Vol. 87, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1979 Pages 416-423

BENZO[a]PYRENE-7,8-DIHYDRODIOL: SELECTIVE BINDING TO SINGLE STRANDED DNA AND INACTIVATION OF ØX174 DNA INFECTIVITY

W. T. Hsu*†, D. Sagher*, E. J. Lin*, R. G. Harvey††, P. P. Fu††, and S. B. Weiss*†

*The Franklin McLean Memorial Research Institute, †The Departments of Biochemistry and Microbiology, and ††The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637

Received February 2, 1979

SUMMARY

When single-stranded ØX174 DNA is exposed to certain dihydrodiol derivatives of benzo[a]pyrene and benz[a]anthracene, inhibition of viral DNA infectivity is observed. Binding studies with labeled trans-7,8-dihydrodiol of benzo[a]pyrene and anti-benzo-[a]pyrene-7,8-dihydrodiol-9,10-epoxide indicate that the diol preferentially reacts with single-stranded DNA, whereas the diolepoxide reacts equally well with both single- and double-stranded DNA, as well as with RNA. Also, the diol and diolepoxide derivatives show a marked difference in their capacity to complex with specific deoxyhomopolymers, i.e., Poly dI. These observations suggest that the diol and diolepoxide derivatives recognize different binding sites in nucleic acids, and that the diol derivative may play an important role in mutagenesis and carcinogenesis induced by polycyclic aromatic hydrocarbons.

It is now generally accepted that polycyclic aromatic hydrocarbons (PAH)[1] in order to exert their carcinogenic, mutagenic, and toxic effects require metabolic activation to reactive intermediates which bind covalently to nucleic acids in vivo (1). Benzo[a]-pyrene (BP) has been most intensively studied, and trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-BP (anti-BPDE) has been implicated as the active metabolite involved (1). Analogous diolepoxide derivatives have been identified as the likely active forms of other carcinogenic PAH (2-10). We now provide evidence for the

[1] Abbreviations: PAH, polycyclic aromatic hydrocarbon(s); BP, benzo[a]pyrene; anti-BPDE, (±)-trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-BP; 7,8-BPD, trans-7,8-dihydroxy-7,8-dihydro-BP; cis-7,8-BPD, cis-7,8-dihydroxy-7,8-dihydro-BP; 9,10-BPD, trans-9,10-dihydroxy-9,10-dihydro-BP; 4,5-BPD, trans-4,5-dihydroxy-4,5-dihydro-BP; BA, benz[a]anthracene; 1,2-BAD, trans-1,2-dihydroxy-1,2-dihydro-BA; 3,4-BAD, trans-3,4-dihydroxy-3,4-dihydro-BA; 10,11-BAD, trans-10,11-dihydroxy-10,11-dihydro-BA; 7,8-H_4BPD, trans-7,8-dihydroxy-7,8,9,10-tetrahydro-BP; 9,10-H_4BPD, trans-9,10-dihydroxy-7,8,9,10-tetrahydro-BP; 9,10-BePD, trans-9,10-dihydroxy-9,10-dihydrobenzo[e]pyrene.

0006-291X/79/060416-08$01.00/0

Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.

direct binding of BP-7,8-dihydrodiol (7,8-BPD) to nucleic acids, without metabolic transformation to anti-BPDE, and discuss the implications of this finding for the biological activity of 7,8-BPD and BP.

In prior studies we developed a phage assay system which employs infectious viral DNA and RNA for the determination of the comparative reactivities of PAH metabolites with nucleic acids (11). In the experimental procedure designated Method B (11), the infectious viral nucleic acid is pretreated with the hydrocarbon derivative and unreacted PAH is removed prior to infection of Escherichia coli spheroplasts. Certain reactive PAH derivatives were shown to inhibit the capacity of the infectious macromolecules to direct progeny virus formation in the spheroplasts in this system; anti-BPDE was the most potent inhibitor tested (11). It was demonstrated that anti-BPDE efficiently alkylates ØX174 DNA, a single alkylation event sufficing to totally inhibit viral replication (12). The inhibitory activity of other PAH derivatives correlated with their activity as mutagenic and carcinogenic agents. Although these experiments suggested that 7,8-BPD could interact directly with nucleic acid, it was suspected that this might be an experimental artifact since this appeared improbable on chemical grounds. We now find that 7,8-BPD does directly inactivate infectious ØX DNA, even at relatively low concentrations, and that certain other PAH dihydrodiols are similarly active. Moreover, 7,8-BPD preferentially reacts with single-stranded DNA, whereas anti-BPDE reacts equally well with both single- and double-stranded DNA as well as with RNA.

