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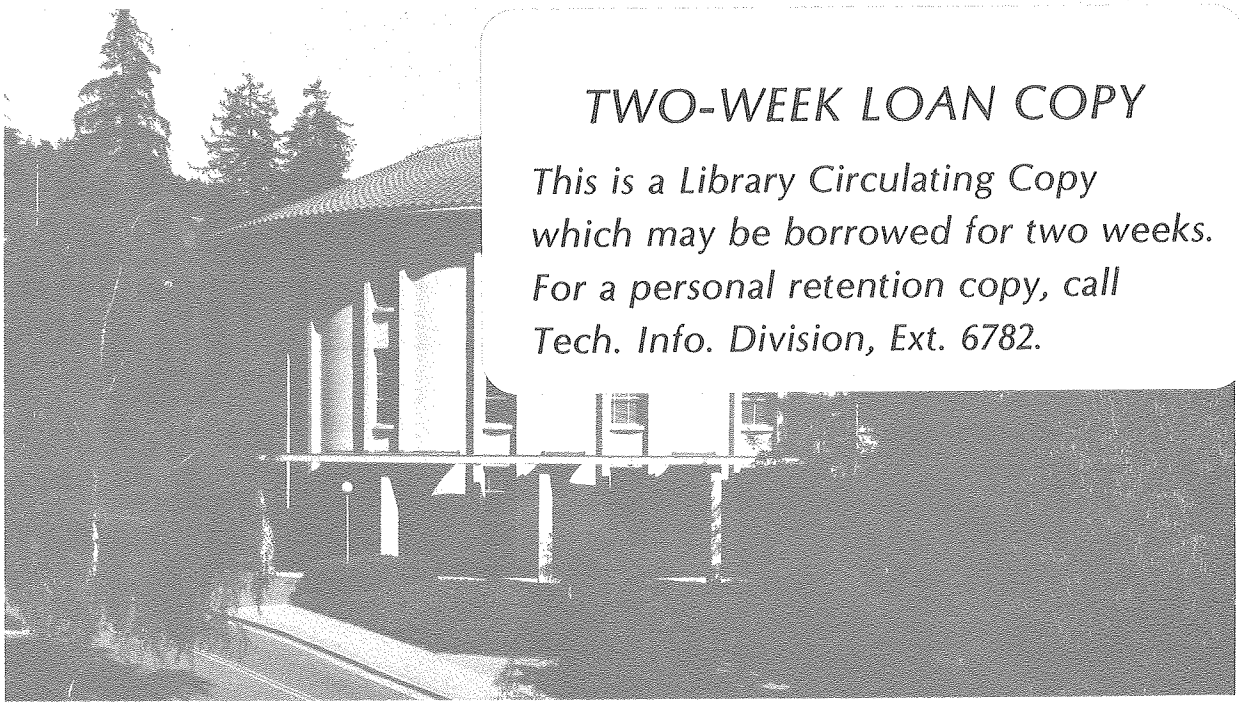

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DISCLAIMER

Biochemistry

Modulation by Ionic Strength and Superhelicity of Benzo[a]pyrene Diol Epoxide Induced DNA Alkylation and Unwinding

(SV40 DNA/DNA relaxing enzyme/unwinding angle/gel electrophoresis/high-pressure liquid chromatography)

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Abbreviations: BaP diol epoxide, (±)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.

ABSTRACT

Superhelical and partially relaxed SV40 DNA were reacted *in vitro* with (±)7β, 8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP diol epoxide). The modified DNA contained N^2 guanine and N^6 adenine hydrocarbon adducts in the ratio 86:14. Superhelical SV40 DNA was approximately 6% more susceptible to modification than partially relaxed viral DNA. Counterions inhibited DNA alkylation by up to 90%, Mg^{2+} being 50-fold more effective than Na^+. The sensitivty of covalent binding to helix stability is consistent with a reaction complex in which BaP diol epoxide is intercalated.

The superhelical density of the modified DNA substrates was determined electrophoretically relative to partially relaxed standards and an unwinding angle for the hydrocarbon adducts was calculated. The angle was dependent upon the superhelicity of the DNA molecule and ranged from 330° to 30°. This data indicates that the modified base pairs are disrupted and, in the presence of torsional strain, act as centers for the further denaturation of up to 8 adjacent base pairs. In the absence of such strain the alkylation sites have an ordered structure with the attached hydrocarbon probably oriented in the minor or major groove of the helix.

INTRODUCTION

Benzo[a]pyrene is a widespread environmental pollutant and a potent carcinogen (1). In mammalian cells it undergoes metabolic activation to the ultimate carcinogen BaP diol epoxide (2). This metabolite is responsible for the majority of benzo[a]pyrene originated DNA and RNA alkylation events (3,4). The interaction with DNA is of particular interest, since a preponderance of evidence indicates that carcinogen modification of DNA initiates the transformation process (5-7). The primary adduct present in DNA consists of a linkage between the C-10 position of the hydrocarbon and the exocyclic amino group of guanine (3,8-10).

We have probed the physical structure of the guanine adduct and the reaction complex which precedes it using BaP diol epoxide treated SV40 DNA. Taking advantage of the topological constraints in a covalently closed DNA molecule, we have determined the unwinding angle of the alkylation sites in both superhelical and partially relaxed SV40 DNA and the effect of torsional strain on the physical binding of BaP diol epoxide to the double helix. We have also investigated the effect of counterions on DNA alkylation by the hydrocarbon.

MATERIALS AND METHODS

BaP Diol Epoxide. Crystalline BaP diol epoxide was synthesized essentially by the method of McCaustland and Engel (11). [^{3}H]-BaP diol epoxide, 1.5 mg/ml in 19:1 tetrahydrofuran-triethylamine, was also synthesized according to published procedures (12). It had a specific activity of 1.23 C_i/mmole. The BaP diol epoxide stocks were stored at -70° and dilutions were made with dimethyl sulfoxide.

