HIGH MUTAGENICITY OF METABOLICALLY ACTIVATED CHRYSENE 1,2 DIHYDRODIOL: EVIDENCE FOR BAY REGION ACTIVATION OF CHRYSENE

A. W. Wood, W. Levin, D. Ryan, P. E. Thomas, H. Yagi[+], H. D. Mah[+], D. R. Thakker[+], D. M. Jerina[+] and A. H. Conney

Department of Biochemistry and Drug Metabolism
Hoffmann-La Roche Inc., Nutley, New Jersey 07110

and

[+]Section on Oxidation Mechanisms, Laboratory of Chemistry,
National Institutes of Arthritis, Metabolism and Digestive Diseases
National Institutes of Health, Bethesda, Maryland 20014

Received August 17,1977

SUMMARY: Chrysene and the 3 metabolically possible vicinal trans dihydrodiols of chrysene were tested for mutagenicity towards S. typhimurium strain TA100 in the presence of hepatic microsomes or a highly purified hepatic microsomal monooxygenase system. The products formed during the metabolic activation of chrysene 1,2-dihydrodiol were more than 20 times as mutagenic to the bacteria than the metabolites formed from chrysene, chrysene 3,4-dihydrodiol or chrysene 5,6-dihydrodiol. When the double bond in the 3,4-position of chrysene 1,2-dihydrodiol was saturated, the resulting tetrahydrodiol could not be metabolically activated. These results, which strongly suggest that chrysene 1,2-dihydrodiol is activated by metabolism to either or both of the diastereomeric chrysene 1,2-diol-3,4-epoxides, provide additional support for the bay region theory of polycyclic hydrocarbon carcinogenicity.

Introduction

Many cancer-causing chemicals exert their carcinogenic effects only after metabolism to chemically reactive metabolites (ultimate carcinogens) that bind to critical cellular constituents (1-4). The carcinogenicity and widespread occurrence of many polycyclic hydrocarbons in man's environment have prompted numerous studies directed toward the identification of the

---

Abbreviations: Benzo[a]pyrene 7,8-dihydrodiol, trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene; benzo[a]pyrene 7,8-diol-9,10-epoxide, either or both diastereomers of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene derived from benzo[a]pyrene 7,8-dihydrodiol; benz[a]anthracene 3,4-dihydrodiol, trans-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene; benz[a]anthracene 3,4-diol-1,2-epoxide, either or both diastereomers of 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene derived from benz[a]anthracene 3,4-dihydrodiol; chrysene 1,2-dihydrodiol, trans-1,2-dihydroxy-1,2-dihydrochrysene; chrysene 3,4- and 5,6-dihydrodiols, other trans-dihydrodiols of chrysene; chrysene 1,2-diol-3,4-epoxide, either or both diastereomers of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrochrysene derived from chrysene 1,2-dihydrodiol; chrysene H_4 1,2-diol, trans-1,2-dihydroxy-1,2,3,4-tetrahydrochrysene.

ISSN 0006-291X

ultimately reactive metabolites of the parent hydrocarbons. The exceptional chemical and biological activities - including binding to nucleic acid (5-9), mutagenicity (10-13) and carcinogenicity (14) - of one or both diastereomers of benzo[a]pyrene 7,8-diol-9,10-epoxide have led to the conclusion that this metabolite is the principal ultimate carcinogen of benzo[a]pyrene.

