# AN INVESTIGATION OF THE DEGRADATION OF CYTOCHROME P-450 HEMOPROTEINS USING SDS GEL ELECTROPHORESIS*

Kathryn M. Ivanetich and Jean J. Bradshaw

Department of Medical Biochemistry
University of Cape Town Medical School
Observatory, Cape, South Africa

Received June 13, 1977

SUMMARY: Treatment with fluroxene or allyl-iso-propylacetamide of rats induced for elevated levels of cytochromes P-450 results in markedly decreased levels of hepatic microsomal cytochromes P-450 and heme as determined by spectral assay but in unchanged levels of cytochromes P-450 as determined by SDS gel electrophoresis. Since SDS gel electrophoresis does not detect changes in the heme of cytochromes P-450, it is concluded that fluroxene and AIA do not chemically degrade the apoproteins of cytochromes P-450.

The volatile anesthetic agent fluroxene (2,2,2-trifluoroethyl vinyl ether) and the porphyrogenic compound AIA are known to degrade the heme moiety of one or more of the cytochrome P-450 hemoproteins without affecting the levels of other microsomal enzymes (1-3)[1]. The heme moiety of cytochrome P-450 alone appears to be degraded by fluroxene following phenobarbital induction, whereas the heme of cytochrome P-448 is preferentially degraded in 3-methylcholanthrene induced animals (4). AIA apparently degrades the heme of cytochrome P-450 following induction by either phenobarbital or 3-methylcholanthrene (2). Although several different cytochrome P-450 hemoproteins have been separated by SDS gel electrophoresis on the basis of differences in their apoproteins (5,6), the apoprotein and holoprotein of type P-450 cytochromes are not separable from one another by SDS gel electrophoresis (6). Never-

* Supported by grants from the South African Medical Research Council, the University of Cape Town Staff Research Fund and Harry Crossley Research Fund.

[1] Abbreviations used are AIA, allyl-iso-propylacetamide; SDS, sodium dodecylsulfate.





ISSN 0006-291X

theless, Baird et al. (7) have based conclusions on the identity of the cytochromes P-450 susceptible to degradation by AIA on changes in SDS gel electrophoresis patterns.

The present paper demonstrates that although SDS gel electrophoresis can not be utilized for estimation of the relative amounts of cytochrome P-450 hemoproteins following degradation of the heme moiety of these enzymes, this technique can provide information on the integrity of the apoprotein.

## EXPERIMENTAL:

Groups of 6 to 8 male Wistar rats of similar weights (190 ± 5 g) were induced with sodium phenobarbital or 3-methylcholanthrene and were starved overnight after the last injection (8). The following morning, some of the animals received fluroxene intraperitoneally or AIA by subcutaneous injection. In the case of phenobarbital induced rats, the animals received 2 ml/kg fluroxene or 200 mg/kg AIA and were killed 55 ± 5 min thereafter. In the case of 3-methylcholanthrene induction, the rats received 5 ml/kg fluroxene and were killed 85 ± 5 min after the fluroxene treatment. Hepatic microsomes were isolated by gel filtration on Sepharose 2B (9) in 0.15 M KCl - 0.02 M Tris-HCl, pH 7.4. Microsomal protein was determined by the method of Lowry et al. (10) as modified by Chaykin (11). The levels of type P-450 cytochromes and heme in hepatic microsomes were assayed by the method of Omura and Sato (12).

SDS gel electrophoresis was performed on 5.6% polyacrylamide gels containing 0.1% SDS, essentially as described by Welton and Aust (5). Bromophenol blue (0.01%) was used as tracking dye. The developed gels were stained with Coomassie blue (13) or with a benzidine stain (14) to locate protein or heme. Cytochrome P-450 hemoproteins were localized by the above stains in conjunction with molecular weight determinations (5). The gels stained with Coomassie blue were scanned at 550 nm using a Varian Techtron model 635 spectrophotometer with a gel-scanning attachment. Areas of chromatogram peaks were calculated by a Unicam SP 88 computing integrator.

## RESULTS AND DISCUSSION:

The ability of fluroxene and AIA to degrade the heme moiety of cytochrome P-450 hemoproteins is confirmed in Table 1. Based on absorbance measurements, treatment of phenobarbital and 3-methyl-

Table 1. The effects of fluroxene and AIA on hepatic microsomal cytochrome P-450 hemoproteins[a]

| Induction | Additional treatment | Cytochrome P-450[b] (nmol/mg mic. protein) | Heme[b] (nmol/mg mic. protein) | Cytochrome P-450 hemoprotein bands[c] % of total area Peak 1 | Peak 2 | Peak 3 | Peak 4 | Total area (arbitrary units) |
|---|---|---|---|---|---|---|---|---|
| PB | None | 3.01±.04 | 3.27±.49 | 41±2 | 29±1 | 23±1 | 8±1 | 38±5 |
| PB | Fluroxene | 1.79±.39 | 2.20±.42 | 40±3 | 25±3 | 27±4 | 8±1 | 32±3 |
| PB | None | 2.06±.03 | 2.80±.08 | 45±2 | 30±2 | 17±4 | 8±1 | 16±3 |
| PB | AIA | 0.63±.05 | 1.19±.05 | 45±1 | 27±2 | 21±2 | 8±1 | 19±3 |
| MC | None | 2.27±.07 | 3.19±.01 | 22 | 45 | 24±1 | 10±1 | 29±5 |
| MC | Fluroxene | 1.06±.03 | 2.10±.05 | 22±2 | 46±3 | 23±1 | 11±1 | 26±4 |

Means and standard deviations reported represent at least three sets of experiments performed with different preparations of hepatic microsomes.

[a] Abbreviations used are mic., microsomal; PB, phenobarbital; MC, 3-methylcholanthrene.