SV40 DNA. Superhelical SV40 (form I) DNA was isolated from infected TC-7 African green monkey cells by a modification of the Hirt extraction as

essentially described by Hallick et al. (13). This DNA contained a small amount of nicked circular (form II) DNA present as an impurity.

Partially relaxed SV40 DNA standards were prepared by incubating 1-2 µg of form I DNA with 5-20 E.U. of DNA relaxing enzyme (Bethesda Research) in 20 mM Tris-HCl (pH 8.0)-0.5 mM EDTA-2.5 mM $MgCl_2$ for 5-20 min at 37°. The DNA was stored at 4° in the presence of 0.02 M EDTA. Preparative relaxation of 18.8 µg of SV40 form I DNA was conducted in 940 µl of the above buffer containing 140 E.U. of DNA relaxing enzyme for 15 min at 37°. After stopping the reaction with 50 µl of 0.2 M EDTA, the DNA was ethanol precipitated and dissolved in 20 mM Tris-HCl (pH 8.0)-0.5 mM EDTA prior to reaction with BaP diol epoxide.

DNA Alkylation and Adduct Characterization. SV40 DNA was modified with BaP diol epoxide in 20 mM Tris-HCl (pH 8.0)-0.5 mM EDTA-10% dimethyl sulfoxide at the indicated molar reaction ratios (BaP diol epoxide/DNA mononucleotide). Reaction mixtures were incubated in the dark at 37° for at least 90 min. Alkylation was quantified by radioactivity counting using SV40 [^{14}C]-DNA and [^{3}H]-BaP diol epoxide. The modified DNA was diluted to 1.0 ml with 20 mM Tris-HCl (pH 8.0)-0.5 mM EDTA-0.5 M NaCl and extracted 3 times with 1.0 ml of ethyl acetate. After adding 30 µg of carrier calf thymus DNA (Sigma), the DNA was precipitated from the aqueous phase by addition of 2.0 ml of NaCl-saturated ethanol and by storage overnight at -20°. The DNA was pelleted, washed, and resuspended in 1.0 ml of NaCl-saturated 70% ethanol. It was then collected on a Millipore 0.45 µ HAWP filter, combusted and counted. Binding data was calculated assuming

SV40 DNA contained 5200 base pairs (14) and had a molecular weight of 3.6×10^6 daltons (15).

Hydrocarbon adducts, enzymatically released from alkylated DNA, were isolated by Sephadex LH-20 chromatography and further resolved by highpressure liquid chromatography as described by Straub et al. (8).

Gel Electrophoresis. Vertical 1.4% agarose (Bio-Rad) slab gels (13.3 x 13.0 x 0.5 cm) were prepared and run in 40 mM Tris-HCl (pH 7.9)-5 mM sodium acetate-1 mM EDTA at 50 V for 12-16 hr. Composite 2% polyacrylamide-0.5% agarose vertical slab gels (22 x 13.0 x 0.3 cm) were prepared and run in 36 mM Tris-30 mM NaH_2PO_4 (pH 7.7)-1 mM EDTA at 100 V for 72 hr with air cooling. Gels were stained for at least 1 hr at 4° in electrophoresis buffer containing 0.5 µg/ml ethidium bromide. The fluorescent DNA bands were illuminated from below with short wavelength uv (Ultra Violet Products, Inc., San Gabriel, CA) and photographed on Polaroid type 665 positive/negative film through a Corning 3-69 filter. Tracings of the negatives were obtained with a Schoeffel model SD3000 spectrodensitometer.

RESULTS

Alkylation of SV40 DNA. The distribution of stable covalent adducts in BaP diol epoxide modified superhelical SV40 DNA was determined by high-pressure liquid chromatography of the modified mononucleosides (Fig. 1). The fluorescent peaks, derived from BaP diol epoxide modified calf thymus DNA carrier, had been previously characterized (8) and permitted identification of the radioactive adducts from SV40 DNA. Only 4 adducts were obtained, corresponding to trans addition of the exocyclic amino groups of guanine and adenine to the C-10 position of the enantiomeric hydrocarbon. There was no detectable reaction with cytosine or thymine.

Slightly less than 1% of the initial alkylation events generated single strand scissions (data not shown). Two mechanisms, phosphotriester hydrolysis (16) and depurination strand scission (17), have been proposed to account for

this phenomenon. Our investigtion, which will be published elsewhere, indicates that the formation of apurinic sites precedes DNA nicking. No positive evidence was found for phosphotriester formation. The initial alkylation event responsible for strand scission may occur at the N-7 position of guanine (18).

The adduct distribution for both superhelical and partially relaxed SV40 DNA is summarized in Table 1. Nearly 90% of BaP diol epoxide alkylation occurred on guanine. Stereoselectivity was exhibited in the modification of both purines and this suggests that the hydrocarbon is oriented within the helix at the time of reaction. The decrease in guanine binding and overall stereoselectivity obtained with superhelical relative to relaxed DNA might be significant and could result from a slight reaction of BaP diol epoxide with naturally occurring single-stranded regions in the superhelical DNA (20). The even greater differences between SV40 and calf thymus DNA probably arise from the 20-fold greater level of modification employed in this study.

The extent of reaction of viral DNA with BaP diol epoxide was modulated by the conformation of the double helix. When the helix was stabilized by addition of counterions to the solvent, there was a significant inhibition of BaP diol epoxide alkylation. The inhibition of binding by Na^+ and Mg^{2+} ions is shown in Fig. 2 and is comparable to similar data for the photobinding of psoralen derivatives (21), agents known to intercalate and crosslink DNA. By shielding the phosphates these ions wind the helix and decrease base pair separation (22). At the highest concentrations tested, both ions inhibited BaP diol epoxide modification of DNA by about 90%. Hydrocarbon binding was far more sensitive to Mg^{2+} than Na^+, reflecting greater stabilization of the double helix by Mg^{2+} relative to Na^+ (23). The differential inhibition by the two ions argues that they reduce binding by changing the conformation of DNA rather than by interacting with BaP diol epoxide.

