MOLECULAR PHARMACOLOGY, 14, 215-219

SHORT COMMUNICATION

Differences between Nuclear and Microsomal Cytochrome P-450 in Uninduced and Induced Rat Liver

ELEANOR ROGAN AND ERCOLE CAVALIERI

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

(Received May 11, 1977)
(Accepted September 12, 1977)

SUMMARY

ROGAN, ELEANOR & CAVALIERI, ERCOLE (1978) Differences between nuclear and microsomal cytochrome P-450 in uninduced and induced rat liver. *Mol. Pharmacol.*, 14, 215-219.

Cytochrome P-450 in rat liver nuclei was distinguishable from that in the corresponding liver microsomes. Characterization was carried out using carbon monoxide and ethyl isocyanide binding difference spectra. Nuclear cytochrome P-450 in 3-methylcholanthrene-induced animals had different spectral properties from the nuclear cytochrome P-450 in uninduced and phenobarbital-induced animals. These spectral data demonstrate that hepatic nuclear cytochrome P-450 differs from the microsomal species.

Cytochrome P-450, the terminal oxidase of monooxygenase systems (1), has been shown to be a heterogeneous enzyme in both normal rat liver microsomes and microsomes from rats treated with a variety of drugs (2-7). Spectral characteristics (8, 9) and catalytic activities (10) have been used to differentiate the effects of various inducers. Multiple forms of microsomal cytochromes P-448 and P-450 also have been detected and characterized by spectral properties, chromatographic properties, lipid content, molecular weight, and antigenicity (2-7).

Cytochrome P-450 is also present in rat liver nuclei (11) and is inducible by MC[1] and PB (12). Nuclear monooxygenase enzymes may well play a critical role in carcinogenesis because of their capacity to induce binding of aromatic hydrocarbons to DNA (12). Such hydrocarbons induce a different form of microsomal cytochrome P-450 having enhanced specificity for hydrocarbons (13). Therefore determination of the effects of inducers on the form of nuclear cytochrome P-450 is of great interest. Because differences between nuclear and microsomal monooxygenase activities have already been observed (12), comparison of cytochromes P-450 from these organelles is also of interest. With these objectives, we investigated the reduced carbon monoxide and ethyl isocyanide difference spectra of nuclear cytochrome P-450 in uninduced and PB- and MC-induced rat liver nuclei.

Nuclei (14) and microsomes (15) were prepared from the livers of ten 3-week-old male Sprague-Dawley rats (the Eppley colony). Nuclear membranes were prepared from nuclei by the DNase digestion method of Kay *et al* (16). Monooxygenase activities were induced by intraperitoneal injection of MC in olive oil, 25 mg/kg of body weight, 24 hr before killing or by

This work was supported by Grant 1 RO1 CA14826 from the National Institutes of Health and by Public Health Service Contract NO1 CP33278 with the Division of Cancer Cause and Prevention, National Cancer Institute.

[1] The abbreviations used are: MC, 3-methylcholanthrene; PB, phenobarbital.

0026-895X/78/0141-0215$02.00/0

twice daily intraperitoneal injections of sodium PB in olive oil, 37 mg/kg of body weight, for 3 days. These conditions induced optimal levels of cytochrome P-450 in our animals. Protein was determined by the method of Lowry *et al.* (17). Cytochrome P-450 was measured in three to seven different preparations by its carbon monoxide binding difference spectrum after reduction with sodium dithionite on an Aminco DW-2 spectrophotometer according to Estabrook *et al.* (18), except that the buffer used was 0.23 M Tris–50% glycerol, pH 7.5. Ethyl isocyanide (Sigma) binding difference spectra were measured for three replicate preparations as described by Sladek and Mannering (19), except that the 0.23 M Tris–50% glycerol buffer, pH 7.5, was used. Aryl hydrocarbon hydroxylase was assayed (20) in each preparation of nuclear membranes and microsomes to confirm that the preparations contained monooxygenase activity. The possibility that nuclear membranes and microsomes were contaminated with hemoglobin was eliminated by measuring the difference spectrum between a CO-treated and an untreated sample; a peak at 420 nm indicated the presence of hemoglobin. A maximum of 1% as much hemoglobin as cytochrome P-450 was found in microsomes, and less than that in nuclear membranes.