MATERIALS AND METHODS

Assay Procedure: The preparation of ØX174 DNA and the assay of its infectivity were as previously described (11). Modification of ØX DNA infectivity by binding with BP derivatives was determined by the "Method B" assay procedure (11). The binding reaction mixture (0.1 ml) contained 10 µg of single-stranded ØX DNA and different amounts of a single BP derivative, as indicated. After 10 min incubation at 25°, DNA was precipitated with ethanol, washed with acetone, dried, and dissolved in Tris-EDTA. Infectivity of the treated DNA was assayed by incubation with E. coli spheroplasts and plating on agar plates, with E. coli HF4714 used for plaque development. The values of percent inhibition represent an average for triplicate plates.

Materials: [^{3}H]7,8-BPD and [^{3}H]anti-BPDE were synthesized under NCI Contract CP-033387 by the Midwest Research Institute, Kansas City, Mo., and further purified by Dr. Shen Yang, Uniformed Services University of the Health Sciences, Bethesda, Md. The following compounds were synthesized by the procedures described in the references cited: 7,8-BPD (1a,13), anti- and syn-BPDE (1a,14), 4,5-BPD (15), cis-7,8-

BPD (1a), 1,2-BAD (1a,16), 3,4-BAD (1a,16), 10,11-BAD (1a,13), and 9,10-BePD (17). 9,10-BPD was synthesized from 7,8-dihydro-BP (18) by a procedure analogous to that employed to prepare 7,8-BPD. 7,8-H_4BPD and 9,10-H_4BPD were synthesized from the corresponding dibenzoates (1a) by reaction with sodium methoxide in methanol.

RESULTS AND DISCUSSION

Treatment of the ØX174 DNA with 7,8-BPD inactivated the viral DNA infectivity, even at relatively low diol concentrations, but 7,8-BPD proved less effective than anti-BPDE (Fig. 1A). As the concentration of the PAH compound is increased, maximum inhibition by 7,8-BPD reaches a constant level of about 60-70%, whereas inhibition by anti-BPDE is nearly complete (99.8%).

When single-stranded ØX DNA was incubated with [^{3}H]7,8-BPD, precipitated with ethanol, and washed to remove free dihydrodiol, radioactivity was found associated with the viral DNA (Fig. 1B). With increasing amounts of labeled 7,8-BPD in the binding reaction mixture, the level of radioactivity bound to DNA also increased; at the same time, inhibition of viral-DNA infectivity was observed, although complete inactivation did not occur even at molar binding ratios greater than 15.[2] It seems unlikely that 7,8-BPD exchange from the bound complex occurred, since incubation of [^{3}H]BPD-DNA in 3% formaldehyde at 63°, or in the presence of nonradioactive 7,8-BPD, did not significantly change the amount of labeled complex recovered.

When we compared the properties of labeled 7,8-BPD with those of anti-BPDE, in regard to binding with various nucleic acids, we observed some important differences in the reactivity of the two BP derivatives (Table 1). Whereas anti-BPDE reacted with both single- and double-stranded DNAs and with RNA, 7,8-BPD bound extensively only to single-stranded DNA. Among the synthetic polymers examined, poly dI and denatured poly dG were most reactive with [^{3}H]7,8-BPD; reaction with poly dC, poly rI, and poly rAUG was also observed, but to a much lesser extent. On the other hand, [^{3}H]anti-BPDE reacted poorly with poly dI and with most of the other synthetic nucleic acids, except for poly dC and those polymers containing guanine, with which binding was extensive (19-22). The marked preference of 7,8-BPD for

[2]Molar ratios of 7,8-BPD bound to ØX DNA were calculated from the data of Fig. 1B as previously described (12).