Unwinding of SV40 DNA. The unwinding angle associated with BaP diol epoxide alkylation sites was determined using superhelical and partially relaxed SV40 DNA. These alkylated substrates were electrophoresed under conditions where their mean superhelicity ($\bar{\tau}$) could be calibrated relative to unreacted partially relaxed SV40 DNA standards. The standards were resolved into a series of bands, each differing from its neighbor by one superturn. Superhelical SV40 DNA (initial $\bar{\tau}$ = -21.0) was analyzed on a composite agarose-polyacrylamide gel (Fig. 3A) and partially relaxed SV40 DNA (initial $\bar{\tau}$ = -6.56) was analyzed on an agarose gel (Fig. 3B). The level of alkylation was determined by radioactivity counting of reaction aliquots after removal of free hydrocarbon.

BaP diol epoxide alkylation of both DNA substrates (1.5-44 adducts/genome) led to a loss of fine structure and a reduction in electrophoretic mobility. The decrease in mobility is interpreted as a loss of superturns directly resulting from local unwinding of the DNA helix by hydrocarbon adducts. The loss of fine structure reflects the inability of either gel system to resolve the component DNA bands which result from the presence of a Gaussian distribution of adducts on each parental band. Thus each band in the unreacted DNA is converted into a set of bands too closely spaced to be resolved. These bands differ in superhelicity by the BaP diol epoxide unwinding angle. Both effects are due to covalently bound BaP diol epoxide, since the electrophoretic pattern of DNA treated under identical conditions with 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene was unchanged (data not shown).

The magnitude of both effects was greater for superhelical than partially relaxed DNA. This was apparent when the mean number of superhelical turns was plotted as a function of the number of adducts per genome for the two DNA substrates (Fig. 4). The decrease in slope of the curve for superhelical DNA

indicates that hydrocarbon induced unwinding is a function of superhelical density at high $\bar{\tau}$. The nearly linear curve obtained for partially relaxed DNA indicates that unwinding is almost independent of superhelicity at low $\bar{\tau}$.

Table 2 summarizes the data from the unwinding experiments and lists the incremental unwinding angles and open base pairs per adduct for superhelical and partially relaxed DNA. The extremely large unwinding angles obtained with superhelical DNA reflect localized denaturation at the site of alkylation. The degree of denaturation is primarily determined by the superhelicity of the DNA molecule and is probably enhanced by the low ionic strength of the electrophoresis buffer. The release of torsional strain on a per adduct basis decreased with superhelicity and ranged from 4.5-2.2%. The single-stranded regions adjacent to the hydrocarbon adducts are unlikely to be stabilized by intrastrand hairpins, since BaP diol epoxide modification of the SV40 genome is a random event, even at low reaction levels (24).

In the absence of significant torsional strain, the unwinding angle plateaued at about 31° suggesting a distinct structure for the hydrocarbon DNA complex. The most likely physical structure involves destabilization or disruption of the modified guanine-cytosine base pair and localization of the hydrocarbon in either the minor or major groove (see Discussion). Consistent with this model is the increased electrophoretic mobility of BaP diol epoxide modified form II DNA in agarose gels. In Fig. 5, where the quantity of DNA in the form II band remained nearly constant, this effect is seen without the added complication of band overloading. By acting as flexible "hinges", the alkylation sites are believed to decrease the intrinsic viscosity of the nicked circular DNA and increase its mobility. Similar changes have been observed with

dichlorodiammineplatinum (25). Intercalating agents act in the opposite fashion (26). They stabilize base pairs, lengthen and stiffen the helix, and increase the intrinsic viscosity of DNA.

An examination of Table 2 demonstrates that BaP diol epoxide alkylation of SV40 DNA is also modulated by torsional strain. At identical molar reaction ratios the modification of superhelical SV40 DNA was consistently greater than partially relaxed DNA. Torsional strain present in superhelical DNA facilitates any binding mode which unwinds the helix. The reduction of superhelical turns adds an extra favorable free energy term to the binding and enhances affinity relative to relaxed (or covalently closed relaxed) DNA. For BaP diol epoxide, binding was approximately 6% greater with superhelical relative to relaxed DNA. This is comparable to the 10% enhancement of reactivity seen with the intercalating agents psoralen (21) and bleomycin (27).

The relative affinity of a molecule for superhelical and relaxed forms of a DNA is a function of its unwinding angle and is given by the relationsh°hip (27)

$$\nu_s/\nu_r = e^{-A\phi/kT} \qquad (1)$$

where ϕ is the unwinding angle, A is the torsional free energy change per degree of unwinding, and ν_s/ν_r is the partition of the molecule between superhelical and relaxed forms of DNA. For SV40 DNA $A = -8.6 \times 10^{-24}$ cal/degree (28-30). If BaP diol epoxide denatures superhelical DNA upon physical binding, just as it does when covalently bound, a high partition ratio would be expected. At a molar reaction ratio of 0.02 the difference between the average adduct unwinding angles in superhelical and partially relaxed DNA is 215°. From equation (1) if ϕ = 215° then $\nu_s/\nu_r = 6.7$. This estimate is far greater than the value of 1.07 actually obtained (Table 2) and indicates that BaP diol epoxide disrupts base pairing only after covalent linkage. The experimental partition ratio is

consistent with a small unwinding angle accompanying the physical binding of BaP diol epoxide to both superhelical and relaxed DNA.

