mation of the peptide bonds. This is similar to the biosynthesis of typical peptide antibiotics (Kurahashi, 1974) and in contrast to that of penicillins and cephalosporins (Abraham, 1974), which are believed to be formed by modification of a precursor peptide.

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We gratefully acknowledge Dr. S. Suzuki of this Institute for his interest. We are also indebted to Mr. K. Muraoka of Hikari Kogyo Co., Ltd., for performing the emission spectrography.

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Conformation of DNA Modified with a Dihydrodiol Epoxide Derivative of Benzo[*a*]pyrene†

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ABSTRACT: The conformation of calf thymus DNA modified by reaction with (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, which binds covalently mainly to the 2-amino group of guanosine residues, was studied. With samples in which 1.5 or 2.2% of the bases were modified, there was a slight decrease in T_m during heat denaturation and a slight increase in susceptibility to the single strand specific nuclease S_1. In a DNA sample in which 4.5% of the bases were modified, there was an appreciable decrease in T_m and a marked increase in susceptibility to S_1 nuclease. The kinetics of the reaction of the modified DNAs with formaldehyde provided evidence for locally destabilized regions ranging from 1 to 7 base plates, depending on the extent of modification. Alkaline and neutral sucrose gradient analyses revealed no evidence for strand breakage in the 1.5 and 2.2% modified samples, although single-strand breaks were found in the 4.5% modified sample. Taken together, these results suggest that DNA molecules containing a covalently bound benzo[*a*]pyrene derivative have an altered conformation characterized by small localized regions which are destabilized and easily denatured. The conformational changes associated with the covalent binding of the benzo[*a*]pyrene derivative to native DNA appear to be different from, and less marked, than those associated with the covalent binding of *N*-2-acetylaminofluorene to native DNA.

There is strong evidence that the covalent binding of chemical carcinogens to cellular macromolecules, and in particular to nucleic acids, appears to be a prerequisite for their action (Brookes and Lawley, 1964; Goshman and Heidelberger, 1967; Brookes and Heidelberger, 1969; Duncan et al., 1969; Miller, 1970; Gelboin et al., 1972). Most carcinogens, including polycyclic aromatic hydrocarbons (PAH[1]), require metabolic

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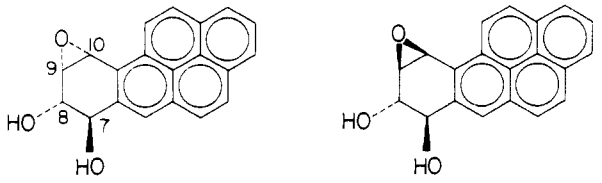
[1] Abbreviations used: PAH, polycyclic aromatic hydrocarbons; BP, benzo[*a*]pyrene; BPDE, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; AAF, *N*-2-acetylaminofluorene; BP-DNA and BP-G, designate DNA or G modified by reaction with BPDE; T_m, the temperature at which the DNA has attained 50% of its maximal hyperchromicity during heat denaturation; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.



I II

FIGURE 1: Structures of compound I, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, and compound II, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

activation before covalent binding takes place. The microsomal enzyme system, aryl hydrocarbon hydroxylase, oxidizes PAHs to a variety of derivatives (Jerina and Daly, 1974). Several lines of evidence suggest that in vivo the major reactive intermediate in the case of benzo[*a*]pyrene (BP) is a 7,8-diol 9,10-epoxide (BPDE) metabolite (Borgen et al., 1973; Sims et al., 1974; Daudel et al., 1975; King et al., 1976; Ivanovic et al., 1976; Weinstein et al., 1976).