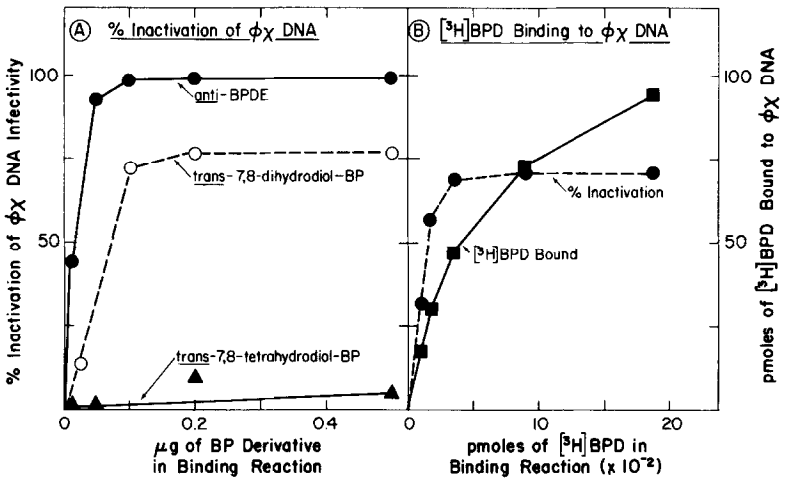

Fig. 1. Effect of various BP derivatives on ϕX DNA infectivity and the binding of [^{3}H]BPD to viral DNA. (A) The preparation of ϕX174 DNA and the assay of its infectivity are described in Materials and Methods. (B) Various amounts of [^{3}H]BPD, as indicated, were incubated with 10 μg of single-stranded ϕX DNA, and the treated DNA was isolated and assayed for infectivity as described in (A). The amount of BPD bound to DNA was determined by counting of samples of the treated DNA, following precipitation and washing, in a Nuclear Chicago Mark III scintillation counter. The [^{3}H]7,8-BPD used in these experiments had a specific activity of 202 cpm/pmole.

binding to single-stranded DNA and most especially to poly dI, compared with binding to duplex DNA and RNA, appears to be a unique property of the dihydrodiol. These results suggest that 7,8-BPD and anti-BPDE recognize different DNA binding sites, and that, in vivo, the dihydrodiol is limited to reacting with single-stranded regions in DNA molecules when such regions are available, i.e., during replication and repair.

Whether other PAH dihydrodiols and structurally related compounds might similarly bind covalently to viral nucleic acids was also investigated. The experimental findings are summarized in Table 2. The cis stereoisomer of 7,8-BPD inhibits viral replication only slightly less effectively than 7,8-BPD. The BA 1,2- and 3,4-dihydrodiols also bind relatively efficiently to ϕX DNA. On the other hand, the BP - 4,5- and 9,10-dihydrodiols, BA-10,11-dihydrodiol, benzo[e] pyrene-9,10-dihydrodiol, and 9-HO-BP exhibited no significant activity. Although 7,8-H_4BPD, the saturated analog of 7,8-BPD, also proved inactive, weak activity was shown by the other tetrahydro-BP alcohols 9,10-H_4BPD and 10-HO-H_4BP.These findings clearly indicate that 7,8-BPD is not unique among PAH dihydrodiol metabolites in its capacity to alter DNA expres-

Table 1. Binding of [^{3}H] BPD and [^{3}H] anti-BPDE to Natural and Synthetic Nucleic Acids.