Reduced carbon monoxide binding difference spectra obtained for microsomal and nuclear cytochrome P-450 from uninduced, PB-induced, and MC-induced animals are presented in Fig. 1 and summarized in Table 1. In each case the microsomal cytochrome showed the expected spectral maximum, i.e., 450 nm for uninduced and PB-induced and 448 nm for MC-induced animals, and contained little material absorbing around 420 nm; the shoulder observed for PB- and MC-induced cytochrome and the small peak observed for uninduced cytochrome centered at about 422 nm.

The spectra of nuclear cytochrome P-450 were significantly different from those of the corresponding microsomal cytochrome for every set of nuclear membranes and microsomes prepared from a group of animals. These observations show clearly

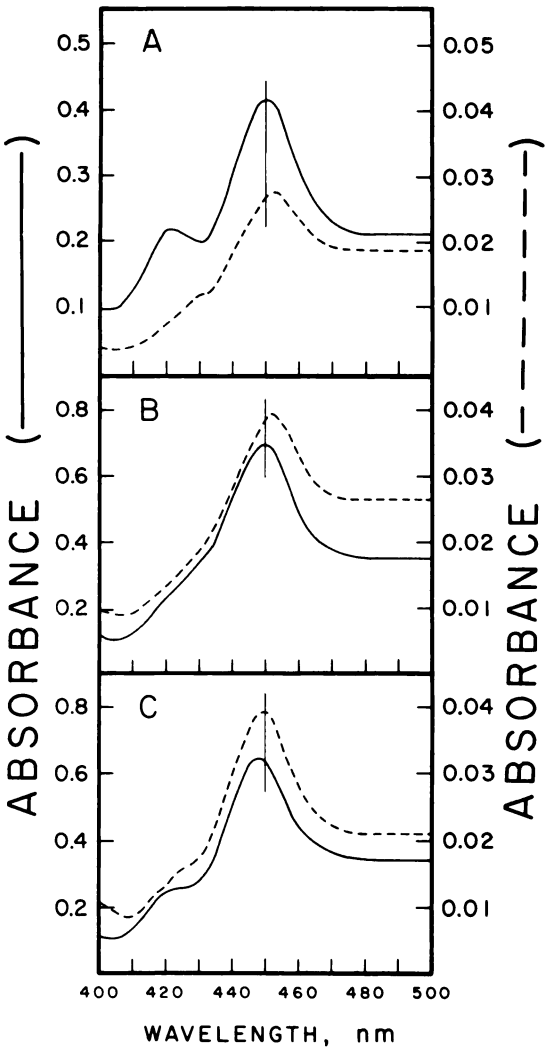

FIG. 1. *CO binding difference spectra of hepatic microsomes* (——) *and nuclear membranes* (– – –)

A. Uninduced (7.1, 6.0). B. PB-induced (1.7, 3.4). C. MC-induced (3.6, 1.5). Numbers in parentheses are concentrations of protein in milligrams per milliliter for microsomes first, then nuclear membranes.

that monooxygenase activities in nuclei do not result from contamination with endoplasmic reticulum. The spectrum obtained with uninduced nuclear membranes (Fig. 1A) had a peak at 452 nm. After induction with PB (Fig. 1B) the nuclear cytochrome again peaked at 452 nm. Induction with MC (Fig. 1C) gave rise to nuclear cytochrome with a peak at 450 nm instead of the 448 nm expected

from all studies with liver microsomal cytochrome. The CO binding spectra of nuclear membranes were identical with spectra obtained with whole nuclei, except that nuclei exhibited a peak at about 420 nm, resulting from contamination with hemoglobin.

Ethyl isocyanide binding difference spectra are shown in Fig. 2. Nuclear cytochrome was clearly different from microsomal cytochrome. The spectral data are summarized in Table 1. The ratio of A_{455}:A_{430} for nuclear cytochrome was much less than 1.0 for uninduced and PB-induced animals, while the ratio after MC induction was about 1.0. The ratio in microsomes was considerably higher. These spectral characteristics for uninduced and PB-induced nuclear cytochrome P-450 were identical, both in the positions of the peaks (452 nm with CO; 430 and 457–458 nm with ethyl isocyanide) and in the A_{455}:A_{430} ratio. The microsomal cytochrome P-450 from these animals also could not be differentiated, although the A_{455}:A_{430} ratio in uninduced microsomes was slightly lower than in those induced by PB. The position of the "430 nm" peak at 428–429 nm was consistent. This discrepancy between these data and the 430 nm previously reported (3) does not seem significant. Placement of the upper peak at 454 nm for PB-induced and at 452 nm for MC-induced microsomes and the A_{455}:A_{430} ratios agree well with the observations of Ryan *et al.* (3). MC-induced nuclear cytochrome was clearly distinguishable from the uninduced and PB-induced species, both in the positions of the CO and ethyl isocyanide peaks and in the A_{455}:A_{430} ratio. The differences in the 430 and 455 nm chromophores determined for the various nuclear and microsomal preparations have been suggested to be the result of structural differences in the vicinity of the prosthetic group of the cytochromes P-450 (21).