Two stereoisomers of BPDE, designated I and II (Weinstein et al., 1976), have been synthesized (Yagi et al., 1975; McCaustland and Engel, 1975; Beland and Harvey, 1976) (Figure 1). Both isomers were found to be mutagenic to bacteria (Malaveille et al., 1975; Wislocki et al., 1976a,b); however, isomer I was much more mutagenic to mammalian cells than isomer II (Huberman et al., 1976). Each isomer consisted of a mixture of the (+) and (−) enantiomers. More recently the (+) and (−) enantiomers of isomer I have been prepared by resolution of 7,8-BP-diol (Nakanishi et al., 1977). Evidence was obtained by high-pressure liquid chromatographic studies of their respective nucleoside adducts that the major RNA adduct formed in vivo is derived from the (−)-7,8-diol enantiomer via BPDE isomer I (Nakanishi et al., 1977). This evidence for stereoselective in vivo nucleic acid binding activity of isomer I BPDE is consistent with metabolism studies of Yang et al. (1976). The data do not indicate, however, that this compound is the exclusive derivative of BP responsible for in vivo nucleic acid binding (Weinstein et al., 1976, and unpublished studies). The complete structure and absolute stereochemistry of the guanosine-BP adduct formed from the reaction of isomer I with RNA has been elucidated (Weinstein et al., 1976; Jeffrey et al., 1976; Nakanishi et al., 1977). The 10 position of the BPDE moiety is linked to the 2-amino group of guanine. There is also evidence that the major derivative of [^{3}H]BP bound to cellular DNA in vivo has the same structure (Jeffrey et al., unpublished studies). It appears likely, therefore, that this guanine adduct is important in the mechanism of BP carcinogenicity. Koreeda et al. (1976) have obtained evidence that, in the reaction of BPDE (±) isomer II with poly(G), the BP residue is also linked via its 10 position to the 2-amino group of guanine.

The covalent binding of a carcinogen to native DNA may introduce changes not only in the DNA's primary structure, but also in the three-dimensional conformation of the helix at the sites of carcinogen modification. In our previous studies with the hepatic carcinogen *N*-2-acetylaminofluorene (AAF) which binds mainly to the C-8 position of guanine, we demonstrated that the covalent binding of this bulky carcinogen is associated with major conformational alterations in various modified nucleic acids including oligonucleotides (Grunberger et al., 1970, 1974; Nelson et al., 1971), RNA (Grunberger and Weinstein, 1971; Fujimura et al., 1972; Pulkrabek et al., 1974), and native DNA (Levine et al., 1974; Yamasaki et al., 1977). We proposed a specific three-dimensional conformation at the sites of AAF modification which we named the "base displacement model" (Levine et al., 1974; Weinstein and Grunberger, 1974; Grunberger and Weinstein, 1976). Separate studies of AAF modified DNA (Fuchs and Daune, 1974; Fuchs et al., 1976) have led to a similar model. It is of course of considerable interest to show whether this model, or possibly some other conformational change, applies to nucleic acids modified by the diol epoxide derivative of BP.

In the present study we have modified native calf thymus DNA by in vitro reaction with isomer I of BPDE and then studied certain physical properties of the modified DNA. The properties studied have included heat denaturation, formaldehyde denaturation, susceptibility to the single strand specific nuclease S_1, and sedimentation in cesium chloride density gradients and neutral and alkaline sucrose density gradients. The results obtained are contrasted with the properties of AAF-modified DNA and certain tentative hypotheses are made regarding the conformational changes in DNA associated with benzo[*a*]pyrene carcinogenesis.

Experimental Section

Materials. Nonradioactive (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) was kindly supplied by Dr. R. G. Harvey. Native calf thymus DNA was from Worthington Co., Freehold, N.J., and had the following characteristics: protein content <0.1%, hyperchromicity 42% and $s_{20,w}$ = 22. S_1 endonuclease (EC 3.1.4.X.) from *Aspergillus oryzae* was purchased from Miles Co., Elkhart, Ind.

Methods. The reaction with BPDE was carried out at 37 °C in the dark in a solution containing 0.05 M sodium cacodylate buffer (pH 7.1), 33% ethanol, and 0.01 to 0.5 mg of BPDE per mL. The final concentration of DNA was 10 A_{260} units per mL. Incubation time was for 60 min and, in contrast to previous studies done in acetone-water (1:1) mixtures (Jennette et al., 1977), further incubation did not result in higher modification. The unreacted BP derivatives were removed by repeated (10 times) extraction with ether and DNA was precipitated and reprecipitated with ethanol until the ratio of A_{350}/A_{260} remained constant. The amount of BP derivative bound to DNA was calculated from the formula:

$$[\text{BP-G}] = \frac{A_{350}}{\epsilon_{350}\text{BP-G}}$$

$$[\text{DNA}] = \frac{A_{260} - 0.18A_{350}}{\epsilon_{260}\text{DNA}}$$

$$\%\ \text{modification} = \frac{[\text{BP-G}]}{[\text{DNA}]} \times 100$$

The correction factor 0.18 for A_{260} contributed by the bound BP moiety and ϵ_{350}BP-G (2.9×10^4) were established experimentally (Weinstein et al., 1976). ϵ_{260} for native DNA was taken as 6.65×10^3 (Chargaff and Lipshitz, 1953). The formula has been verified in separate experiments using [^{3}H]BPDE (V. Ivanovic, unpublished results). The base specificity of the binding of BPDE was determined by enzymatic hydrolysis of DNA and high-pressure liquid chromatography of the modified deoxyribonucleotide (Jennette et al., 1977). It was found that, under the conditions employed in the present study, only the BP–deoxyguanosine adduct was detected. Recent studies with BPDE-modified DNA indicate that, as with BPDE-modified RNA, the BP moiety is on the 2-amino group of guanine (Jeffrey et al., unpublished studies). Heat-denatured DNA was prepared by heating native DNA at 100 °C for 5 min in water and then by rapid cooling on ice. Tem-

perature melting studies were performed in 0.042 M sodium borate buffer (pH 9.0) or in 0.01 M sodium cacodylate (pH 7.1) using a Gilford type 240 automatic recording spectrophotometer, equipped with a Gilford thermoprogrammer type 2527. S_1 nuclease digestion was performed essentially according to Vogt (1973) in buffer containing 0.03 M sodium acetate (pH 4.6), 0.05 M NaCl and 1×10^{-3} M $ZnSO_4$. Each incubation mixture contained, in a final volume of 0.3 mL, 0.1 mg DNA and 5 units of the enzyme. The incubation was carried out at 45 °C for 0–240 min and terminated by chilling. Then 0.25 mg of carrier DNA and 0.6 mL of 10% perchloric acid were immediately added. Samples were centrifuged at 4000 rpm for 10 min and the concentration of acid-soluble material was determined by measuring the absorbance at 260 nm. The dynamic structure of the BP-modified DNA was studied by measuring the kinetics of unwinding induced by formaldehyde as previously described (Utiyama and Doty, 1971; Von Hippel and Wong, 1971). To one A_{260} unit of each DNA sample in 0.042 M sodium borate buffer (pH 9.0), 50 °C, formaldehyde was added to a final concentration of 1 M and the increase of absorbance at 251 nm was recorded. The number of open base plates was then calculated from the initial rates of the unwinding reactions according to Von Hippel and Wong (1971). Cesium chloride density gradient analyses were performed as follows. Approximately 10 A_{260} units of each DNA sample was mixed with 13.5 mL of cesium chloride solution (1.26 g/mL) containing 0.01 M Tris-HCl (pH 7.5) and centrifuged at 40 000 rpm for 60 h at 20 °C in a Beckman Ti 50 rotor. The gradients were then fractionated by a Buchler Auto Densi-Flow apparatus and absorbance at 260 nm was determined on a Gilford type 240 spectrophotometer. Alkaline sucrose density gradient centrifugations were performed essentially as described by Studier (1965). DNA samples (1 A_{260} of each) were applied in a 0.1 mL volume of 0.2 M NaOH to the top of a 5 to 20% linear sucrose gradient in 0.1 M NaOH and 0.9 M NaCl, and then centrifuged in a Beckman SW 41 rotor at 40 000 rpm for 240 min at 20 °C. Fractions were collected and analyzed for A_{260} absorbance as described above. Neutral sucrose density gradients were performed as follows. One A_{260} unit of each DNA sample in 0.1 mL of water was applied to the top of a 10 to 30% linear sucrose density gradient in 0.01 M sodium cacodylate (pH 7.1) buffer containing 0.1 M NaCl and 1×10^{-3} M EDTA and centrifuged in a Beckman SW 41 rotor at 40 000 rpm for 300 min at 20 °C.

In all analyses BP-modified samples were compared with a control sample of native DNA which was incubated and repurified under the same conditions as those used for the BPDE-modification reaction, with the exception that in the control the BPDE was omitted. The control sample had essentially the same physical properties as the original sample of DNA, thus indicating that the incubation procedure, itself, did not cause significant denaturation.