DISCUSSION

Microenvironment of the Reaction Complex. Intercalation of BaP diol epoxide prior to covalent binding with guanine was proposed by Meehan and Straub (19) to explain the stereoselectivity of the reaction. Our data support this hypothesis. The inhibition of BaP diol epoxide alkylation by Na^+ and Mg^{2+} is readily explainable in terms of an increase in the free energy barrier to intercalation while the enhancement of covalent binding by superhelicity is of the order expected for an intercalating agent. Although binding of hydrocarbon within the minor groove prior to reaction with guanine cannot be ruled out, the formation of such a complex would lack both the stacking interaction provided by intercalation and the electrostatic interactions characteristic of external binding.

Microenvironment of the Guanine Adduct. It is important when determining the unwinding angle of BaP diol epoxide modified guanine that the electrophoretic pattern observed actually represent the unwinding of the helix by this adduct and not strand scission or depurination. At a molar reaction ratio of 0.10, approximately 1 alkylation event out of 150 will give rise to depurination or strand scission over a 24 hr period (unpublished data). This is equivalent to 0.20 nicks or apurinic sites in an SV40 DNA molecule containing 30 adducts. The local unwinding associated with depurination is insignificant given the infrequency of the event. The effect of strand scission, however, is far more dramatic since it leads to an entire loss of superhelical turns. Prior to electrophoretic analysis, strand scission removes DNA molecules from the unwinding angle determination. Preferential removal of DNA molecules with higher than mean values of superhelicity or alkylation may occur. As discussed

by Wiesehahn and Hearst (31), these phenomena exert slight opposing effects on the calculated unwinding angle which can probably be neglected.

Strand scission during the time span of electrophoresis could lead to a significant overestimation of the unwinding angle by decreasing the mobility of the DNA. The probability of strand scission increases with the number of adducts. In Fig. 3 the tailing of covalently closed DNA bands at higher molar reaction ratios is probably due to nicking during electrophoresis. The effect of nicking on the unwinding angle calculations was minimized for partially relaxed DNA by measuring the mobility in terms of the most intense band, which was resolvable up to a molar reaction ratio of 0.10. The same approach was precluded for superhelical DNA by the loss of band structure at very low levels of modification, and the unwinding angles calculated from this substrate are therefore overestimated at higher molar reaction ratios. The magnitude of this error is indicated by a comparison of the incremental unwinding angles from superhelical and partially relaxed SV40 DNA samples with similar torsional strain, i.e. 59° and 35°, respectively, at $\tau = -6$.

The lower intrinsic viscosity of modified DNA and the single-stranded character of the alkylation sites in superhelical DNA will partially offset the effect of nicking by enhancing the electrophoretic mobility of covalently closed DNA. In both cases, however, the enhancement is minor. The 10% greater mobility of alkylated form II DNA relative to control (Fig. 5) is attributed to an increased flexibility. Given the more compact structure of superhelical and partially relaxed DNA, the increased flexibility will exert a proportionately smaller effect on the mobility of these molecules. The single-stranded alkylation sites in superhelical DNA will likely collapse in the low ionic

strength electrophoresis buffer, thus shortening the molecules and increasing their mobility. Here, again, the effect is minor since even at a molar reaction ratio of 0.15 only 3% of the base pairs are denatured.

The reaction with adenine complicates analysis of the unwinding angle data. If the unwinding angle of the adenine adduct is significantly different from the guanine adduct, then an average value will be obtained and interpretation could be misleading. Fortunately, there is no reason to expect a large difference. Since the N^6 position of adenine is involved in base pairing, binding of BaP diol epoxide to that site will probably disrupt the adenine-thymine base pair. In superhelical DNA, where torsional strain amplifies the unwinding, both adducts should have identical unwinding angles. In partially relaxed DNA, the two unwinding angles may differ slightly but should both reflect local denaturation.

While this work was in progress Drinkwater et al. (32) published a study on the removal and reversal of superturns in SV40 DNA by BaP diol epoxide and N-acetoxy-2-acetylaminofluorene. By employing short reaction times and rapid electrophoretic analysis, strand scission was minimized and recoiling of the covalently closed DNA was observed at high adduct levels. From the level of binding required to cause comigration of superhelical DNA with nicked circular DNA an average unwinding angle of 22° was calculated for the two carcinogens. This value is below the range of unwinding angles reported here and may be due to the extensive alkylation required for comigration of DNA forms I and II (~500 adducts per genome). At high modification the alkylation sites may interact and so possess an altered conformation with a reduced unwinding angle. Extensive alkylation may also change the spectrum of adducts as well as alter the electrophoretic mobility of the DNA through phenomena other than unwinding. These factors could reduce apparent relaxation.

Recently, fluorescence quenching and linear dichroism studies have ruled out intercalation of covalently bound BaP diol epoxide (33,34). Taking into account our unwinding data, it is apparent that while the alkylation sites are denatured under conditions of strain (i.e. superhelicity, extremes of pH, high temperature, etc.), these sites possess an ordered structure under physiological conditions. In vivo, BaP diol epoxide modification of DNA probably unwinds the helix by 30-35°. The major perturbation induced by covalent binding and the probable cause of unwinding is disruption of the modified guanine-cytosine base pair. The hydrocarbon could reside in the minor groove since the exocyclic amino group of guanine is found there. Computer modeling indicates that only minor perturbation of the helix is caused by this orientation of the adduct (35). Alternatively, the hydrocarbon could reside in the more exposed major groove; that would require a 180° rotation of the modified guanine about its glycosidic linkage.

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FIGURE LEGENDS

Figure 1. High-pressure liquid chromatography profile of co-injection of [^{3}H]-BaP diol epoxide SV40 DNA adducts plus unlabeled BaP diol epoxide calf thymus DNA adducts. The form I viral DNA was reacted with [^{3}H]-BaP diol epoxide at a molar reaction ratio of 0.04. Peaks 3, 5, and 6 are deoxyguanosine adducts, peaks 7, 8, and 9 are deoxyadenosine adducts, and peaks 2 and 4 are deoxycytidine adducts. Peak 1 and part of peak 4 consist consist of 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene. The two early eluting radioactive peaks probably represent BaP diol epoxide solvent addition products.