The different spectral maxima indicate a structural form of nuclear cytochrome P-450 different from the microsomal cytochrome P-450. Other reports of different cytochromes P-450 have been made. Rat kidney cortex microsomes contain cytochrome P-454 (22). The absorption maximum of cytochrome P-450 from rat lung microsomes shifts from 453 nm in uninduced to 451 nm in MC-induced animals (23).

The different spectral characteristics with MC-induced nuclear cytochrome also indicate that MC induces a form of cytochrome P-450 in nuclei different from that in uninduced and PB-induced nuclei. Since nuclear cytochrome P-450 is induced 6–7-fold by MC (12) (Fig. 1), at least 80% of the cytochrome present is the newly synthesized, induced form. Therefore the spectrum must reflect the maximum of the induced form. Previous studies with microsomal cytochrome have consistently

TABLE 1

Spectral characteristics of cytochrome P-450

Carbon monoxide and ethyl isocyanide binding difference spectra were measured as described in the text. The concentration of protein in the cuvettes ranged from 1.5 to 7.1 mg/ml. The concentration of ethyl isocyanide was 10 μM. Data are from Figs. 1 and 2.

Source of cytochrome P-450	CO difference spectral peak	Ethyl isocyanide difference spectrum	
		Peaks	A_{455}:A_{430}
	nm	*nm*	
Uninduced			
Nuclear membranes	452	430, 457	0.17–0.40
Microsomes	450	429, 454	0.83–0.98
PB-induced			
Nuclear membranes	452	430, 458	0.26–0.36
Microsomes	450	428, 454	0.95–1.12
MC-induced			
Nuclear membranes	450	430, 453	0.96–1.05
Microsomes	448	429, 452	1.45–1.65

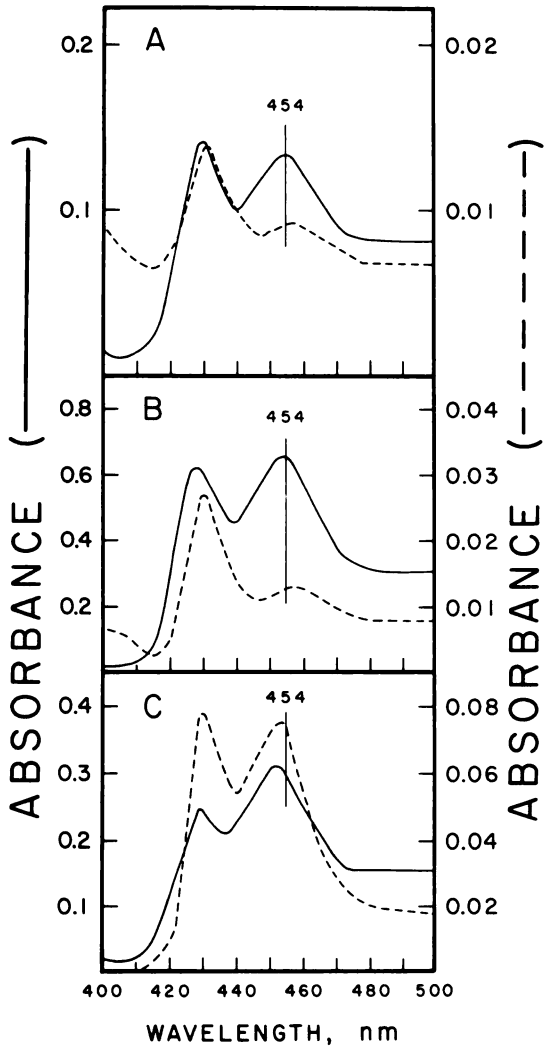

FIG. 2. *Ethyl isocyanide binding difference spectra of hepatic microsomes* (——) *and nuclear membranes* (- - -)

A. Uninduced (2.5, 1.5). B. PB-induced (2.5, 1.6). C. MC-induced (2.3, 2.2). Numbers in parentheses are concentrations of protein in milligrams per milliliter for microsomes first, then nuclear membranes. The concentration of ethyl isocyanide was 10 mM.

demonstrated that cytochromes P-448 and P-450 represent distinct, though heterogeneous, forms with distinguishable physical properties (3, 24) as well as enzyme activities (19). The position of the spectral maximum appears to be an intrinsic characteristic of each cytochrome, rather than an effect of the environment in which the cytochrome resides (25). The induction by a polycyclic hydrocarbon of nuclear cytochrome P-450 that differs spectrally from microsomal cytochrome P-448 is an indication that the cytochrome itself may determine the differences in monooxygenase metabolism observed between microsomes and nuclei (26–28).

