Vol. 114, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1983 Pages 8-13

BASE SEQUENCE SELECTIVITY IN BINDING OF AROMATIC HYDROCARBONS WITH SYNTHETIC POLYNUCLEOTIDES

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Received May 24, 1983

The interactions between DOBP (3) and calf thymus DNA as well as four synthetic polynucleotides, poly(dA-dT), polydA:polydT, poly(dG-dC), and polydG:polydC, were investigated by spectroscopic techniques. It was found that the binding of 3 with poly(dA-dT) is favored appreciably over other synthetic polynucleotides and DNA. The results suggest that the initial association of carcinogenic BPDE (2) with DNA may take place preferentially at certain specific base sequences in DNA.

The ubiquitous precarcinogen benzo(a)pyrene (BP, 1) is metabolically activated to (+)-7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene ((+)-BPDE, 2), which may react subsequently with cellular macromolecules to alter their biological functions and lead to the carcinogenic transformation of cells.[1] Polynuclear aromatic hydrocarbons are known to be solubilized by DNA,[2] and recent work from several laboratories indicates that some of the solubilized hydrocarbons are intercalated between the base pairs of DNA.[3,4] In order to determine the possible selectivity of intercalative association of metabolically activated carcinogens with specific base sequences, we investigated the spectroscopic properties of (±)-7r,8t-dihydroxy-7,8,9,10-tetrahydrobenzo(a)-pyrene (DOBP, 3) in the presence of calf thymus DNA and four synthetic polynucleotides, poly(dA-dT), polydA:polydT, poly(dG-dC), and polydG:polydC. DOBP was chosen as the model compound for BPDE because of its structural similarity to BPDE and its ability to intercalate into DNA.[5]

Abbreviations: BP, benzo(a)pyrene; BPDE, (±)-7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; DOBP, (±)-7r,8t-dihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene; DNA, deoxyribonucleic acid; poly(dA-dT), polydeoxyadenylic-deoxythymidylic acid; polydA:polydT, polydeoxyadenylic-polydeoxythymidylic acid; poly(dG-dC), polydeoxyguanylic-deoxycytidylic acid; polydG:polydC, polydeoxyguanylic-polydeoxycytidylic acid.

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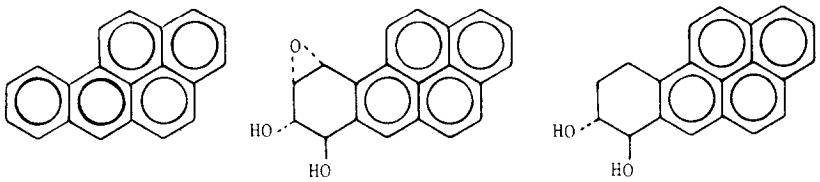

1 2 3

EXPERIMENTAL

High molecular weight calf thymus DNA (hyperchromicity >33%, Sigma) and synthetic polynucleotides (P-L Biochemicals) were used as received. DOBP was prepared according to the method of McCaustland and Engel.[6] All other chemical reagents and organic solvents used were of the highest purity available.

Stock solutions of DOBP with final concentrations of 140.0 μM and 1.4 μM were prepared in dry dimethyl sulfoxide and in buffer A (10^{-4} M Na_2HPO_4, 10^{-4} NaH_2PO_4, 0.1 M NaCl, pH 7.0) containing DMSO (2.5%, v/v), respectively. Stock solutions of polynucleotides (2.0-3.0 mM in phosphate units) were prepared in buffer A containing DMSO (2.5%, v/v). Samples for spectroscopic examination were prepared by mixing these polynucleotide and hydrocarbon stock solutions to obtain the desired polynucleotide concentrations while maintaining a constant DOBP concentration of 1.4 μM and a constant DMSO content of 2.5% (v/v). All sample solutions were deoxygenated by bubbling with high purity nitrogen (Union Carbide, Linde division) and sealed prior to spectral measurements.

