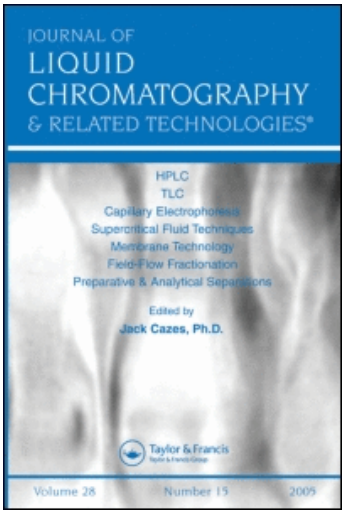

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Raineri, Ronald , Poiley, Judith A. , Hillesund, Tove and Pienta, Roman J.(1979) 'A Comparison of Benzo(A)Pyrene-4,5-Epoxide Hydrase Activity in Hamster Embryo Cells, Hepatocytes and Livers Using High-Pressure Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 2: 4, 577 — 587
To link to this Article: DOI: 10.1080/01483917908060086
URL: http://dx.doi.org/10.1080/01483917908060086

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(4), 577-587 (1979)

A COMPARISON OF BENZO(A)PYRENE-4,5-EPOXIDE HYDRASE ACTIVITY IN HAMSTER EMBRYO CELLS, HEPATOCYTES AND LIVERS USING HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

The benzo(a)pyrene-4,5-epoxide (BP-4,5-epoxide) hydrase activities of intact hamster hepatocytes and embryo cells, and homogenates of these cells as well as of adult liver are compared. The product of the epoxide hydrase (EH) reaction, trans-4,5-dihydro-4,5-dihydroxybenzo(a)pyrene (BP-4,5-diol), was isolated by high-pressure liquid chromatography (HPLC) with a Waters Bondapak C_{18}/Corasil column and acetonitrile-water as the mobile phase. Using this procedure to determine BP-4,5-epoxide hydrase activity in intact cells, it was found that 266 nmoles of BP-4,5-diol/10^6 cells were produced by hepatocytes while no diol formation was detected with embryo cells. EH activity in the intact hepatocytes was 8-fold greater than in hepatocyte homogenates.

INTRODUCTION

Although the hamster cell transformation assay can detect many carcinogens, the chemical transformation of cells in culture often requires the addition of enzyme systems to metabolically activate the chemical being tested (1). Therefore, it is important to determine first if the activation system (i.e., "feeder layer" cells and isolated enzyme systems such as liver microsome preparations) is able to metabolize carcinogens. A number of metabolic reactions, including the EH reaction, which has been measured with a variety of epoxide substrates, namely styrene oxide (2), the K-region epoxide of 3-methylcholanthrene (3,4) and BP-4,5-epoxide (5-7), may be used for this purpose.

Enzymes prepared from disrupted cells may be used to determine the metabolic capabilities of cells. However, a more valid measure of metabolic events can be obtained only by using intact cells which are metabolically functional. We report here the EH activity in cultured cells using as a substrate, the K-region epoxide of the widespread carcinogen BP. Using a modification of previously published HPLC procedures for the separation of BP metabolites (5,8), we measured the EH activity in intact hamster hepatocytes and embryo cells, as well as in enzyme preparations from hamster hepatocytes, embryo cells and liver.

EXPERIMENTAL

Apparatus

A Spectra-Physics Model 3500B Gradient Liquid Chromatograph equipped with a Model 770 Variable Wavelength Detector (Spectra-Physics, Santa Clara, CA) and a Waters Bondapak C_{18}/Corasil column (2' x 1/8") (Waters Associates, Inc., Framingham, MA) was used in all experiments.

Materials

BP-4,5-dihydrodiol, BP-4,5-epoxide, 5-hydroxy-BP and randomly labeled BP-4,5-epoxide-[^{3}H], (438 mCi/mmole) were generously provided by the NCI Carcinogenesis Research Program, Bethesda, MD. Syrian golden hamsters of the Graffi strain were supplied by Dr. Robert J. Huebner of the Viral Oncology Program at the Frederick Cancer Research Center. Dulbecco's modified Eagle's medium (Microbiological Associates, Inc., Walkersville, MD), supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Rockville, MD) and 2 mM L-glutamine, was used as the growth medium.