Results

Heat Denaturation Studies. Figure 2A shows that increasing extents of modification of native DNA with BPDE led to a progressive decrease in T_m during heat denaturation. The data presented in Figure 2 were obtained when the heat denaturation was done at pH 9.0, but similar results were also obtained at pH 7.1. It is apparent that the decrease in T_m was not linearly proportional to the percent of bases modified by the BP derivative. When less than 1% of the bases were modified, no significant decrease in T_m could be detected (data not shown). In the range of 1 to 2% modification of the bases, the decrease in T_m was about 0.75 °C per each 1% modification.

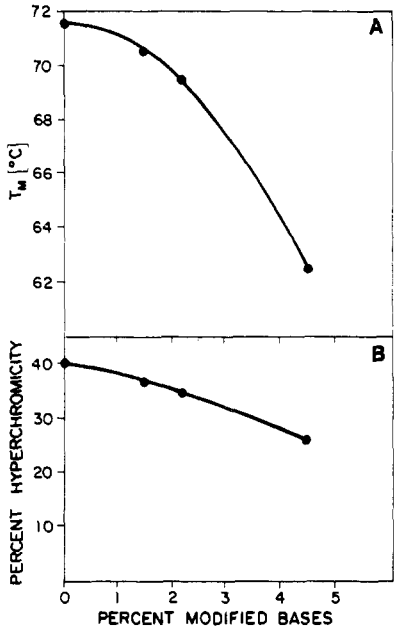


FIGURE 2: Effect of modification of DNA on T_m (A) and hyperchromicity (B). Each DNA sample (1 A_{260}) in 0.042 M sodium borate (pH 9.0) was melted in a thermocell of a Gilford type 240 recording spectrophotometer equipped with type 2527 Thermoprogrammer. Temperature was increased at a rate of 1 °C per min and absorption at 260 nm was recorded by a Gilford type 5040A recorder. Data were plotted against the percentage of modified bases.

In DNA with a 4.5% modification, the T_m was lowered by about 1.7 °C per each 1% modification. The decrease in T_m was associated with a decrease in the total hyperchromicity observed during heat denaturation (Figure 2B), suggesting that portions of the DNA duplex had undergone localized denaturation as a consequence of modification by BPDE.

Kinetics of Formaldehyde Unwinding. To more accurately assess the extent of denaturation in the BP-modified DNA samples, we applied the formaldehyde unwinding method of Utiyama and Doty (1971). This method has the advantage that it provides information about the dynamic structure of DNA and can be used to actually calculate the number of open base pairs. It is based on following the kinetics of the reaction of formaldehyde with the nucleic acid bases, measured by the associated increase in absorbance at 251 nm. Since formaldehyde reacts preferentially with bases in "open" regions of the helix, it can be used as a chemical probe for determining the number of "open" base pairs in double helical DNA at temperatures below the T_m of the DNA. Figure 3 plots the increase in absorbance at 251 nm with time, following the addition of formaldehyde to equal concentrations of various types of DNA. It is apparent that the 1.5% BP-modified DNA sample was slightly more reactive than the control native DNA, the 2.2% BP-modified DNA was somewhat more reactive, and the 4.5% sample was quite reactive.

In Table I we have listed the initial rate constants (K_0) of unwinding for native, heat denatured, and BP-modified DNA samples. The relative fraction of "open" base plates in native DNA at a given temperature [$\theta_n(T)$] is represented by the ratio of the initial rate constants of native and denatured molecules [K_0(n-DNA)/K_0(d-DNA)]. The value 0.013 for the control DNA (Table I) is similar to that obtained by other investigators (Utiyama and Doty, 1971; Fuchs and Daune, 1973). Corresponding ratios for BP-modified DNA [$\theta_{BP}(T)$], calculated from the ratio of the initial rate constants of modified and denatured molecules [K_0(BP-DNA)/K_0(d-DNA)],

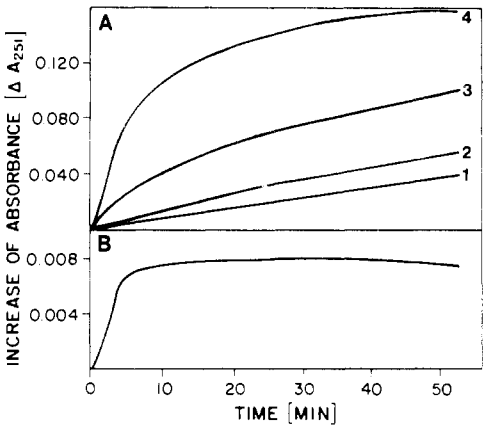


FIGURE 3: Kinetics of formaldehyde unwinding of DNA samples. Increase in optical density of DNA at 251 nm was followed in reaction mixtures containing 0.042 M sodium borate (pH 9.0)-1 M formaldehyde; (A) 1, native DNA; 2, 1.5% BP-modified DNA; 3, 2.2% BP-modified DNA; 4, 4.5% BP-modified DNA; (B) heat-denatured DNA.

