

Mapping the B-A conformational transition along plasmid DNA

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ABSTRACT

A simple method is presented to monitor conformational isomerizations along genomic DNA. We illustrate properties of the method with the B-A conformational transition induced by ethanol in linearized pUC19 plasmid DNA. At various ethanol concentrations, the DNA was irradiated with ultraviolet light, transferred to a restriction endonuclease buffer and the irradiated DNA was cleaved by 17 restriction endonucleases. The irradiation damaged DNA and the damage blocked the restrictase cleavage. The amount of uncleaved, i.e. damaged, DNA depended on the concentration of ethanol in a characteristic S-shape way typical of the cooperative B-A transition. The transition beginning and midpoint were determined for each restriction endonuclease. These data map the B-A transition along the whole polylinker of pUC19 DNA and six evenly distributed recognition sequences within the rest of the plasmid. The transition midpoints fell within the B-A transition region of the plasmid simultaneously determined by CD spectroscopy. The present method complements the previous methods used to study the B-A transition. It can be employed to analyze multikilobase regions of genomic DNA whose restriction endonuclease cleavage fragments can be separated and quantified on agarose gels.

INTRODUCTION

DNA can adopt two major double-stranded conformations, B-form and A-form (1). The A-form is very interesting because it is an almost constitutive conformation of double-stranded regions in RNA (2). DNA adopts the B-form in aqueous solution (3,4), while the A-form is induced by spore proteins (5), in the presence of ethanol (6,7), methanol (8),

some other proteins (9–14), ligands (15) and cations (16–18) in aqueous solution. The proteins include polymerases (9,10,13) that makes the A-form especially interesting from the biological point of view. A-form type base stacking is exhibited by guanine and cytosine runs within the B-type sugar–phosphate backbone of some molecules of DNA (19). This makes the B-form/A-form hybrid interesting from the evolutionary point of view because it could help DNA to substitute for RNA as the gene carrier during the RNA world to DNA world transition period (19,20). It is likely that the most ancient phenomena, e.g. transcription, still make use of the B-to-A transition (9,10,19,20). Hence, there are good reasons to study the A-form and the B-to-A transition not only for structural but also for molecular biology and molecular evolution reasons.

The most frequently used method to study the B-A transition is CD spectroscopy in solution (6,7,16,4). Other methods (21–24) are much less frequently used. CD spectroscopy is an almost ideal method for this purpose until one needs to follow the B-A transition locally along discrete parts of genomic DNA. Then, CD spectroscopy is less useful because it provides an integral signal from all parts of the molecule. In addition, CD spectroscopy cannot be used to study the B-A transition in complex biological contexts, e.g. in cell nuclei. Both of these shortcomings are overcome by a novel method based on DNA irradiation with ultraviolet (UV) light and detection of the photoproducts by restriction endonucleases. Here, we describe the method, demonstrate that it works and make use of it to study the B-A transition in linearized pUC19 DNA.

MATERIALS AND METHODS

Materials

Plasmid pUC19 was either purified as described previously (25) or bought from MBI Fermentas (Vilnius, Lithuania). Restriction endonucleases were purchased from the following producers: MBI Fermentas (Ecl136II, EcoRI, Eco88I, EheI, NdeI, PaeI, SacI, SalI, ScaI, SspI and XbaI), New England Biolabs (BamHI, KpnI, PstI, XmnI; Beverly, MA) and Promega (HindIII, NarI; Madison, WI).

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DNA linearization

Plasmid DNA was linearized with an excess of appropriate restriction endonuclease (SspI, Eco88I or XmnI) and purified by phenol plus phenol/chloroform extraction to remove all the proteins, especially the used restriction endonuclease. DNA was ethanol precipitated, washed twice with 80% EtOH and dissolved in 1 mM EDTA (pH 7.2) as a stock solution.

UV light irradiation

DNA samples (0.2–0.25 μg in a final volume of 20 μl) in 0.1 mM EDTA plus a necessary concentration of EtOH [usually 50–60–65–70–75–80–85% (v/v)] were irradiated with a set of four 15 W germicidal bulbs (predominantly emitting UV light at 254 nm; Philips) at an incident fluence of 15 kJ m^{-2} as determined by the IL254 germicidal photometer (International Light Inc., Newburyport, MA). To prevent volume and EtOH concentration changes during the irradiation, the samples were irradiated in 1.5 ml Eppendorf tubes immersed in a precooled ethanol bath (-20°C), which ensured that the sample temperature did not exceed -12°C at the end of irradiation. The risk of possible precipitation of DNA in tubes with the highest EtOH concentration was minimized by reducing time interval between sample preparation and the start of irradiation to 5 min (necessary to precool the samples).