Figure 2. Inhibition of BaP diol epoxide alkylation of SV40 DNA by NaCl and $MgCl_2$. SV40 form I [^{14}C]-DNA was reacted with [^{3}H]-BaP diol epoxide at a molar reaction ratio of 0.60 in the presence of the indicated concentrations of (●) NaCl or (○) $MgCl_2$. The DNA control contained 197 adducts/genome.

Figure 3. (A) Polyacrylamide slab gel of superhelical SV40 DNA reacted with BaP diol epoxide. SV40 form I [^{14}C]-DNA was reacted for 90 min at 37° with [^{3}H]-BaP diol epoxide at molar reaction ratios of (a) 0.002, (b) 0, (c) 0.005, (d) 0.01, (f) 0.02, (g) 0.04, (i) 0.06, (j) 0.10, and (l) 0.15. Aliquots were electrophoresed downward for 72 hr at 100 V on a 2% polyacrylamide-0.5% agarose slab gel. Tracks e, h, and k contained partially relaxed form I SV40 DNA. The top band in each track is form II SV40 DNA. (B) Agarose slab gel of partially relaxed SV40 DNA reacted with BaP diol epoxide. Partially relaxed SV40 form I [^{14}C]-DNA was reacted with [^{3}H]-BaP diol epoxide at molar reaction ratios of (c) 0, (d) 0.02, (f)

0.04, (g) 0.06, (i) 0.10 and (j) 0.15. After 3 hr at 37° aliquots were electrophoresed downward for 16 hr at 50 V on a 1.4% agarose slab gel. Tracks b, e, h, and k contained a mixture of SV40 DNA incubated for 15 min and for 20 min with DNA relaxing enzyme. Track a contained superhelical DNA. The top band in each track is unresolved low molecular weight cellular DNA; the second band is form II viral DNA.

Figure 4. Removal of superhelical turns by BaP diol epoxide adducts in (a) superhelical and (b) partially relaxed SV40 DNA.

Figure 5. BaP diol epoxide alkylation of SV40 DNA monitored by agarose gel electrophoresis. SV40 form I DNA (8.4 µg/ml) was reacted at 37° with BaP diol epoxide at a molar reaction ratio of 0.60. Aliquots of 50 µl were taken at the indicated times into 10 µl of 2.5 M 2-mercaptoethanol to stop the reaction. The alkylated DNA was electrophoresed downward at 50 V for 12 hr on a 1.4% agarose slab gel. The decrease in mobility and broadening of the form I DNA band are attributed to local unwinding of the helix by BaP diol epoxide adducts and to strand scission during the electrophoretic analysis.

Table 1

Stereoselectivity in the Reaction of BaP Diol Epoxide with DNA

Sample	% guanine	Isomer ratio*	% adenine	Isomer ratio*
Superhelical SV40 DNA	86.0	1:3.03	14.0	1:0.44
Partially relaxed SV40 DNA	86.9	1:3.38	13.1	1:0.38
Calf thymus DNA†	90.0	1:21.5	10.0	1:1

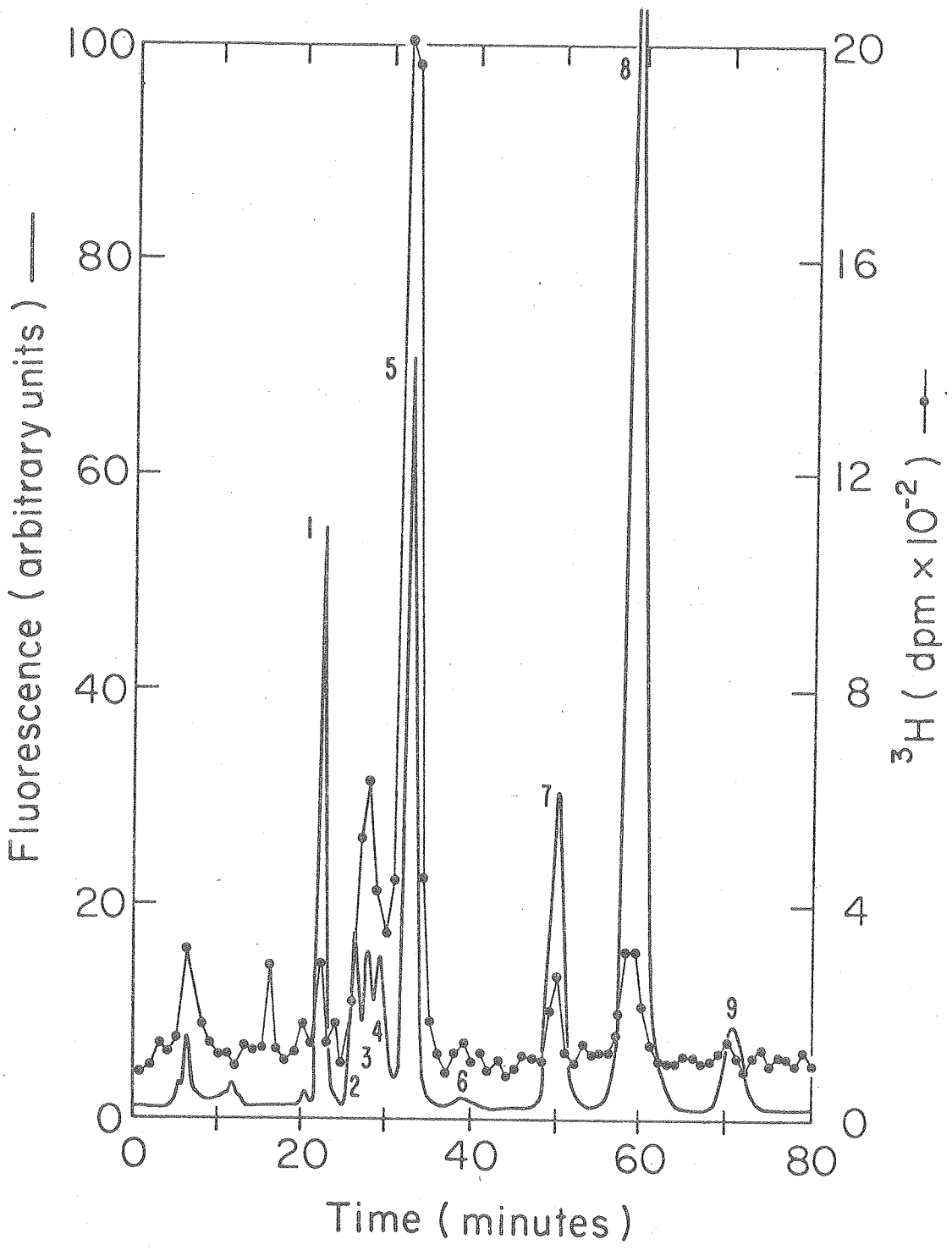
*The isomer ratio refers to the ratio of (-) to (+) BaP diol epoxide alkylation products.