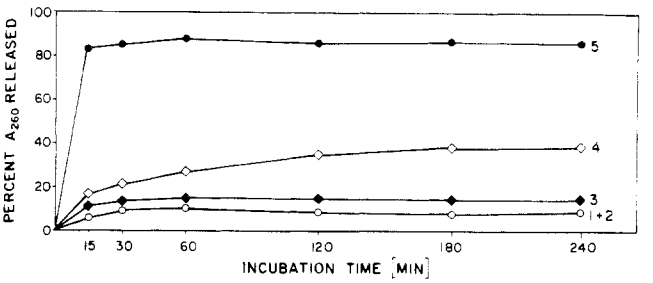


FIGURE 4: Kinetics of hydrolysis of DNA samples by S_1 endonuclease from *Aspergillus oryzae*. The incubation mixtures contained (final volume 0.3 mL): 0.05 M sodium acetate (pH 4.6), 0.05 M NaCl, 1×10^{-3} M $ZnSO_4$, 0.1 mg of DNA, and 5 units of S_1 nuclease. Incubation was carried out at 45 °C and terminated by chilling; then 0.25 mg of DNA was added as a carrier and nonhydrolyzed DNA was immediately precipitated with 0.6 mL of 10% perchloric acid. Samples were centrifuged and A_{260} was determined in supernatants. (○—○) Control DNA (1) and 1.5% BP-modified DNA (2); (♦—♦) 2.2% BP-modified DNA (3); (◇—◇) 4.5% BP-modified DNA (4); (●—●) heat denatured DNA (5).

TABLE I: Calculation of the Number of Open Base Plates $\chi(T)$ Introduced by a Single BP Derivative Bound to DNA.

DNA	$K_0 \times 10^4$/min	$\theta_n(T)$	$\theta_{BP}(T)$	$\chi(T)$
Heat denatured	1600			
Native, mock modified	21	0.013		
1.5% BP-modified	37		0.023	0-1
2.2% BP-modified	102		0.063	2-3
4.5% BP-modified	490		0.306	6-7

represent the relative fraction of open base plates in the BP-modified samples. All of these values were higher than those for the control native DNA sample (0.013) and rose from 0.023 for the 1.5% modified sample to 0.063 for the 2.2% sample, to 0.306 for the 4.5% sample. The differences in the relative fractions of open base plates between BP-modified samples and the control native DNA $[\theta_{BP}(T) - \theta_n(T)]$ can be attributed to the increment in locally denatured regions induced by covalently bound carcinogen. The average number of "open" base plates $[\chi(T)]$ induced by a single base modification was calculated from the following equation:

$$\chi(T) = \frac{\theta_{BP}(T) - \theta_n(T)}{\% \text{ modified bases}}$$

The results indicate that this value was 0-1 with the 1.5% modified sample, 2-3 with the 2.2% sample, and 6-7 with the 4.5% modified sample. Thus, only with the highly modified sample did the binding of the BP derivative produce large regions of "open" base plates. These results are in marked contrast to those obtained by Fuchs and Daune (1973) with AAF modified DNA. (See also Discussion.)

S_1 Nuclease Digestion. A separate approach to determine whether covalent binding of the BP derivative to native DNA produced localized single stranded regions in the DNA molecule was to incubate the DNA samples with S_1 endonuclease from *Aspergillus oryzae*, an enzyme which, under appropriate conditions, will cleave single but not double stranded regions of DNA. Figure 4 indicates that, under the conditions used, a sample of DNA that had been previously completely heat denatured was rapidly and almost completely digested during a 240-min incubation. The BP-DNA sample that contained a 1.5% modification was identical with the control in its resistance to S_1 digestion, whereas that which contained a 2.2% modification was slightly more susceptible to S_1 digestion (15% digestion at 240 min). The 4.5% modified sample showed a marked increase in susceptibility to digestion with about 40% hydrolysis at 240 min.