Since diol epoxides may be responsible for the carcinogenicity of other polycyclic hydrocarbons, we have developed a theory based on perturbational molecular orbital calculations to predict which diol epoxides of a number of polycyclic hydrocarbons will have high biological activity (15-17). The calculations suggest that the formation of a bay region epoxide on a saturated angular benzo-ring is a principal determinant of polycyclic hydrocarbon carcinogenicity. As the initial test of the bay region theory we synthesized three diastereomeric pairs of diol epoxides of benz[a]anthracene (18) and showed that the 3,4-diol-1,2-epoxides, in which the epoxide forms part of the bay region, were from one to three orders of magnitude more mutagenic to bacterial and mammalian cells than were the other two pairs of diol epoxides (19). In addition, we have shown that benz[a]anthracene 3,4-dihydrodiol, the immediate metabolic precursor of the bay region diol epoxide, is 10 times more mutagenic to bacteria in the presence of a microsomal monooxygenase system (20) and 10 to 20 times more tumorigenic toward mice (21) than is benz[a]anthracene or the four other possible trans dihydrodiols of benz[a]anthracene.

While the high biological activities of benzo[a]pyrene 7,8-diol-9,10-epoxides, benz[a]anthracene 3,4-diol-1,2-epoxides, and their trans dihydrodiol precursors are consistent with the bay region theory, validation of this concept is dependent on the demonstration that the bay region diol epoxides and the precursor dihydrodiols of a number of carcinogenic polycyclic hydrocarbons have high biological activity. Toward this end we have synthesized (22) the three metabolically possible trans dihydrodiols of the carcinogen chrysene (Fig. 1). This communication reports that chrysene 1,2-dihydrodiol, the potential metabolic precursor of the chrysene 1,2-diol-3,4-epoxides in which

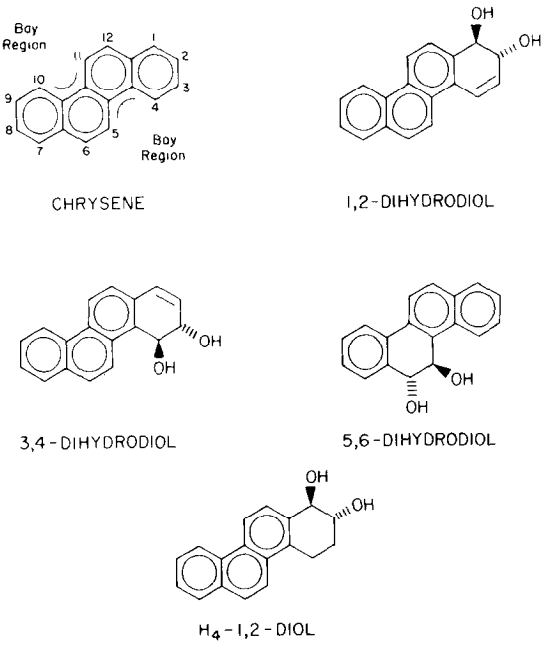

Figure 1. Structure of chrysene, the three vicinal trans dihydrodiols of chrysene and chrysene H_4-1,2-diol. Since chrysene is a symmetric hydrocarbon both bay regions are identical and only the 1,2-, 3,4- and 5,6-dihydrodiols are possible. Stereochemistry is relative.

the epoxide ring forms part of the bay region, is metabolized to exceptionally mutagenic products.

Materials and Methods: Chrysene (95% pure) was obtained from Aldrich Chemical Co. and purified by several recrystalizations from benzene to obtain material melting at 256°. The 1,2-, 3,4- and 5,6-dihydrodiols of chrysene and the H_4-1,2-diol of chrysene were synthesized by unequivocal chemical procedures and the structures of the analytically pure compounds were confirmed by nuclear magnetic resonance spectrometry (22). The hydrocarbons were stored at -90° and dissolved in anhydrous dimethyl sulfoxide immediately before use. Other commercially available biochemicals were obtained from Sigma Chemical Co., St. Louis, Mo. or Sedary Research Labs, Ontario, Canada. Strains TA98 and TA100 of histidine dependent S. typhimurium (23) were obtained from Dr. B. Ames, University of California, Berkeley, and cultured as described (19). Hepatic microsomes were obtained from immature male Long Evans rats pretreated with Aroclor 1254 (Monsanto) (24). Cytochrome P-450 was purified from these microsomes as described (24) and stored at -90° until use. Metabolic activation experiments with microsomes followed the procedure of Ames (23,25) with the exception that 1 unit of glucose-6-phosphate dehydrogenase was added to each reaction and the complete incubation mixture was incubated for 5 min at 37° before addition of the top agar. Experiments with the