†The data for calf thymus DNA was obtained from Meehan and Straub (19).

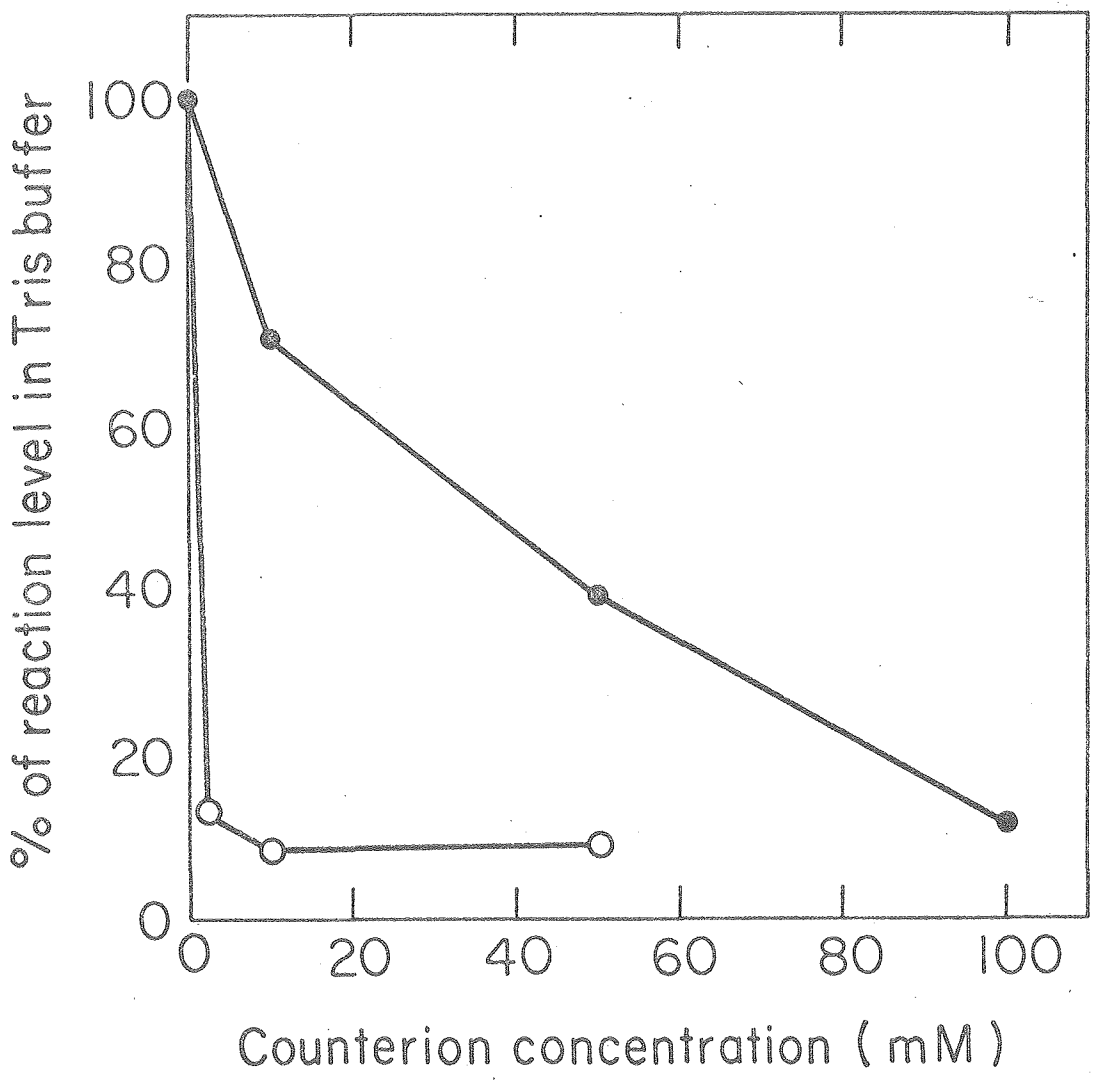
Table 2.