Uv/vis spectra were recorded with a Cary 219 spectrophotometer. Interference in hydrocarbon absorption by polynucleotide was eliminated by placing a hydrocarbon-free polynucleotide solution of identical concentration in the reference compartment for each sample examined. Fluorescence spectra were recorded with a Perkin-Elmer MPF-4 spectrofluorimeter equipped with a corrected spectrum unit and a thermostatic sample compartment. The intensity of exciting light for samples of DOBP containing varying concentrations of polynucleotides was kept constant by exciting the samples at 349 nm, the isosbestic point (Figure 1). Fluorescence lifetimes were determined by using the time-correlated single-photon counting technique described by Robbins.[7]

RESULTS

The intercalation of pyrene derivatives into DNA causes a red and hypochromic shift in their absorption spectra, as well as quenching of their fluorescence.[2,8,9] We found that the interaction of DOBP (λ_{max} = 344.5 nm) with increasing concentrations of polynucleotides leads to the formation of a new absorption band at 353.5 nm corresponding to a ground state complex and an isosbestic point at 349 nm (Figure 1). The equilibrium constant for formation of a molecular complex may be determined from the absorption spectra of the chromophore in the presence of varying concentrations of a complexing agent by using either a modified Hildebrand-Benesi or a simple algebraic regression analysis of the spectral data.[10] The equilibrium constants calculated by these

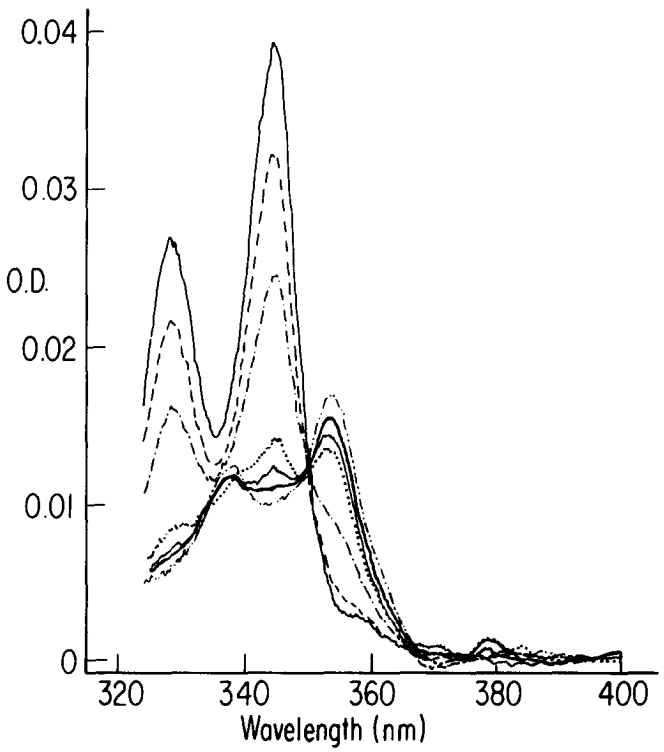

Figure 1. Uv/visible absorption spectra of DOBP in buffer A containing 2.5% DMSO and varying concentrations of poly(dA-dT). [DOBP] = 1.4×10^{-6} M; poly(dA-dT) = 0.00(——), 0.03(---), 0.12(-·-·), 0.48(····), 0.86(▬▬), 1.53(▬▬), and 2.01 mM(-··-··).

methods for DOBP interaction with DNA or poly(dA-dT) are listed in column 2 of the Table. Since the absorption spectra of DOBP were measured in high dilution ($\sim 10^{-6}$ M, Figure 1), and the sensitivity of our instrument is ±0.001 OD unit, the disparity in the values of K_{eq} obtained from two different methods of analysis (Table) may be attributed to the experimental uncertainty of our measurements.

Since the fluorescence of DOBP is quenched by intercalative association with polynucleotides, K_{eq} may also be analyzed spectrofluorimetrically by the Stern-Volmer method, i.e., $K_{eq} = K_{sv}$.[4] The result obtained from the quenching of DOBP fluorescence by poly(dA-dT) is graphically presented in Figure 2. The values determined for K_{sv} are listed in column 3 of the Table. The spectrofluorimetric analysis affords a higher experimental accuracy than analyses based on absorption data. However, the equivalence of K_{eq} and K_{sv} is valid

TABLE
PHYSICAL BINDING OF DOBP TO POLYNUCLEOTIDES

Polynucleotide	K_{eq} $(M^{-1})^a$	K_{sv} $(M^{-1})^b$	τ_{DOBP} (ns)^c
Poly(dA-dT)	5600 ± 800[d] 4200 ± 600[e]	5300 ± 500	122 ± 2[f]
Poly(dG-dC)	--	870 ± 80	120 ± 2[g]
Calf thymus DNA	910 ± 110[d] 750 ± 90[e]	740 ± 70	118 ± 7[h]
PolydG:polydC	--	530 ± 70	--
PolydA:polydT	--	<100	--

