 in the standard diet group (treated groups refer to unbalanced diet fed groups and DP6-treated groups). Table 2 lists the effects of unbalanced diets and DP6 treatment on the incorporation of [^3H] δ ALA into cyt. P-450. The interpretation must be carried out on the basis of the corrected values.

In all experimental groups the incorporation of precursor was higher for the higher- M_r cyt. P-450 isozyme, P-450 PB_{3a}. Based on our characterizations of ligand binding, catalytic activity, amino acid composition and N-terminal sequences ([11] and unpublished), this protein can be compared with the cytochromes P-450 purified by Botelho et

Table 1
Body weight and hepatic cytochrome P-450 content of liver microsomes from rats fed standard or unbalanced diets with or without DP6

	St ^a	HL	Lp	St + P	HL + P	Lp + P
Body weight (g)	296 ± 7 ^b	329 ± 8	127 ± 6	297 ± 9	320 ± 11	129 ± 7
Cytochrome P-450 (nmol/whole liver)	253 ± 25	287 ± 18	68 ± 6	747 ± 88	1328 ± 97	233 ± 11
(nmol/mg microsomal protein)	0.925 ± 0.084	1.027 ± 0.061	0.720 ± 0.104	1.684 ± 0.061	1.959 ± 0.079	1.450 ± 0.146
Correction factor ^c	1	1.02	0.63	2.94	4.86	2.13

^a Rats were fed standard diet (St) or unbalanced diets (HL, high-lipid; Lp, low-protein) with (+ P) or without Phenoclor DP6

^b Means ± SD from 8 animals are shown

^c Correction factor: (body wt St × total liver P-450 treated)/(body wt treated × total liver P-450 St)

Table 2

Effect of unbalanced diet and DP6 treatment on the incorporation of [^3H] δALA into cytochrome P-450

Group	Cytochrome P-450 (dpm/nmol)					
	Uncorrected values			Corrected values		
	AOS P-450 ^a	P-450 UT ₅₀	P-450 PB _{3a}	AOS P-450	P-450 UT ₅₀	P-450 PB _{3a}
St ^b	42492 ^c $\pm$ 3806	33846 $\pm$ 2309	56258 $\pm$ 4308	42000	34000	56000
HL	43068 $\pm$ 2968	33420 $\pm$ 2030	55348 $\pm$ 3785	43000	34000	56000
Lp	1118 $\pm$ 103	288 $\pm$ 90	2141 $\pm$ 129	700	200	14000
St + P	29006 $\pm$ 2202	15208 $\pm$ 1304	36155 $\pm$ 2628	85000	45000	106000
HL + P	15856 $\pm$ 1185	8415 $\pm$ 597	20079 $\pm$ 1504	77000	41000	98000
Lp + P	20591 $\pm$ 1692	19025 $\pm$ 1008	33036 $\pm$ 2466	44000	40000	70000

^a AOS P-450: total cytochrome P-450 eluted from ω -aminooctyl-Sepharose by Emulgen 911 as indicated in section 2^b See footnotes to table 1^c Means $\pm$ SD of 4 determinations are shown

al. [2], cyt. P-450 UT₅₀ being identical or very similar to cytochrome P-450₁ [11]. From the decay of radioactivity in the heme and the protein moieties, Parkinson and co-workers [22], studying cyt. P-450 turnover in Arochlor 1254-treated immature male long Evans rats, assumed that two kinetically distinct populations of cyt. P-450a may exist. The half-life calculated for cyt. P-450a from the slow kinetics of radioactivity decay was 40% longer than that calculated from the decay of radioactivity in cyt. P-450b. In the present experiments the incorporation of [^3H] δALA was 40% lower in cyt. P-450 UT₅₀ than in cyt. P-450 PB_{3a} in untreated rats. From this it may be concluded that cyt. P-450 UT₅₀ isolated from untreated rats corresponds to the slow kinetic population of cyt. P-450a described by Parkinson et al. [23]. The results also suggest that a new population of cyt. P-450 UT₅₀ with a fast kinetic incorporation of radioactivity appears after treatment by inducers. With high-lipid diet incorporation into the enzymic forms of cyt. P-450 remained unchanged compared with the standard diet group. However, the low-protein diet strongly decreased incorporation of the precursor in both isoenzymes and AOS P-450. Compared with the standard diet group incorporation was only 1.6% in AOS P-450, 0.5% in P-450 UT₅₀ and 25% in P-450 PB_{3a}. This decrease in synthesis may explain the decrease in total liver cyt. P-450 to one quarter of that present during the standard diet (table 1). The treatment with Phenoclor DP6 increases the incorporation of

[^3H] δALA into AOS cyt. P-450 up to 2-fold. The synthesis of cyt. P-450 PB_{3a} shows a larger increase (90%) than that of cyt. P-450 UT₅₀ (32%). Parkinson et al. [23] showed that cyt. P-450b was highly induced by 2,2',4,4',5,5'-hexachlorobiphenyl and that the induction reached a maximum 5 days after PCB treatment and remained maximally induced for 2 weeks after treatment. 2,2',4,4',5,5'-Hexachlorobiphenyl is a major constituent of Phenoclor DP6 and the induction of cyt. P-450b can be related to the high induction in demethylase activity by this PCB [23].

The increased incorporation of precursor in cyt. P-450 UT₅₀ may be due either to an increase in the rate of synthesis of the population of cyt. P-450 UT₅₀ found in untreated rats or to the appearance of a new population of cyt. P-450 UT₅₀ with fast kinetic parameters. We have previously shown [14] that the catalytic activities were quite different between cyt. P-450 UT₅₀ isolated from untreated animals and cyt. P-450 UT₅₀ from DP6-treated rats whereas the catalytic activities of cyt. P-450 PB_{3a} remained largely unchanged on DP6 treatment. When Ryan and co-workers [24] isolated P-450a from immature male Long Evans rats pretreated with Aroclor 1254, phenobarbital or 3-methylcholanthrene, they found considerable variations in the specific activities of benzo(a)-pyrene hydroxylase, ethoxycoumarin-O-deethylase and zoxazolamine hydroxylase [24]. Although these authors did not purify cyt. P-450a from the livers of untreated rats, one explanation for the

variation in activity they observed in induced rats may be the different extents to which individual members of a cyt. P-450a family are increased following inducer treatment. Our results support the existence of a new population of cyt. P-450 UT₅₀. The increases in precursor incorporation into cyt. P-450 isoenzymes by Phenoclor DP6 were not modified by high-lipid diet. In the group fed a low protein + DP6 diet the incorporation of [³H]- δ ALA is slightly increased (20%) compared with animals on the standard diet and not treated with inducers. However, when the influence of DP6 in the animals fed the low-protein diet was studied the increase caused by DP6 was 200-fold for cyt. P-450 UT₅₀ and 5-fold for cyt. P-450 PB_{3a}. Thus it can be concluded that low-protein diet strongly decreases the synthesis of cyt. P-450 UT₅₀ and P-450 PB_{3a}, but treatment with DP6 restores a normal synthesis rate to both enzymic forms.

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