Unwinding of Superhelical and Partially Relaxed SV40 DNA by BaP Diol Epoxide

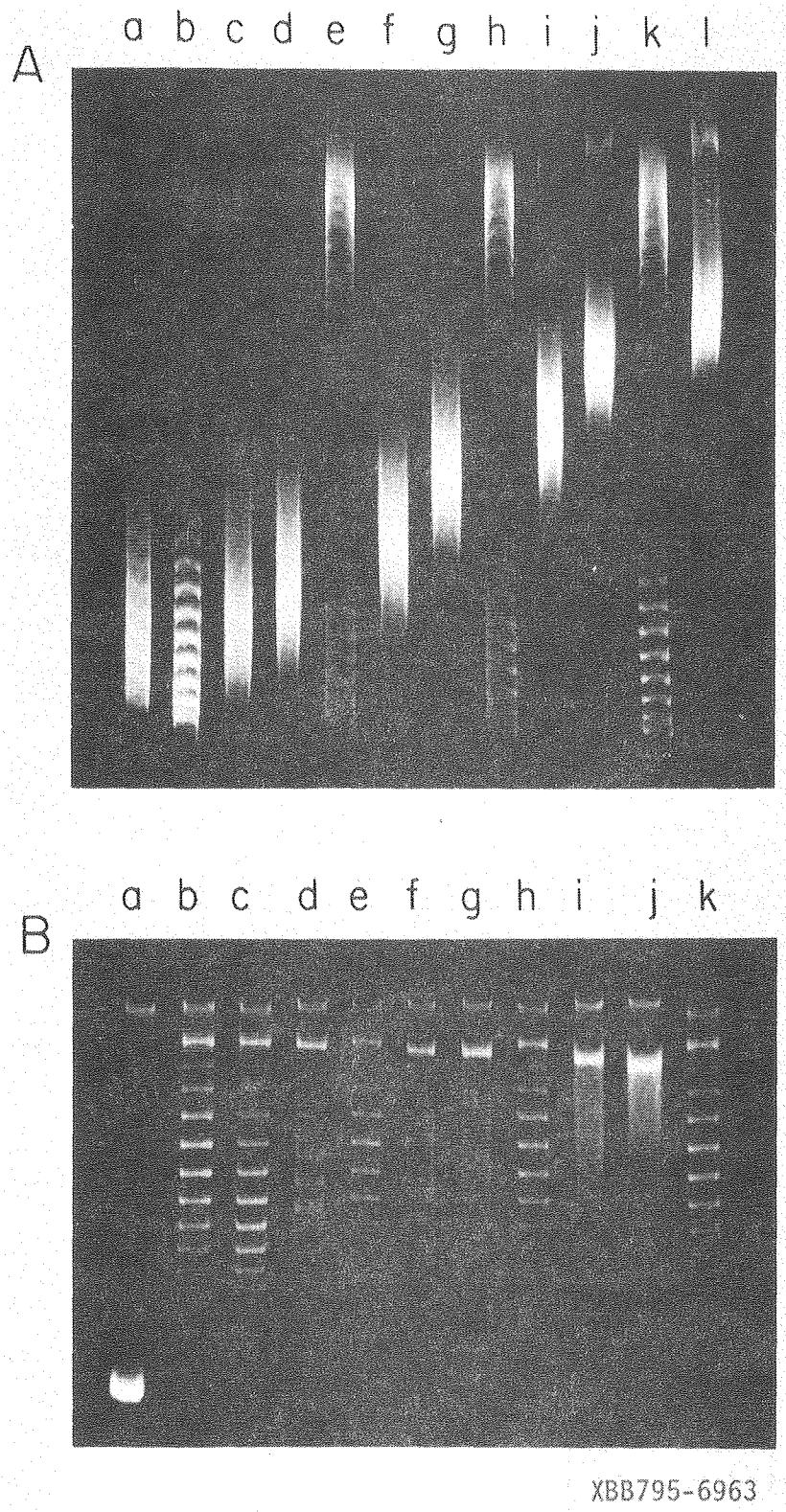
Molar Ratio	Adducts per Genome	Decrease in $\bar{\tau}$	Incremental Relaxation per Adduct	
			Open base plates	Unwinding angle
Superhelical DNA ($\bar{\tau}$ = 21.0)				
0.005	1.54	1.4	9.1	328°
0.01	3.53	3.0	8.0	290
0.02	7.10	4.9	5.3	192
0.04	13.2	7.9	4.9	177
0.06	20.7	10.5	3.5	125
0.10	30.5	12.7	2.2	81
0.15	43.9	14.9	1.6	59
Relaxed DNA ($\bar{\tau}$ = 6.56)				
0.02	6.62	0.64	0.97	35.1°
0.04	12.9	1.20	0.88	31.8
0.06	18.6	1.70	0.87	31.4
0.10	29.7	2.63	0.84	30.3


Fluorescence (arbitrary units)
^3H (dpm x 10^{-2})
Time (minutes)
0
20
40
60
80
100
4
8
12
16
20
1
2
3
4
5
6
7
8
9