Preparation of Cells, Cell Homogenates and Liver 9,000 x g Supernatants

Hamster embryo cells and hepatocytes were prepared from Syrian golden hamsters as described elsewhere (9,10). Pelleted cells were homogenized in 0.05 M Tris buffer, pH 7.5 containing 0.25 M sucrose, with a Dounce homogenizer. Hamster liver 9,000 x g supernatants were prepared by homogenizing livers (8-10 week old animals) in 0.05 M Tris buffer (pH 7.5) containing 0.25 M sucrose, with a motor drive teflon-glass Potter-Elvehjem homogenizer followed by centrifugation of the homogenate at 1,000 x g for 15 min. The supernatant was then centrifuged at 9,000 x g for 20 min. Protein concentrations were determined by the method of Lowry et al (11).

Determination of BP-4,5-Epoxide Hydrase Activity in Liver and Cellular Enzyme Preparations and in Intact Cells

The reaction mixture and extraction procedure were similar to those used by Nesnow and Heidelberger (4) for determination of EH in enzyme preparations, except that we used tritiated BP-4,5-epoxide in place of unlabeled 3-methylcholanthrene-11,12-epoxide as the substrate. BP-4,5-epoxide was stored in tetrahydrofuran and before each experiment, an aliquot was evaporated to dryness under N_2 and dissolved in dimethylsulfoxide (DMSO). For determination of EH

activity in 9000 x g fractions, the enzyme preparations were added to 0.1 M sodium phosphate buffer, pH 8.0 (total volume, 0.44 ml) and preincubated at 37°C with shaking for 1 min. After BP-4,5-epoxide-[^{3}H] (0.4 μCi) was added in 10 μl of DMSO, the incubation was continued for 5 min unless otherwise stated.

For the determination of EH activity in intact cells, 2.5 x 10^5 hamster embryo cells or 5 x 10^4 hamster hepatocytes were treated for 20 min with 88 μM BP-4,5-epoxide-[^{3}H] (1.1 μCi) in a total volume of 1 ml of Dulbecco's modified Eagle's medium without serum in 60 mm^2 dishes. The sensitivity of this procedure may be enhanced by increasing the ratio of radiolabeled to unlabeled BP-4,5-epoxide which in these experiments is only 1:45.

Following incubation, the cells were scraped in the medium, immediately placed on ice and extracted twice with 4 volumes of methylene chloride. After the extract was evaporated to dryness under nitrogen, the residue was dissolved in 0.4 ml of methanol, and the product, BP-4,5-diol was isolated using the HPLC procedure described below.

RESULTS

Isolation of Benzo(a)pyrene-4,5-diol

The BP-4,5-diol was separated from BP-4,5-epoxide and BP using a Waters Bondapak C_{18}/Corasil column and an acetonitrile-water elution system (Figure 1a). The reaction product, BP-4,5-diol was eluted rapidly (within 3 min) with 50% acetonitrile in water. After 2 min, a linear gradient (25%/min) to 100% acetonitrile was initiated. Separation of BP-4,5-diol from 5-hydroxy-BP, a potential by-product formed spontaneously from BP-4,5-epoxide, is shown in Figure 1b.