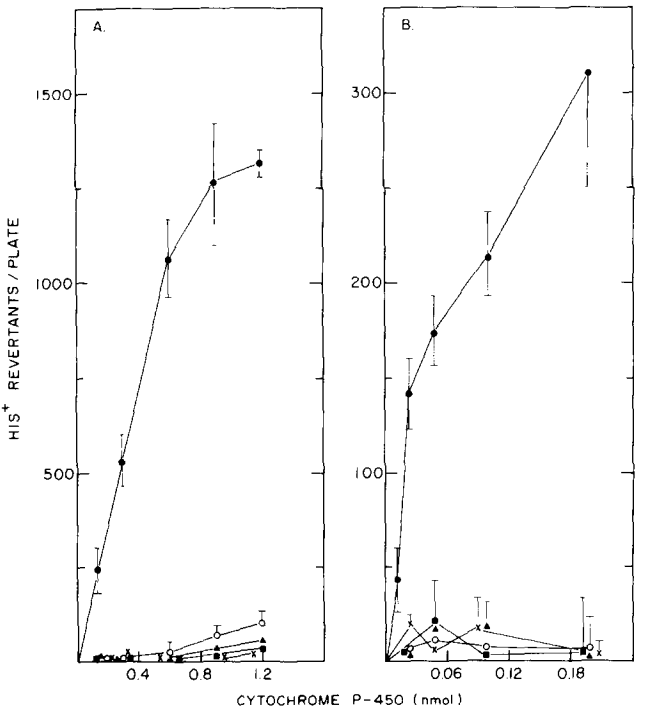

Figure 2. Effect of microsomal cytochrome P-450 concentration (A) and highly purified cytochrome P-450 concentration (B) on the metabolism of chrysene and its dihydrodiols to products mutagenic to strain TA100 of S. typhimurium. In (A), 125 nmol of each hydrocarbon was incubated. In (B), 37.5 nmol of each hydrocarbon was incubated. The specific content of cytochrome P-450 in the microsomes was 3.98 nmol hemoprotein per mg protein. Background mutation frequencies of 80 revertants/plate have been subtracted from the data which is presented as the mean and standard deviation of 3 replicates. Key to symbols: O, chrysene; ●, 1,2-dihydrodiol; ▲, 3,4-dihydrodiol; ■5,6-dihydrodiol; X, H_4-1,2-diol.

purified monooxygenase system were conducted as described for the activation of the benz[a]anthracene dihydrodiols (20) except that 300 units of purified cytochrome c reductase (26) were used. After incubation of the histidine dependent bacteria with either enzyme system, 2 ml of top agar was added to the reaction and the entire mixture was poured onto histidine deficient agar Petri dishes. Mutations were assessed after a two day incubation of the plates by counting the macroscopic colonies of bacteria. All experiments were performed in triplicate.

RESULTS: The mutagenic activities of metabolically activated chrysene and its 3 dihydrodiols are plotted as a function of the amount of microsomal monooxygenase in the reaction mixtures (Fig. 2A). Mutations increased linearly