[b] Determined by method of Omura & Sato (12).

[c] Determined by SDS gel electrophoresis.

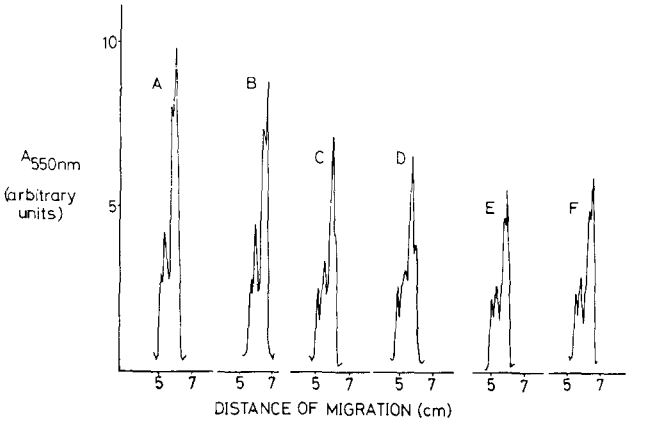


Figure 1. SDS gel electrophoresis patterns obtained with hepatic microsomes from animals pretreated with (A) phenobarbital, (B) phenobarbital plus fluroxene, (C) 3-methylcholanthrene, (D) 3-methylcholanthrene plus fluroxene, (E) phenobarbital, (F) phenobarbital plus AIA. (A),(B): 3.5 mg microsomal protein; (C)-(F): 3.0 mg microsomal protein.

cholanthrene induced animals with fluroxene results in a decrease of approximately 1.2 nmol/mg microsomal protein in the levels of hepatic microsomal cytochromes P-450 and heme. Similarly, treatment of phenobarbital induced animals with AIA results in a decrease of 1.6 nmol/mg microsomal protein in the levels of hepatic microsomal cytochromes P-450 and heme (Table 1).

The scans of the cytochrome P-450 band patterns obtained following SDS gel electrophoresis of hepatic microsomes from induced animals treated or not with fluroxene or AIA are shown in Figure 1. The treatment of phenobarbital or 3-methylcholanthrene induced animals with fluroxene or of phenobarbital induced animals with AIA results in no change in the pattern or in the relative areas of the cytochrome P-450 hemoprotein bands (Fig. 1, Table 1). In addition, the treatment of induced animals with fluroxene or AIA does not appreciably alter the sum of the areas of all of the cytochrome P-450 hemoprotein peaks (Table 1).

These results indicate that neither fluroxene nor AIA gives rise to appreciable alteration in the SDS electrophoretograms of cytochromes P-450, although the heme prosthetic group of these enzymes is degraded to a significant extent.

Since the apoprotein and hemoprotein of cytochromes P-450 are not separable by SDS gel electrophoresis (6), it is not possible to detect changes in the heme of cytochromes P-450 by this technique. The inability of fluroxene and AIA to alter the electrophoretograms of cytochromes P-450 suggests that these chemicals do not degrade the apoproteins of cytochromes P-450. In addition, the inability of fluroxene and AIA to convert cytochrome P-450 to cytochrome P-420 suggests that these compounds do not considerably alter the conformation of the native protein.

It is concluded that the SDS gel electrophoretic patterns obtained after degradation of the heme moiety of type P-450 cytochromes do not reflect the levels of the intact cytochrome P-450 hemoproteins in the hepatic microsomes *in vitro*. Furthermore, since fluroxene and AIA considerably degrade heme without affecting the SDS electrophoresis of cytochromes P-450, it is concluded that these compounds do not chemically degrade the apoprotein moieties of cytochromes P-450. It would appear that the attempt by Baird *et al*. (7) to utilize SDS gel electrophoresis to identify the cytochrome P-450 hemoprotein degraded by AIA is invalid.

ACKNOWLEDGEMENT: We would like to gratefully acknowledge the advice and assistance of Professor John E. Kench in preparing this manuscript.

REFERENCES:

1. Ivanetich, K.M., Marsh, J.A., Bradshaw, J.J., and Kaminsky, L.S. (1975) Biochem. Pharmac., 24, 1933-1936.

2. Levin, W., Jacobson, M., and Kuntzman, R. (1972) Arch. Biochem. Biophys., 148, 262-269.
3. De Matteis, F. (1971) Biochem. J., 124, 767-777.
4. Bradshaw, J.J. (1977) M.Sc. Thesis, University of Cape Town Medical School.
5. Welton, A.F., and Aust, S.D. (1974) Biochem. Biophys. Res. Commun., 56, 898-905.
6. Haugen, D.A., van der Hoeven, T.A., and Coon, M.J. (1975) J. Biol. Chem., 250, 3567-3570.
7. Baird, M.B., Birnbaum, L.S., Samis, H.V., Massie, H.R., and Zimmerman, J.A. (1976) Biochem. Pharmac., 25, 2416-2418.
8. Ivanetich, K.M., Bradshaw, J.J., Marsh, J.A., Harrison, G.G., and Kaminsky, L.S. (1976) Biochem. Pharmac., 25, 773-778.
9. Tangen, O., Jonsson, J., and Orrenius, S. (1973) Analyt. Biochem., 54, 597-603.
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275.
11. Chaykin, S. (1966) Biochemistry Laboratory Techniques, p.20, Wiley, New York.
12. Omura, T., and Sato, R. (1964) J. Biol. Chem., 239, 2370-2378.
13. Fairbanks, G., Steck, T.L., and Wallach, D.F.H. (1971) Biochemistry, 10, 2606-2617.
14. Clausen, J. (1968) in Laboratory Techniques in Biochemistry and Molecular Biology, v.1. (Work, T.S., and Work, E., eds.), North Holland Publishers, Amsterdam, p. 535.