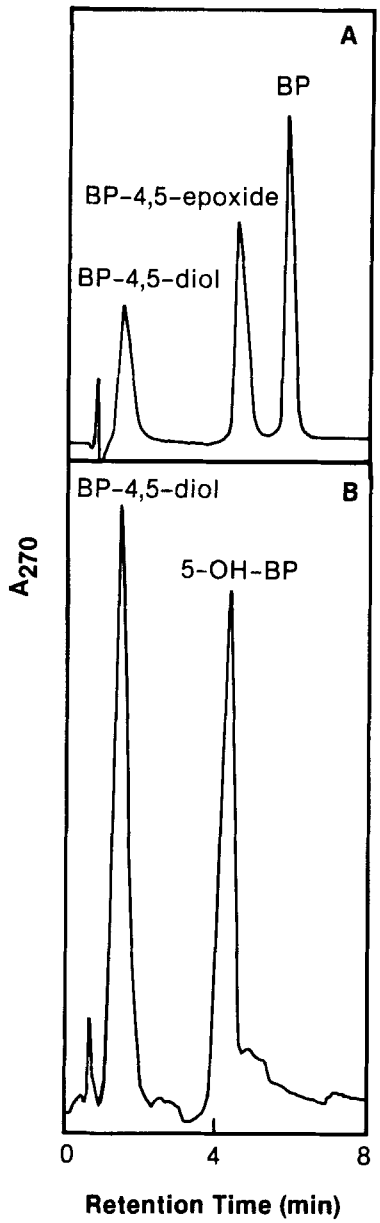

FIGURE 1

A. Separation of BP-4,5-diol from BP-4,5-epoxide and BP. Conditions: Column, Waters Bondapak C_{18}/Corasil (2' x 1/8"); elution solvent system, acetonitrile-water; gradient conditions, a) 50% acetonitrile for 2 min b) gradient of 25%/min to 100% acetonitrile; flow rate, 2 ml/min; temperature, ambient.

B. Chromatogram of mixture of BP-4,5-epoxide and 5-hydroxy-BP. Conditions were the same as those used in Figure 1A.

Determination of Epoxide Hydrase Activity in Enzyme Preparations from Hamster Liver, Hamster Embryo Cells and Hepatocytes and in Intact Hamster Embryo Cells and Hepatocytes

Enzyme preparations were incubated with varying concentrations of BP-4,5-epoxide [^{3}H] and the product of the EH reaction, BP-4,5-diol, was quantified by measurement of radioactivity present in the diol peak which was isolated by HPLC. Using an authentic BP-4,5-diol standard, 80% of the product was recovered.

A 9,000 x g supernatant from hamster livers was used as the source of enzymes to determine the effect of substrate concentration, incubation time and protein concentration on enzyme activity. Saturation of the enzyme occurred when substrate concentrations of approximately 25 μM or more were used in the presence of 0.2 mg of protein (Figure 2). Figure 3 shows that the rate of formation of the enzymatically produced BP-4,5-diol was linear for up to 5 min.

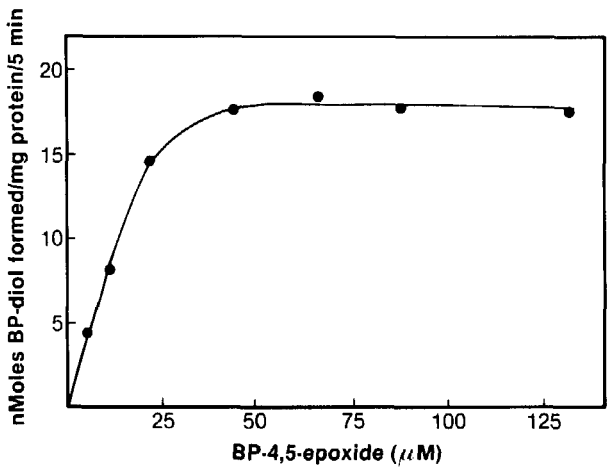

FIGURE 2

Effect of substrate concentration on the rate of formation of BP-4,5-diol. Reaction mixtures (total vol. 0.45 ml) contained varying amounts of substrate and 0.2 mg of protein from a 9,000 x g supernatant from homogenized hamster livers and were incubated for 5 minutes at 37°C.