Restriction endonuclease cleavage

Immediately after the irradiation, the DNA samples were dried out in SpeedVac SC110 (Savant, Holbrook, NY), dissolved in water and complemented with the respective 10 \times concentrated cleavage buffer and the restriction endonuclease (20–30 U per μg of DNA; final volume 10 μl). Cleavage proceeded for 2 h at the optimum temperature (37°C), and digestion was terminated by the addition of EDTA and the electrophoresis loading buffer.

Electrophoresis and densitometry

DNA samples were electrophoresed in 1.0% agarose gels in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.1), stained for 30 min in ethidium bromide (1 $\mu\text{g}/\text{ml}$) and extensively washed in distilled water. The gels were visualized under UV transilluminator (TM36 Model, Ultra-Violet Products Inc., San Gabriel, CA) and photographed with an orange filter. The negatives were quantified by densitometry using Personal Densitometer SI, Model 375A and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

CD spectroscopy

CD spectra were measured using a Jobin-Yvon Mark VI dichrograph in 0.1, 0.5 and 1 cm pathlength Hellma cells, and placed in a thermostatted holder. The precise sample concentration was determined from its absorption at 260 nm measured at low salt and room temperature using the molar extinction coefficient of $6500 \text{ M}^{-1} \text{ cm}^{-1}$. The UV absorption spectra were measured on a UNICAM 5625 UV/VIS spectrometer. Circular dichroism was expressed as the difference in the molar absorption of the left- and right-handed circularly polarized light, $\Delta\epsilon$, in units of $\text{M}^{-1} \text{ cm}^{-1}$. The molarity (M) was related to the nucleoside residues in the DNA samples.

A sample of pUC19 linearized with Eco88I was dissolved in 1.0 mM sodium phosphate buffer + 0.1 mM EDTA, pH 7. The CD spectrum of this DNA (B-form) was measured in 1 mm pathlength cells. Absolute ethanol was added subsequently to get 55% ethanol concentration in the DNA sample. Owing to an increased volume, the sample was transferred to a 5 mm pathlength cell. Then, the concentration of ethanol was increased up to 82% by gradual aliquot additions of 100% ethanol. DNA concentration was similar in both CD and restrictase experiments around the B-A transition midpoint. Usually, the sample in 82% ethanol was transferred into a 1 cm pathlength cell, and 0.27 mM Na phosphate was added stepwise to decrease ethanol concentration below 55% to check reversibility of the B-A transition.

RESULTS

DNA sample preparation

Induction of the B-A transition by ethanol is not trivial in molecules as large as pUC19 DNA (2686 bp). First, the DNA should be free of proteins to be able to undergo the transition. The presence of restriction endonuclease used to linearize the circular pUC19 DNA may inhibit the transition. That is why we carefully deproteinized the linearized pUC19 DNA by phenol extraction before starting the CD spectroscopy measurements or irradiation by UV light.

Second, the ionic strength should be as low as possible because, otherwise, DNA aggregates at the high ethanol concentrations inducing the A-form. The aggregation also hinders the B-A transition, and intermolecular DNA crosslinks are generated in the course of UV irradiation (26). Third, a chelating agent (e.g. EDTA) should be present in the solution of DNA to bind traces of divalent cations that are almost always present in water stored in glass bottles. Even submicromolar concentrations of divalent cations aggregate DNA at high ethanol concentrations needed to induce the B-A transition. The risk of DNA aggregation was further diminished by using the concentration of DNA as low as possible (10–15 $\mu\text{g}/\text{ml}$ with UV light irradiation and 30–10 $\mu\text{g}/\text{ml}$ with CD spectroscopy).

CD spectroscopy of the B-A transition

For a number of reasons, CD spectroscopy is the most frequently used method to follow the B-A transition in DNA. It followed from our previous experience (27,4), literature data (7) as well as our preliminary experiments with linearized pUC19 DNA that the CD spectra showed no signs of the B-A transition nor any other substantial changes up to 55% ethanol (Figure 1). That is why our advanced experiments were focused on the concentration range between 55 and 85% aqueous ethanol where DNA underwent the B-A transition. The transition was found to be essentially complete at 75% ethanol with our samples of the linearized pUC19 DNA (Figure 1). CD spectrum of the A-form was dominated by a strong positive band close to 270 nm. The B-A transition was highly cooperative and the cooperativity was best reflected by the amplitude of the dominant positive band at 270 nm (7). The negative band at 247.5 nm also reflected the transition and its cooperativity (Figure 1). Midpoint of the transition was at 69% ethanol concentration and its width was about 5.8%. The transition started at $\sim 65\%$ ethanol concentration.