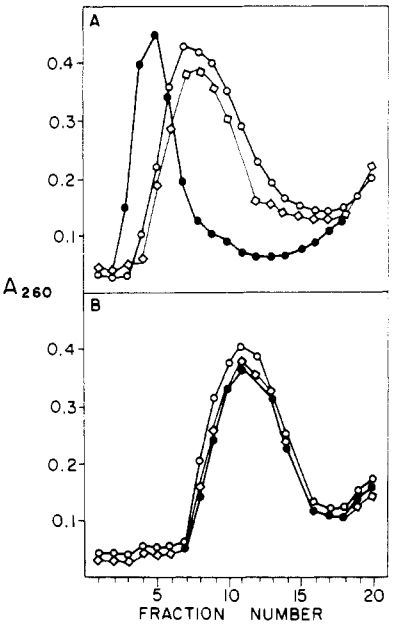


FIGURE 5: Sucrose gradient centrifugations of DNA. (A) Alkaline gradient: 1 A_{260} unit of each DNA sample in 0.1 mL of 0.2 M NaOH was applied to the top of 5-20% sucrose gradient in 0.1 M NaOH and 0.9 M NaCl. Gradients were centrifuged in a Beckman SW 41 rotor at 40 000 rpm for 240 min at 20 °C. Fractions were collected from the top with a Buchler Auto-Densi-Flow and A_{260} was measured on a Gilford type 240 spectrophotometer. (B) Neutral gradient: 1 A_{260} unit of each DNA sample in 0.1 mL was applied to the top of a 10-30% sucrose gradient in 0.01 M sodium cacodylate (pH 7.1), 0.1 M NaCl, and 1×10^{-3} M EDTA. Gradients were centrifuged in a Beckman SW 41 rotor at 40 000 rpm for 300 min at 20 °C and processed as above. (○—○) Control DNA; (◇—◇) 1.5% BP-modified DNA; (●—●) 4.5% BP-modified DNA.

Alkaline and Neutral Sucrose Density Gradients. To establish whether the reaction of native DNA with BPDE introduced any single and/or double strand breaks in the DNA molecules, we analyzed the modified DNA samples by sucrose density gradient centrifugation. The alkaline sucrose gradient profile of the 1.5 and 2.2% (not shown) modified sample was identical with that of the control unmodified DNA (Figure

5A). On the other hand, the 4.5% modified sample sedimented considerably slower, indicating that apparently all of the molecules had undergone chain scissions. These breaks are single stranded in nature since the neutral sucrose gradients (Figure 5B) did not show any differences in sedimentation profiles between the control, the 1.5% and 4.5% modified samples.

Buoyant Density. We used cesium chloride density gradient centrifugation to explore the possibility that the BP-modified DNA might have an altered buoyant density. Figure 6 indicates that the control native DNA, the 1.5% modified and the 4.5% modified samples, had similar profiles with a peak at 1.690 g cm^{-3}, the density characteristic of native calf thymus DNA (Szybalski and Szybalski, 1971). The absence of a significant shift in buoyant density indicates that even in the 4.5% modified samples the manifestations of partial denaturation obtained in the above studies reflect localized regions of denaturation in molecules that are mainly native DNA, rather than the existence of a subpopulation of DNA molecules that are completely denatured.