[a][DOBP] = 1.4 x 10^{-6} M; buffer A/DMSO (2.5%). [b]λ_{ex} = 349 nm. [c][DOBP] = 1.3 x 10^{-6} M; λ_{ex} = 323 nm; τ_{DOBP} = 120 ± 2 ns in absence of polynucleotide. [d]Determined using modified Hildebrand-Benesi method (reference 10). [e]Determined using simple algebraic analysis of data. [f][poly(dA-dT)] = 0.47 - 1.85 x 10^{-3} M (phosphate unit). [g][poly(dG-dC)] = 1.41 x 10^{-3} M (phosphate unit). [h][DNA] = 0.89 - 3.70 x 10^{-3} M (phosphate unit).

only when the quenching of DOBP fluorescence by polynucleotides is a static process involving molecular complexation in the ground state, i.e., when the photoexcited DOBP in the free form does not interact with polynucleotides to undergo a dynamic quenching process. The possibility of dynamic quenching in our studies was precluded by experimental observations that the fluorescence

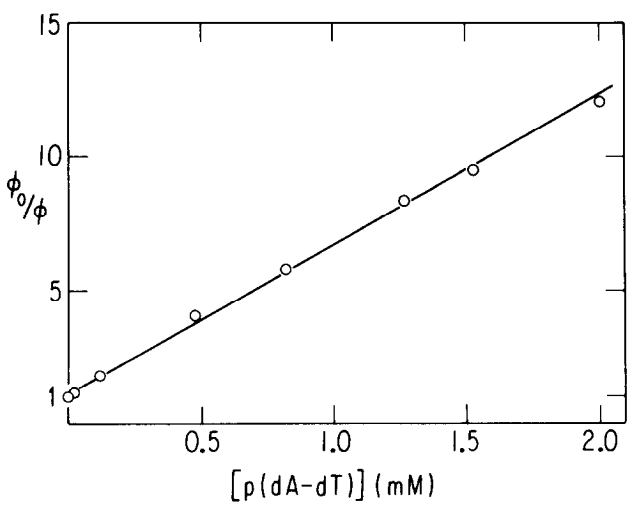

Figure 2. Stern-Volmer plot for quenching of DOBP fluorescence by poly(dA-dT) in buffer A containing 2.5% DMSO. [DOBP] = 1.4x10^{-6} M, poly(dA-dT) = 0.02-2.01 mM; excitation wavelength = 349 nm.

of DOBP exhibits single exponential decay with a non-variant lifetime of 120 ± 9 nsec both in the absence and in the presence of a range of poly(dA-dT) or calf thymus DNA concentrations (Table).

DISCUSSION

The results summarized in the Table indicate that the alternating dA-dT base sequence is the preferential site for the intercalation of metabolically activated BP derivatives; poly dA-poly dT is ineffective for the intercalation, and dG-dC polynucleotides are of intermediate activity. (±)-anti-BPDE is known to react with DNA and polynucleotides at a variety of sites. The major chemical consequences of these interactions include the covalent binding of BPDE to the 2-amino group of guanine[11] and the hydrolysis of phosphodiester linkages, or strand scission. The latter event is supported by the morphological evidence in electron microscopy,[12-14] by enzymatic studies with S_1-endonuclease,[12] and by chemical analysis.[15] Although the mechanism of this phosphodiester hydrolysis is not yet clearly understood, a point of particular interest is that this hydrolysis seems to take place preferentially at dA-dT sites.[12] When the interaction between BPDE and DNA was analyzed by a rapid kinetic technique, it was observed that there was an initial red-shift in the hydrocarbon λ_{max} similar to what we had observed in the interaction between DOBP and polynucleotides.[16] This result indicates that a major portion of BPDE had become intercalated between the base pairs of DNA prior to the formation of final chemical products. Since alternating dA-dT sequences are the preferential sites for the intercalation of DOBP, and the phosphodiester hydrolysis of polynucleotides by BPDE also takes place preferentially at dA-dT sites, the preferential intercalation of BPDE into dA-dT sequences may be an important preliminary event for the phosphodiester hydrolysis. Alternating A-T sequences are known to exist in the promotor region of a variety of genes as well as at the sites of restriction enzyme action, and preferential action of ultimate carcinogens at these sites may play an important role in their biological activities.[17]

Acknowledgement: The authors wish to thank the Louis Block Fund of the University of Chicago and the National Cancer Institute, grant No. CA-10220 for the support of this work. Two of us (T. P. H. and J. W. P.) wish to thank the

National Institute of General Medical Sciences for the traineeships under the Pharmacological Training Grant, GM-07151.