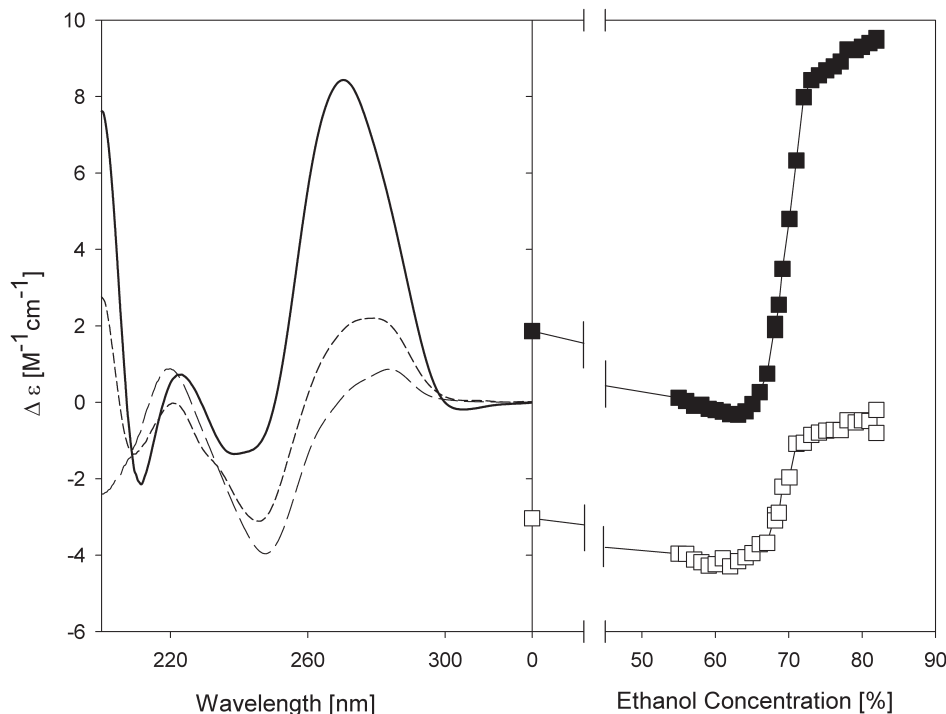


Figure 1. Left: CD spectra of the Eco88I-linearized pUC19 DNA in the B-form in the absence of ethanol (short dashed line), in the presence of 55% ethanol (long dashed line) and the A-form in the presence of 73% ethanol (full line). Right: dependences on the ethanol concentration of the ellipticity at 270 nm (closed squares) and 247.5 nm (open squares).

UV irradiation and changes in fraction of restrictase-resistant DNA during the B-A transition

Samples of linear pUC19 DNA, which were checked by CD spectroscopy to undergo the B-A transition, were irradiated by a dose of 15 kJ m^{-2} known from our previous studies (28–30) to cause an optimum DNA damage. The irradiations were carried out at stepwise ethanol concentrations within 50–85% and the irradiated DNA was cleaved with 17 detection restriction endonucleases. The restriction endonucleases fail to cleave UV-damaged DNA (31). Each experiment was repeated 3–5 times to ensure reproducibility and to find the experimental error of these experiments. We were especially careful regarding reproducibility of the data obtained at ethanol concentrations exceeding 70% where the risk of DNA aggregation was higher than at lower ethanol concentrations.

The dependences of the amount of restriction endonuclease-resistant DNA on ethanol concentration generally showed (Figure 2) abrupt changes within 65 and 75% concentration range of ethanol, which corresponds to the B-A transition range monitored by CD spectroscopy (Figure 1). It is known (24) that the A-form is significantly more resistant to the UV light-induced damage than the B-form. The diminished DNA damage causes the observed decrease of restriction endonuclease-resistant DNA fraction in the course of the B-A transition. The decrease was observed with 16 of 17 analyzed restriction endonucleases. An increase was detected only with SspI, which we attribute to the nucleotide composition of its recognition sequence and surrounding regions that resemble poly(dA).poly(dT) known to be unable to undergo the B-A transition (32). This (A+T)-rich part of the pUC19 DNA seems to isomerize into a B'-form rather than to the A-form due to

the presence of ethanol, and the B'-form is more sensitive to UV light than the canonical B-form (24).

Quantitative differences between the fractions of restriction endonuclease-resistant DNA irradiated in the B- and in the A-form vary within wide limits (Table 1). The Eco88I and KpnI target sites are cleaved completely after irradiation in the A-form by 15 kJ m^{-2} though the same dose of UV irradiation caused a non-negligible damage blocking the restriction endonuclease digestion if the DNA was irradiated in the B-form (Table 1). The effect of UV damage depends on DNA primary structure and conformation (31). However, the changes in restriction cleavage reflect the local conformational transition that can be characterized on the basis of the curves shown in Figures 2 and 3.