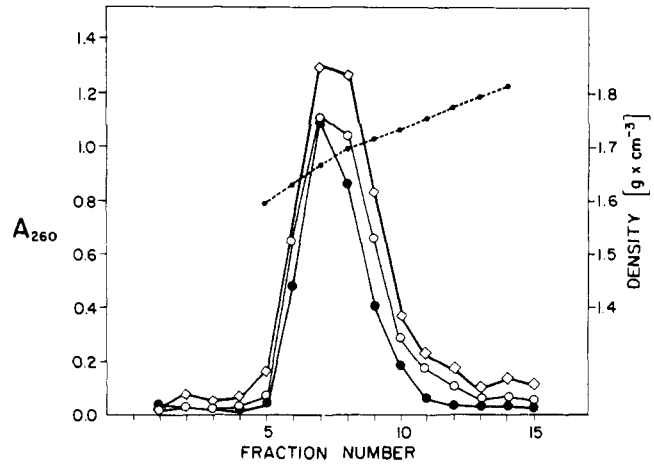


FIGURE 6: Cesium chloride gradient centrifugation of DNA; 10 A_{260} units of each DNA sample were mixed with 13.5 mL of cesium chloride solution (1.26 g/mL) in 0.01 M Tris-HCl (pH 7.5) and centrifuged in a Beckman Ti 50 rotor at 40 000 rpm for 60 h at 20 °C. The samples were processed as described in the legend to Figure 5. (◊—◊) Control DNA; (○—○) DNA with 1.5% modified bases; (●—●) DNA with 4.5% modified bases.

Discussion

It is of interest to contrast the results obtained in the present study with BP-modified DNA to those obtained in previous studies with AAF-modified DNA. With samples of native DNA in which about 1 to 2% of the bases were modified by the BP derivative, there was a 0.75 °C reduction in T_m during heat denaturation per each 1% modification; a value of about 1 to 2 for the average number of "open" base plates associated with each BP modified base, as revealed by the formaldehyde unwinding procedure; and an increase of about 0 to 5% in susceptibility to digestion by S_1 endonuclease. The corresponding values for samples of native DNA in which 1 to 2% of the bases were modified by AAF are: a 1.15 °C reduction in T_m per each 1% modification of the bases (Fuchs and Daune, 1971; Levine et al., 1974; Fuchs et al., 1976); 12 to 13 "open" base plates per each AAF modified base (Fuchs and Daune, 1973, 1974); and an increase of about 15% in digestion by S_1 nuclease (Fuchs, 1975; Yamasaki et al., 1977).

We must stress that these are approximate values, that they assume random distribution of the carcinogen moiety on the DNA strand, and that they assume that all sites of substitution by the same carcinogen are equivalent. Additional studies are required to establish the validity of these assumptions. Nevertheless, the marked differences between BP and AAF modified DNAs suggest that the BP modification produces a less drastic distortion in the conformation of native DNA than that produced by AAF.

A more heavily BP modified sample of DNA, in which 4.5% of the bases were modified, did reveal rather extensive denaturation. This sample, however, also had extensive single strand breaks. The strand breakage could be a consequence of modification of phosphate residues by BPDE, a finding described by Koreeda et al. (1976). Alternatively, the more highly modified sample may have undergone other structural modifications. This aspect requires further study.

Examination of a space-filling model of double-stranded DNA with Watson-Crick geometry indicates that the 2-amino group of deoxyguanosine is relatively exposed in the minor groove of the helix. It is likely, therefore, that BPDE can attack this residue with very little distortion of the native DNA conformation. On the other hand, the C-8 position of deoxyguanosine faces the major groove and because of steric hindrance by the deoxyribose residue, is inaccessible to attack by *N*-acetoxy-AAF, unless there is rotation of the guanine base about the glycosidic bond from the "anti" to the "syn" conformation (Nelson et al., 1971; Grunberger and Weinstein, 1976). The latter aspect, coupled with the evidence that the AAF residue actually becomes inserted into the helix occupying the position of the displaced guanine residue (Nelson et al., 1971; Levine et al., 1974; Grunberger and Weinstein, 1976; Fuchs et al., 1976), explains the marked conformational distortion and rather large localized regions of denaturation associated with AAF modification.

Further studies are required to determine the precise orientation of the BP residue in the DNA helix, following its covalent linkage to the 2-amino group of guanine. Space-filling models suggest that from steric considerations it could reside in the minor groove, although it might "seek" a more hydrophobic environment by actual insertion into the helix. Our data do suggest that one, or a few, base plates are destabilized at sites of BP modification. From this point of view it is of interest that there is evidence that even a less bulky methyl substituent on the 2-amino group of guanine alters its base-pairing potential (Pochon and Michelson, 1969; Ikehara and Hattori, 1971). This impairment in base-pairing might explain our findings, in separate studies, demonstrating that sites of BP modification in DNA interfere with chain elongation during transcription by RNA polymerase (Leffler et al., 1977). The effects of BP modification on the template capacities of nucleic acids in other polymerase reactions are currently under investigation in this laboratory.

The possible in vivo relevance of the present results is discussed in a related paper (Leffler et al., 1977).

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