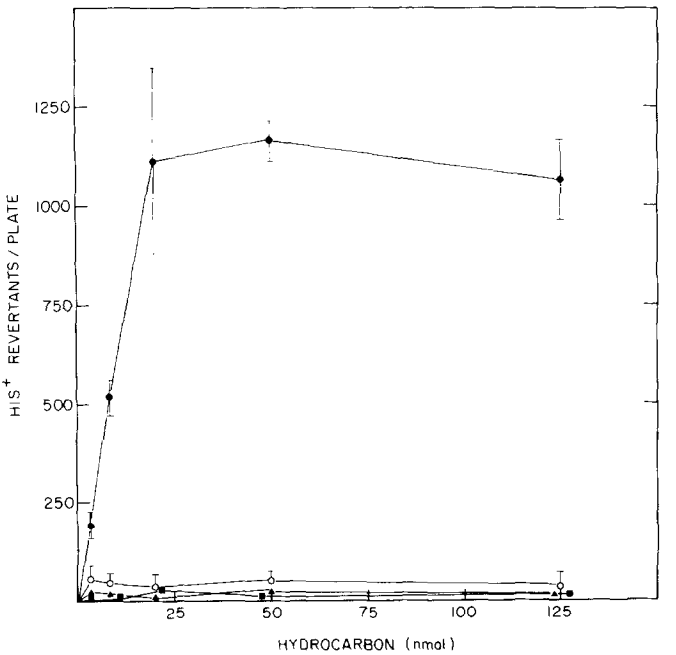

Figure 3. Effect of hydrocarbon concentration on the metabolic activation of chrysene and its dihydrodiols. Hepatic microsomes (0.60 nmol cytochrome P-450 per incubation) were the source of the monooxygenase activity. See Fig. 2 for key to symbols.

over at least a four fold range of enzyme concentration when chrysene 1,2-dihydrodiol was the substrate, and the mutation frequency averaged 1800 histidine revertants per nmol of microsomal cytochrome P-450. In marked contrast to these results, chrysene and the 3,4- and 5,6-dihydrodiols of chrysene as well as the chrysene H_4-1,2-diol showed minimal activation to mutagenic products (< 85 histidine revertants per nmol of cytochrome P-450). Similar results were obtained when the highly purified and reconstituted monooxygenase system was used to metabolize the hydrocarbons (Fig. 2B). The effect of varying the concentrations of the hydrocarbons over a 40-fold range in the presence of a fixed amount of microsomal monooxygenase (0.60 nmol cytochrome P-450) is shown in Fig. 3. Chrysene 1,2-dihydrodiol was activated to mutagenic products when as little as 3 nmol of hydrocarbon was incubated, and the monooxygenase reaction was saturated with respect to substrate when 20 nmol

of the dihydrodiol was present. Chrysene and the other dihydrodiols showed negligible metabolism to mutagenic products over the entire range of substrate concentrations. When tester strain TA98 was used in the metabolic activation studies, chrysene 1,2-dihydrodiol was again the most mutagenic compound (data not shown) although strain TA100 was much more sensitive to the mutagenic activities of the chrysene derivatives under comparable assay conditions.

DISCUSSION: The bay region theory of polycyclic hydrocarbon carcinogenicity predicts that chrysene 1,2-diol-3,4-epoxide is an ultimate carcinogenic metabolite of chrysene. Since only one metabolic step - epoxidation of the adjacent double bond - is needed to convert a dihydrodiol to a diol epoxide, we anticipated that chrysene 1,2-dihydrodiol but not chrysene H_4-1,2-diol would be metabolized to a potent mutagen. The results of the present study indicate that the metabolic product(s) of chrysene 1,2-dihydrodiol are 20 times more mutagenic toward strain TA100 of S. typhimurium than are the metabolites formed from chrysene or chrysene H_4-1,2-diol. The metabolites formed from chrysene 3,4-dihydrodiol and chrysene 5,6-dihydrodiol are even less active than those of the parent hydrocarbon.