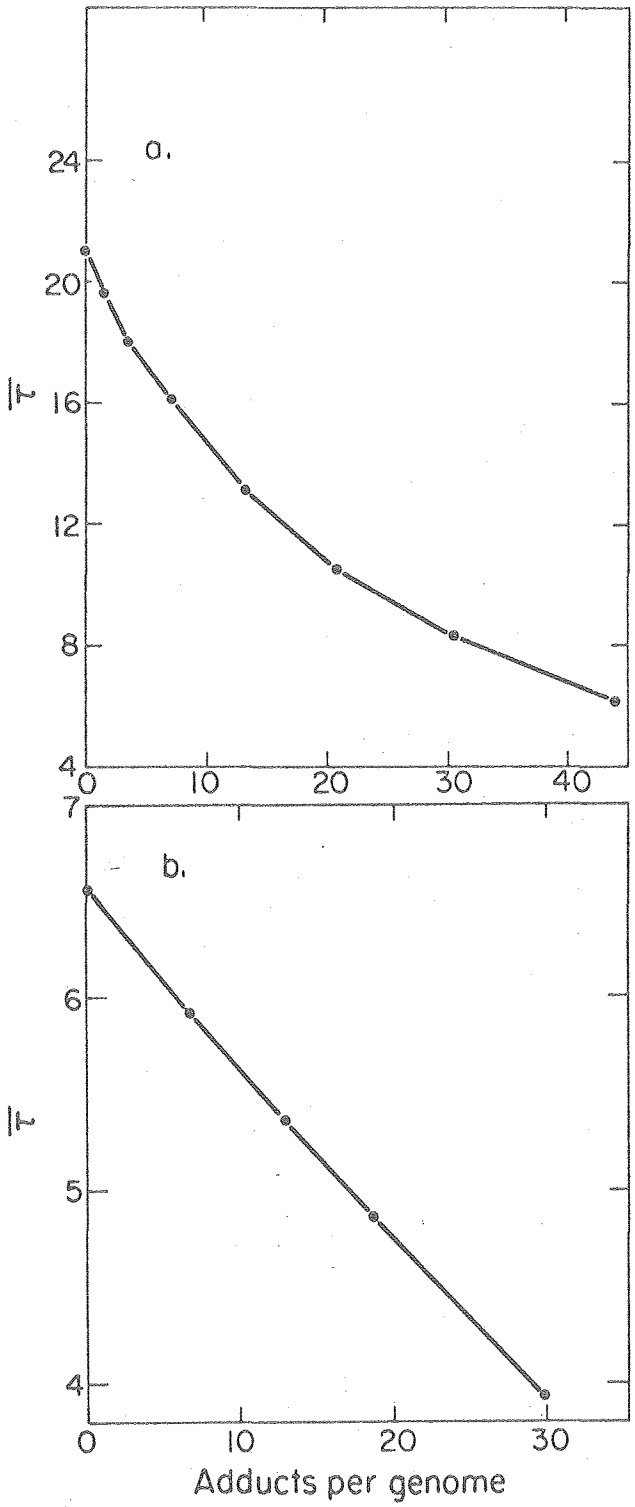
XBL796-4816


% of reaction level in Tris buffer
100
80
60
40
20
0
0
20
40
60
80
100
Counterion concentration (mM)

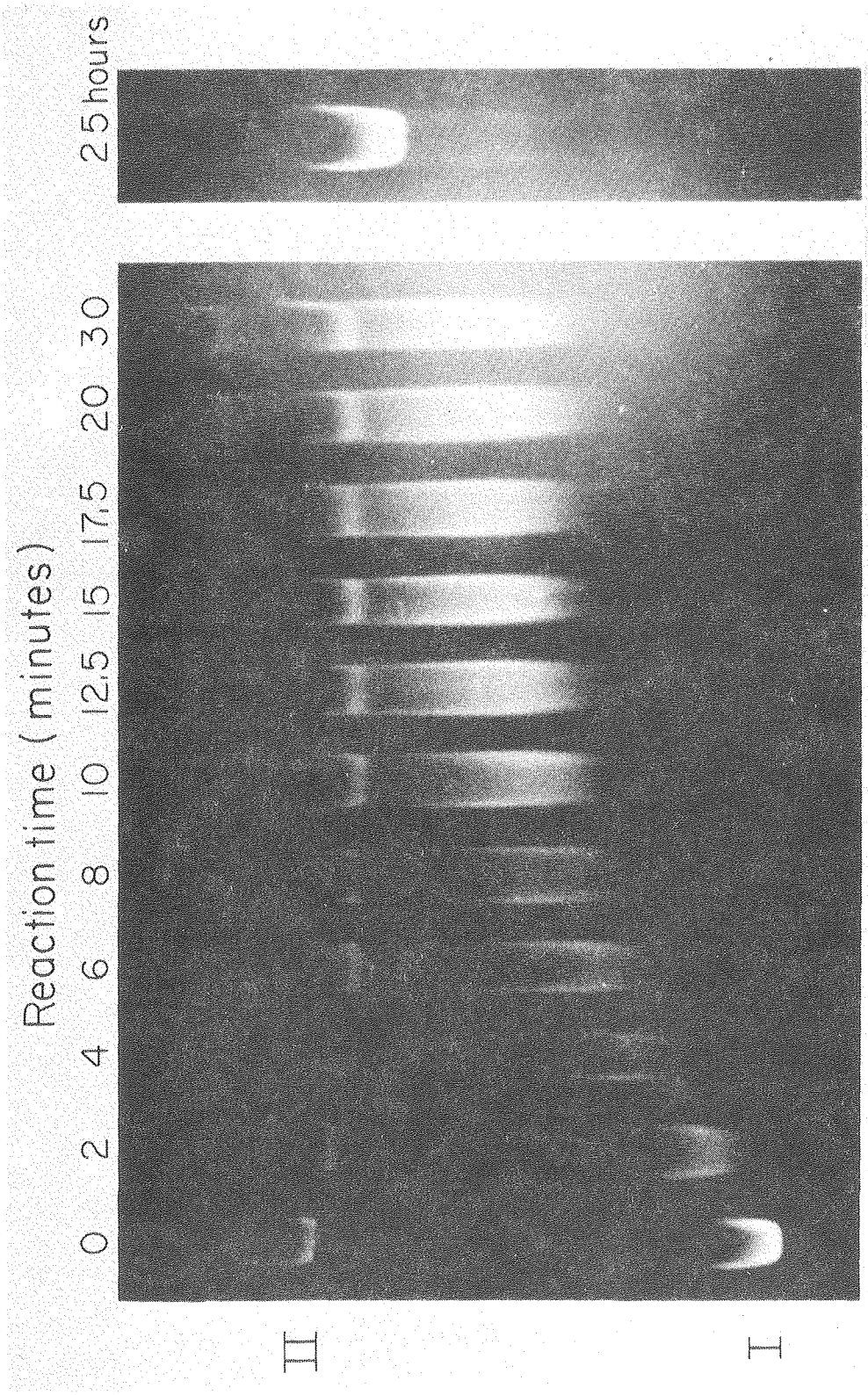
XBL795-4811



XBB795-6963


a.
b.
$\overline{\nu}$
$\overline{\nu}$
24
20
16
12
8
4
7
6
5
4
0
10
20
30
40
0
10
20
30
Adducts per genome

XBL796-4817



XBB796-7856