Nucleic acid		pMoles of BP Derivative Bound to Nucleic Acids	
		[^{3}H] BPD	[^{3}H] anti-BPDE
ØX174 DNA	(single stranded)	187	113
RF Ø174 DNA	(double-stranded)	8	104
T5 DNA	(double-stranded)	4	50
MS2 RNA		6	99
E. coli tRNA		10	64
Poly dG		17	268
Poly dG	(NaOH- and heat-treated)	104	-
Poly dA		4	21
Poly dC		21	83
Poly dI		160	34
Poly rG		27	268
Poly rA		4	19
Poly rC		7	11
Poly rU		5	3
Poly rI		39	15
Poly rAUG		17	139

The binding mixture (0.1 ml) contained 10 µg of DNA or RNA in Tris-EDTA and 0.5 µg of labeled compound as indicated. Incubation and isolation of the treated DNA were as described in Fig. 1. Where indicated, poly dG was heated for 10 min at 80° in 0.46 N NaOH, cooled rapidly in an ice bath, and neutralized with HCl prior to reaction with [^{3}H] BPD. The specific activity of [^{3}H] anti-BPDE was 344 cpm/pmole and [^{3}H]BPD as indicated in Fig. 1B.

sion. It is also clear from the range of activity observed that the structure of the dihydrodiol may markedly influence activity. It would be premature to speculate concerning the detailed mechanism of dihydrodiol-nucleic acid interaction on the basis of the limited evidence available. However, we tentatively suggest that the active dihydrodiols may favor relatively facile dissociation with loss of a hydroxyl group and formation of a particularly stable benzylic or allylic carbonium ion (Fig. 2). The latter

Table 2. Direct Inactivation of ØX 174 DNA Infectivity by BP, BA and BeP Derivatives[a]

Compound	% Inhibition of Infectivity[b]	Compound	% Inhibition of Infectivity[b]
anti-BPDE	99	9,10-H_4BPD	28
syn-BPDE	46	7,8-H_4BPD	0
7,8-BPD	78	10-HO-H_4BP	20
4,5-BPD	7	1,2-BAD	69
9,10-BPD	15	3,4-BAD	65
cis-7,8-BPD	57	10,11-BAD	0
9-HO-BP	4	9,10-BePD	0

[a] ØX174 DNA (10 µg) and 0.5 µg of the PAH derivative were incubated together in 0.1 ml of buffer (pH 7.5) for 10 min. at 25°. The DNA was reisolated as previously described for Method B (11) and assayed in triplicate for infectivity with spheroplasts.

[b] Repeat experiments indicate that differences of 20% in plaque titer are not considered significant.

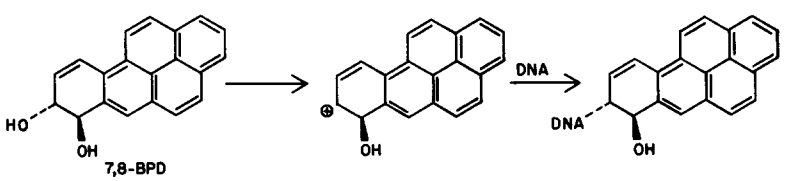

Fig. 2. Mechanism tentatively proposed for covalent binding of 7,8-BPD to viral DNA.

may conceivably alkylate nucleic acid or lose a proton to afford a phenol, an already established pathway (23). Further investigation will be required to determine whether this or some other mechanism is actually involved.

Although dihydrodiols are known to be major metabolites of PAH in vivo (1,24), the capacity of diol intermediates to modify and alter DNA expression directly has not been generally recognized. This capacity is of potential importance with respect to PAH-induced mutagenesis and carcinogenesis. The inability of 7,8-BPD to inactivate viral DNA completely is not understood at present; this observation suggests, however,

that some BPD-bound DNA molecules are replicated for progeny virus formation even at high molar binding ratios. The fidelity of replication, the structures of the BPD-DNA products, and the fate of the dihydrodiol-bound DNA during replication are currently under investigation.

ACKNOWLEDGMENTS

The Franklin McLean Memorial Research Institute is operated by The University of Chicago for the United States Department of Energy under Contract EY-76-C-02-0069. This study was partly supported by a grant from the American Cancer Society (BC-132C) and by funds from the Environmental Protection Agency, Subagreement 77BAN. Daphna Sagher is supported by Training Grant CA-09183 from the National Cancer Institute. We are also grateful to Dr. Shen K. Yang, Uniformed Services University of the Health Sciences, for purification by HPLC of [^{3}H]7,8-BPD and [^{3}H]-anti-BPDE.