Several lines of evidence indicate that chrysene 1,2-diol-3,4-epoxide mediates the high mutagenicity of chrysene 1,2-dihydrodiol. Chrysene 1,2-dihydrodiol was not intrinsically mutagenic and induction of mutations was dependent on the presence of the microsomal fraction of liver and a NADPH generating system (Fig. 2A). Since microsomes contain other enzymes besides the NADPH - dependent monooxygenase system we also utilized a highly purified NADPH - dependent monooxygenase consisting of cytochrome P-450, NADPH-cytochrome c reductase and phosphatidylcholine, to metabolize chrysene and its dihydrodiols (Fig. 2B). Previous studies utilizing this purified monooxygenase system have shown that both benzo[a]pyrene 7,8-dihydrodiol (27,28) and benz[a]anthracene 3,4-dihydrodiol (20) are metabolized to potent bacterial mutagens. In both instances the bay region epoxides of these dihydrodiols are potent intrinsically active mutagenic compounds (10-13,

19). Since the 3,4-double bond is essential for the bay region epoxidation of chrysene 1,2-dihydrodiol we utilized chrysene H_4-1,2-diol, which is saturated in the 3,4-position, to test the occurrence of metabolic activation at positions outside of the bay region. The total lack of induction of mutations when chrysene H_4-1,2-diol was used as a substrate further supports the proposition that the 3,4-epoxide is the activated metabolite of chrysene 1,2-dihydrodiol. Finally, we have previously reported that epoxide hydrase readily inactivates the mutagenic arene oxides of benzo[a]pyrene (27) and benz[a]anthracene (19) but the diol epoxides of both of these hydrocarbons are refractory to epoxide hydrase catalyzed hydration (11,19,27,29). When highly purified epoxide hydrase was added to the purified monooxygenase system, mutations induced by chrysene 1,2-dihydrodiol were diminished by no more than 20% (data not shown). These results are consistent with the formation of a diol epoxide which is a very poor substrate for epoxide hydrase.

The results described above and earlier work from our laboratories (cf. 15-17) indicate that metabolic activation of benzo[a]pyrene, benz[a]anthracene and chrysene to bay region diol epoxides is important for the biological actions of these hydrocarbons. Additional support for the bay region theory has come from recent studies on the metabolic activation of 7,12-dimethylbenz[a]anthracene (30) and from studies on the metabolic activation of the dihydrodiols of 7-methylbenz[a]anthracene (31,32).

References

1. Miller, E. C. and Miller, J. A. (1974) in The Molecular Biology of Cancer, H. Busch ed., pp. 377-402, Academic Press, New York.
2. Jerina, D. M. and Daly, J. W. (1974) Science, 185, 573-582.
3. Sims, P. and Grover, P. L. (1974) Adv. Cancer Research, 20, 165-274.
4. Miller, J. A. and Miller, E. C. (1977) in Biological Reactive Intermediates, Formation, Toxicity and Inactivation, D. J. Jollow, J. Kocsis, R. Snyder and H. Vainio eds., pp. 6-24, Plenum, New York.
5. Sims, P., Grover, P. L., Swaisland, A., Pal, K. and Hewer, A. (1974) Nature, 252, 326-328.
6. Koreeda, M., Moore, P. D., Yagi, H., Yah, H. J. C. and Jerina, D. M. (1976) J. Am. Chem. Soc., 98, 6720-6722.
7. Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. and Nakanishi, K. (1976) Science, 193, 592-595.
8. King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. G. and Brookes, P. (1976) Proc. Natl. Acad. Sci. U.S.A., 73, 2679-2681.