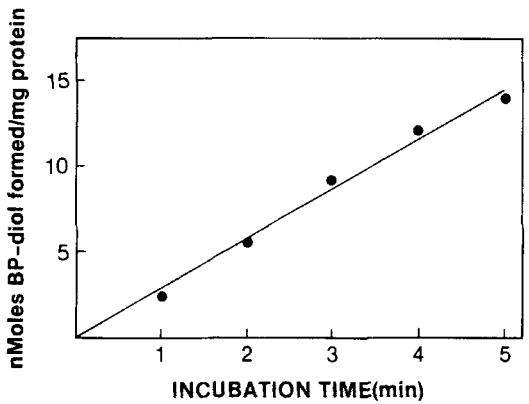

FIGURE 3

Effect of incubation time on the rate of formation of BP-4,5-diol. Incubation conditions were as in Figure 2 except that reaction mixtures contained 88 μM BP-4,5-epoxide.

The effect of protein concentration is shown in Figure 4. A linear rate of BP-4,5-diol formation was observed when up to 0.4 mg of protein was used. When the EH activities of homogenates from cultured hamster hepatocytes and whole hamster embryo cells were compared, the activities were high (8.3 nmoles/mg protein/5 min) in preparations from hepatocytes and very low (0.4 nmoles/mg protein/5 min) in preparations from fibroblasts (Table 1).

To determine the extent of EH activity in cultured cells, hamster hepatocytes and cells from whole embryos were treated with 88 μM of BP-4,5-epoxide-[^{3}H] for 20 min at 37°C, placed on ice and then BP-4,5-diol extracted as described above. Table 1 shows that the EH activity was very high in the intact hepatocytes but no significant activity could be measured in the embryo cells.

We observed that 10^6 hepatocytes contain approximately 0.85 mg protein. This enabled us to compare the EH activities of intact hepatocytes with homogenates of hepatocytes based on their protein content. Further extrapolation of the data in Table 1 to equivalent

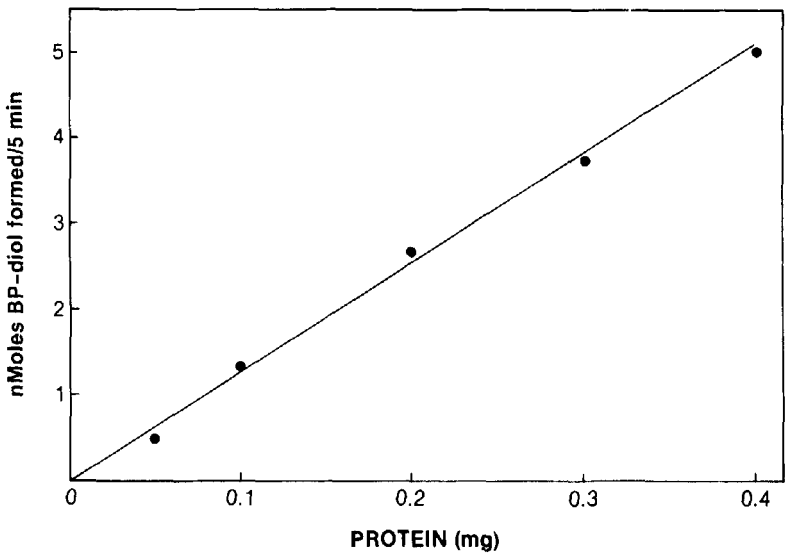

FIGURE 4

Effect of protein concentration on the formation of BP-4,5-diol. Incubation conditions were as in Figure 2 except incubation was for 1, 2, 3, 4, or 5 min.

incubation times showed that hepatocytes treated in culture had eight times greater EH activity than homogenized hepatocyte preparations.

DISCUSSION

The hydration of specific epoxides by EH enzymes has recently been postulated in the formation of the ultimate carcinogen from BP (12), as well as the formation of detoxification products of BP (13). The determination of EH activity in intact cells rather than in enzyme preparations allows mechanistic studies under conditions which are metabolically optimal. A number of methods for the separation of metabolites of BP using HPLC have been reported (8,14,15). The procedure used here is a modification which allows the deter-

TABLE 1

BP-4,5-Epoxide Hydrase Activity in Intact Hamster Embryo Cells and Hamster Hepatocytes or in Cell Homogenates[a]

Preparation	Source	nmoles BP-4,5-diol formed/10^6 cells[b]	nmoles BP-4,5-diol formed/mg protein/5 min
Intact cells	Hamster embryo cells	0 ± 0	0 ± 0[c]
	Hamster hepatocytes	266 ± 31	78 ± 9
Cell homogenates	Hamster embryo cells	-	0.4 ± 0.03[d]
	Hamster hepatocytes	-	8.3 ± 0.00

[a] Values are means ± average deviation from duplicate incubations and have been corrected for 80% product recovery.