REFERENCES

1. Reviews: (a) Harvey, R. G. and Fu, P. P. in "Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, and Metabolism" (1978) Vol. 1, Gelboin, H. V. and Ts'o, P.O.P. Eds., Academic Press, New York, Chap. 6, pp. 133-165; (b) Yang, S. K., Deutsch, J., and Gelboin, H. V., Ibid., Chap. 10, pp. 205-231; (c) Miller, E. C. (1978) Cancer Res., 38, 1479-1496.
2. Slaga, T. J., Huberman, E., Selkirk, J. K., Harvey, R. G., and Bracken, W. M. (1978) Cancer Res., 38, 1699-1704.
3. Levin, W., Thakker, D. R., Wood, A. W., Chang, R. L., Lehr, R. E., Jerina, D. M., and Conney, A. H. (1978) Cancer Res., 38, 1705-1710.
4. Malaveille, C., Tierney, B., Grover, P. L., Sims, P., and Bartsch, H. (1977) Biochem. Biophys. Res. Commun., 75, 427-433.
5. King, H. W.S., Osborne, M. R., and Brookes, P. (1977) Internat. J. Cancer, 20, 564-571.
6. Wood, A. W., Chang, R. L., Levin, W., Lehr, R. E., Schaefer-Ridder, Karle, J. M., Jerina, D. M., and Conney, A. H. (1977) Proc. Natl. Acad. Sci. USA, 74,2746-2750.
7. Ivanovic, V., Geacintov, N. E., Jeffrey, A. M., Fu, P. P., Harvey, R. G., and Weinstein, I. B. (1978), 4, 131-140.
8. Vigny, P., Duquesne, M., Coulomb, H., Tierney, B., Grover, P. L., and Sims, P. (1977) FEBS Lett., 82, 278-282.
9. Moschel, R. C., Baird, W. M., and Dipple, H. (1977) Biochem. Biophys. Res. Commun., 76, 1092-1098.
10. Wood, A. W., Levin, W., Thomas, P. E., Ryan, D., Karle, J. M., Yagi, H., Jerina, D. M., and Conney, A. H. (1978) Cancer Res., 38, 1967-1973.
11. Hsu, W.-T, Harvey, R. G., Lin, E.J.S., and Weiss, S. B. (1977) Proc. Natl. Acad. Sci. USA, 74, 1378-1382.
12. Hsu, W.-T., Lin, E.J.S., Harvey, R. G., and Weiss, S. B. (1977) Proc. Natl. Acad. Sci. USA, 74, 3335-3339.
13. Fu, P. P. and Harvey, R. G. (1977) Tetrahedron Lett. 2059-2062.
14. Beland, F. A. and Harvey, R. G. (1976) J. Chem. Soc. Chem., Commun., 84-85.
15. Harvey, R. G., Goh, S. H., and Cortez, C. (1975) J. Amer. Chem. Soc., 97, 3468-3479.
16. Harvey, R. G. and Sukumaran, K. B. (1977) Tetrahedron Lett. 2387-2391.
17. Harvey, R. G., Lee, H. M., and Shyamasundar, N. (1979) J. Org. Chem., 44, 78-83.
18. Fu, P. P., Lee, H. M., and Harvey, R. G. (1978) Tetrahedron Lett. 551-554.

19. Jennette, K. W., Jeffrey, A. M., Blobstein, S. H., Beland, F. A., Harvey, R. G., and Weinstein, I. B. (1977) Biochem., 16, 932-938.
20. 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.
21. Nakanishi, K., Kasai, H., Cho., H., Harvey, R. G., Jeffrey, A. M., Jennette, K. W., and Weinstein, I. B. (1977) J. Amer. Chem. Soc., 99, 258-260.
22. Jeffrey, A. M., Weinstein, I. B., Jennette, K. W., Grzeskowiak, K., Nakanishi, K., Harvey, R. G., Autrup, H., and Harris, C. (1977) Nature, 269, 348-349.
23. Fu, P. P., Harvey, R. G., and Beland, F. A. (1978) Tetrahedron, 34, 857-866.
24. Sims, P. and Grover, P. L. (1974) Adv. Cancer Res., 20, 165-274.