9. Moore, P. D., Koreeda, M., Wislocki, P. G., Levin, W., Conney, A. H., Yagi, H. and Jerina, D. M. (1977) in Drug Metabolism Concepts, D. M. Jerina ed., pp. 127-154, American Chemical Society, Washington
10. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Jerina, D. M. and Conney, A. H. (1976) Biochem. Biophys. Res. Commun., 68, 1006-1112.
11. Wood, A. W., Wislocki, P. G., Chang, R. L., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M. and Conney, A. H. (1976) Cancer Res. 36, 3358-3366.
12. Newbold, R. F. and Brookes, P. (1976) Nature, 261, 52-54.
13. Huberman, E., Sachs, L., Yang, S. K. and Gelboin, H. V. (1976) Proc. Natl. Acad. Sci. U.S.A., 73, 607-611.
14. Kapitulnik, J., Levin, W., Conney, A. H., Yagi, H. and Jerina, D. M. (1977) Nature, 266, 378-380.
15. Jerina, D. M. and Daly, J. W. (1976) in Drug Metabolism - from Microbe To Man, D. V. Parke and R. L. Smith eds., pp. 13-32, Taylor and Francis Ltd., London
16. Jerina, D. M., Lehr, R. E., Yagi, H., Hernandez, O., Dansette, P. M., Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W. and Conney, A. H. (1976) in In Vitro Metabolic Activation and Mutagenesis Testing, F. J. DeSerres, J. R. Bend, R. M. Philpot eds., pp. 159-177, Elsevier/North Holland Biomedical Press, Amsterdam.
17. Jerina, D. M., Lehr, R., Schaefer-Ridder, M., Yagi, H., Karle, J. M., Thakker, D. R., Wood, A. W., Lu, A. Y. H., Ryan, D., West, S., Levin, W., and Conney, A. H. (1977) in Cold Spring Harbor Conference on Cellular Proliferation: Vol. 4: Origins of Human Cancer, pp. 639-658.
18. Lehr, R. E., Schaefer-Ridder, M. and Jerina, D. M. (1977) Tetrahedron Letter, 539-542.
19. Wood, A. W., Chang, R. L., Levin, W., Lehr, R. E., Schaefer-Ridder, M., Karle, J. M., Jerina, D. M. and Conney, A. H. (1977) Proc. Natl. Acad. Sci. U.S.A., 74, 2746-2750.
20. Wood, A. W., Levin, W., Lu, A. Y. H., Ryan, D., West, S., Lehr, R. E., Schaefer-Ridder, M., Jerina, D. M. and Conney, A. H. (1976) Biochem. Biophys. Res. Commun. 72, 680-686.
21. Wood, A. W., Levin, W., Chang, R. L., Lehr, R. E., Schaefer-Ridder, M., Karle, J. M., Jerina, D. M. and Conney, A. H. (1977) Proc. Natl. Acad. Sci., U.S.A., 74, 3176-3179.
22. Karle, J. M., Mah, H. D., Jerina, D. M. and Yagi, H., submitted for publication.
23. McCann, J., Spingarn, N. W., Kobari, J. and Ames, B. N. (1975) Proc. Natl. Acad. Sci. U.S.A., 72, 979-983.
24. Ryan, D., Thomas, P. E. and Levin, W. (1977) Molecular Pharmacol. 13, 521-532.
25. Ames, B. N., McCann, J. and Yamasaki, E. (1975) Mutation Res. 31, 347-364.
26. Yasukochi, Y. and Masters, B. S. S. (1976) J. Biol. Chem. 251, 5337-5344.
27. Wood, A. W., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M and Conney, A. H. (1976) J. Biol. Chem. 251, 4882-4890.
28. Wood, A. W., Levin, W., Lu, A. Y. H., Ryan, D., West, S. B., Yagi, H., Mah, H. D., Jerina, D. M. and Conney, A. H. (1977) Molecular Pharmacology, in press.
29. Thakker, D. R., Yagi, H., Lu, A. Y. H., Levin, W., Conney, A. H. and Jerina, D. M. (1976) Proc. Natl. Acad. Sci. U.S.A., 73, 3381-3385.
30. Moschel, R. C., Baird, W. M. and Dipple, A. (1977) Biochem. Biophys. Res. Commun. 76, 1092-1098.
31. Malaveille, C., Tierney, B., Grover, P. L., Sims, P. and Bartsch, H. (1977) Biochem. Biophys. Res. Commun. 75, 427-433.
32. Marquardt, H., Baker, S., Tierney, B., Grover, P. L. and Sims, P. (1977) Int. J. Cancer 19, 828-833.