[b] Intact cells were incubated for 20 min as described in "Experimental".

[c] Data extrapolated from column on left based on the finding that one million hepatocytes contain 0.85 mg of protein.

[d] Homogenates from cultured cells were incubated for 5 min as described in "Experimental".

mination of EH in intact living cells but without the interference from cellular materials intrinsic to spectrophotometric methods. In addition, rapid elution and excellent resolution of the reaction product BP-4,5-diol, as well as complete separation of the product from the substrate, BP, and the by-product 5-hydroxy-BP, is achieved.

The metabolism of carcinogens in animal systems varies greatly among species. Differences in metabolism may also exist among various types of preparations of the same tissue. Moreover, metabolites of BP have been shown to differ when intact hamster embryo cells or homogenates are used (16). We have shown that the basal level of EH activity is 8-fold greater in intact hamster hepatocytes than in homogenates of hepatocytes.

When exogenous metabolic activation is used in conjunction with mammalian transformation and microbial mutagenesis systems, it is important to determine both the efficiency of the activation system and how closely the system parallels metabolism in the intact animal. Our observation that EH activity is significantly greater in intact hepatocytes (than in homogenates of cells or liver) illustrates the importance of using intact cells for these kinds of studies.

ACKNOWLEDGEMENTS

We thank Mrs. Corothy M. Cavanaugh and Mrs. Mary K. Ernst for their excellent technical assistance and Mrs. Lana L. Cross for preparation of the manuscript.

Research sponsored by the National Cancer Institute under Contract No. NO1-CO-75380 with Litton Bionetics, Inc.

REFERENCES

1. Poiley, J. A., Pienta, R. J., and Raineri, R., 4th Annual Carcinogenesis Collaborative Conference, Orlando, FL, p. 85, 1976.

2. Oesch, F., Jerina, D. M., and Daly, J., Biochem. Biophys. Acta, 277, 685-691, 1971.

3. Stoming, T. A. and Bresnick, E., Science, 181, 951-952, 1973.

4. Nesnow, S. and Heidelberger, C., Anal. Biochem., 67, 525-530, 1975.

5. Leutz, J. C. and Gelboin, H. V., Arch. Biochem. Biophys., 168, 722-725, 1975.

6. Schmassmann, H. U., Glatt, H. R., and Oesch, F., Anal. Biochem., 74, 94-104, 1976.

7. Bornstein, W. A., Chuang, H., and Bresnick, E., Chem.-Biol. Interactions, 21, 343-346, 1978.

8. Selkirk, J. K., Advances in Chromatography, Giddings, J. C., ed., Marcel Dekker, Inc., New York, NY, 1978, pp. 1-36.

9. Poiley, J. A., Raineri, R., and Pienta, R. J., In preparation.

10. Raineri, R., Poiley, J. A., Hillesund, T., and Pienta, R. J., Chem.-Biol. Interactions, 22, 257-270, 1978.

11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265-275, 1951.

12. Sims, P., Grover, P. L., Swaisland, A., Pal, K., and Hewer, A., Nature, 252, 326-328, 1974.

13. Oesch, F., Kaubisch, N., Jerina, D. M., and Daly, J. W., Biochemistry, 10, 4858-4866, 1971.

14. Selkirk, J. K., Croy, R. G., and Gelboin, H. V., Science, 184, 169-171, 1974.

15. Holder, G., Yagi, H., Dansette, P., Jerina, D. J., Leven, W., Lu, A. Y. H., and Conney, A. H., Proc. Natl. Acad. Sci. U.S., 71, 4356-4360, 1974.

16. Selkirk, J. K., Nature, 270, 604-607, 1977.