REFERENCES

1. For a recent review on the carcinogenic action of polynuclear aromatic hydrocarbons, see B. Pullman, P. O. P. Ts'o, and H. Gelboin (eds), "Carcinogenesis: Fundamental Mechanisms and Environmental Effects," D. Reidel Publishing Co., Boston (1981).
2. E. Boyland and B. Green, Brit. J. Cancer, 16, 507-517 (1962); H. Weil-Malherbe, Biochem. J., 40, 351-368 (1946).
3. N. E. Geacintov, T. Prusik, and J. M. Khosrofian, J. Am. Chem. Soc., 98, 6444-6452 (1976); V. Ibanez, N. E. Geacintov, A. G. Gagliano, S. Brandimarte, and R. G. Harvey, J. Am. Chem. Soc., 102, 5661-5666 (1980); T. Meehan, H. Gamper, and J. F. Becker, J. Biol. Chem., 257, 10479-10485 (1982).
4. M. Shahbaz, R. G. Harvey, A. S. Prakash, T. R. Boal, I. S. Zegar, and P. R. LeBreton, Biochem. Biophys. Res. Comm., 112, 1-7 (1983).
5. T. Kakefuda, H. Mizusawa, C-H. R. Lee, P. Madigan, and R. J. Feldman, in reference 1, p. 389-407.
6. D. G. McCaustland and J. F. Engel, Tetrahedron Letters, 30, 2549-2552 (1975).
7. R. J. Robbins, G. R. Fleming, G. S. Beddard, G. W. Robinson, P. J. Thistlethwaite, and G. J. Woolfe, J. Am. Chem. Soc., 102, 6271-6279 (1980).
8. N. C. Yang, L. K. Ng, S. B. Neoh, and D. Leonov, Biochem. Biophys. Res. Comm., 82, 929-934 (1978).
9. M. E. Hogan, N. Dattagupta, and J. P. Whitlock, Jr., J. Biol. Chem., 256, 4504-4513 (1981).
10. R. Foster, D. Ll. Hammick, and A. A. Wardley, J. Chem. Soc., 3817-3825 (1953); H. A. Benesi and J. H. Hildebrand, J. Am. Chem. Soc., 71, 2703-2707 (1949).
11. A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, J. Am. Chem. Soc., 98, 5714-5715 (1976); I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, and K. Nakanishi, Science, 193, 592-595 (1976).
12. T. Kakefuda and H. Yamamoto, in H. Gelboin and P. O. P. Ts'o (eds), "Polycyclic Hydrocarbons and Cancer," Academic Press, New York, 2, 63-74 (1978).
13. H. Gamper, T. Meehan, K. Straub, A. S.-C. Tung, and M. Calvin, in H. Gelboin and P. O. P. Ts'o (eds), "Polycyclic Hydrocarbons and Cancer," Academic Press, New York, 2, 51-61 (1978).
14. N. R. Drinkwater, J. A. Miller, E. C. Miller, and N. C. Yang, Cancer Research, 38, 3247-3255 (1978).
15. W. A. Haseltine, K.-M. Lo, and A. D. D'Andrea, Science, 209, 929-931 (1980)
16. N. E. Geacintov, V. Ibanez, A. G. Gagliano, H. Yoshida, and R. G. Harvey, Biochem. Biophys. Res. Comm., 92, 1335-1342 (1980); N. E. Geacintov, H. Yoshida, V. Ibanez, and R. G. Harvey, Biochem. Biophys. Res. Comm., 100, 1569-1577 (1981); N. E. Geacintov, H. Yoshida, V. Ibanez, and R. G. Harvey, Biochemistry, 21, 1864-1869 (1982).
17. J. Corden, B. Wasylyk, A. Buchwalder, P. Sassone-Corsi, C. Kedinger, and P. Chambon, Science, 209, 1406-1414 (1980); for a review on Type II DNA restriction enzymes, see P. Modrich, CRC Crit. Rev. Biochem., in press.