REFERENCES

1. Cooper, D. Y., Levin, S., Narasimhulu, S., Rosenthal, O. & Estabrook, R. W. (1965) *Science*, 147, 400–402.
2. Comai, K. & Gaylor, J. L. (1973) *J. Biol. Chem.*, 248, 4947–4955.
3. Ryan, D., Lu, A. Y. H., West, S. & Levin, W. (1975) *J. Biol. Chem.*, 250, 2157–2163.
4. Welton, A. F., O'Neal, F. O., Chaney, L. C. & Aust, S. D. (1975) *J. Biol. Chem.*, 250, 5631–5639.
5. Werringloer, J. & Estabrook, R. W. (1975) *Arch. Biochem. Biophys.*, 167, 270–286.
6. Thomas, P. E., Lu, A. Y. H., Ryan, D., West, S. B., Kawalek, J. & Levin, W. (1976) *Mol. Pharmacol.*, 12, 746–758.
7. Dean, W. L. & Coon, M. J. (1977) *J. Biol. Chem.*, 252, 3255–3261.
8. Alvares, A. P., Schilling, G., Levin, W. & Kuntzman, R. (1967) *Biochem. Biophys. Res. Commun.*, 29, 521–526.
9. Sladek, N. E. & Mannering, G. J. (1969). *Mol. Pharmacol.*, 5, 186–199.
10. Lu, A. Y. H., Kuntzman, R., West, S., Jacobson, M. & Conney, A. H. (1972) *J. Biol. Chem.*, 247, 1727–1734.
11. Kasper, C. B. (1971) *J. Biol. Chem.*, 246, 577–581.
12. Rogan, E. G., Mailander, P. & Cavalieri, E. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, 73, 457–461.
13. Conney, A. H. (1967) *Pharmacol. Rev.*, 19, 317–366.
14. Berezney, R., Macaulay, L. K. & Crane, F. L. (1972) *J. Biol. Chem.*, 247, 5549–5561.
15. Schenkman, J. B. & Cinti, D. L. (1972) *Life Sci.*, 11, P. 2, 247–257.
16. Kay, R. R., Fraser, D. & Johnston, I. R. (1972) *Eur. J. Biochem.*, 30, 145–154.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, 193, 265–275.
18. Estabrook, R. W., Cooper, D. Y. & Rosenthal, O. (1963) *Biochem. Z.*, 338, 741–755.
19. Sladek, N. E. & Mannering, G. J. (1966) *Biochem. Biophys. Res. Commun.*, 24, 668–674.
20. Nebert, D. W. & Gelboin, H. V. (1968) *J. Biol. Chem.*, 243, 6242–6249.
21. Peisach, J. & Mannering, G. J. (1975) *Mol.*

Pharmacol., 11, 818–823.
22. Jakobsson, S., Thor, H. & Orrenius, S. (1970) *Biochem. Biophys. Res. Commun.*, 39, 1073–1080.
23. Capdevila, J., Jakobsson, S. W., Jernström, B., Helia, O. & Orrenius, S. (1975) *Cancer Res.*, 35, 2820–2829.
24. Ryan, D., Lu, A. Y. H., Kawalek, J., West, S. B. & Levin, W. (1975) *Biochem. Biophys. Res. Commun.*, 64, 1134–1141.
25. Orrenius, S. & Ernster, L. (1974) in *Molecular Mechanisms of Oxygen Activation* (Hayaishi, O., ed.), pp. 215–244, Academic Press, New York.
26. Rogan, E., Roth, R., Cavalieri, E., Katomski, P. & Benderson, J. (1977) *Proc. Am. Assoc. Cancer Res.*, 18, 59.
27. Lesko, S. & Ts'o, P. O. P. (1977) *Proc. Am. Assoc. Cancer Res.*, 18, 171.
28. Rogan, E., Roth, R., Katomski, P., Benderson, J. & Cavalieri, E. (1978) *Chem.-Biol. Interact.*, in press.