The B-A transition curves determined by our 'irradiate-and-cut' method were characterized by the ethanol concentration where the fraction of restriction endonuclease-resistant DNA started to decrease, and by the B-A transition midpoint (Table 1; all but the SspI restriction endonuclease). The former values fell within 53 and 74% ethanol concentration and the latter within 67 and 77%. For some restriction endonuclease target sequences in the linear pUC19 DNA, the whole B-A transition was completed within a 5% ethanol concentration range (EcoRI, XmnI, SacI, BamHI, PstI and PaeI), while other sequences (HindIII, NarI, Eco88I, Ecl136II, NdeI and SalI) showed a less cooperative B-A transition (within 10% ethanol concentration range). Transition of the remaining sequences (ScaI, XbaI, KpnI and EheI) fell within 15%. In most cases, the curves had the sigmoidal shapes characteristic for the cooperative B-A transition.

A detailed inspection of the transition midpoints revealed that they differed by <1% EtOH between neighboring four

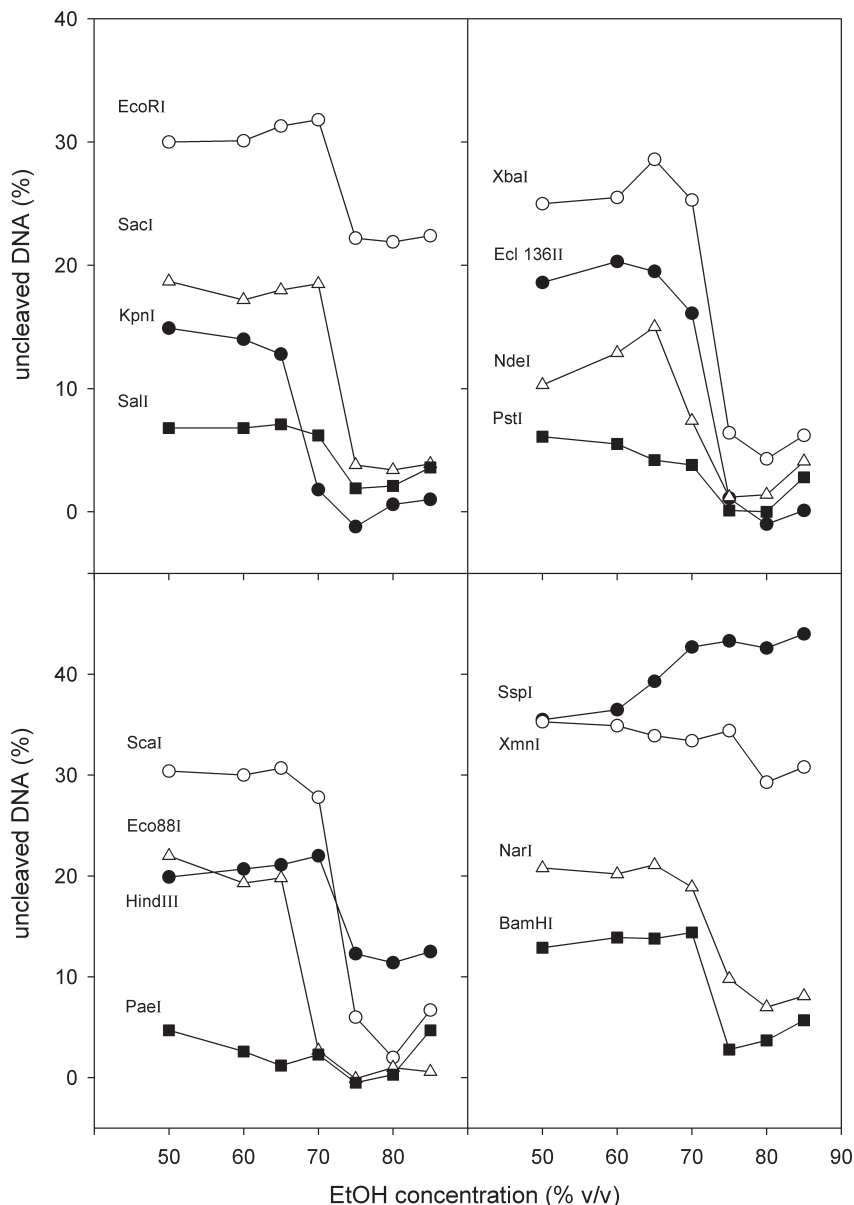


Figure 2. Dependences of the percentage of UV-irradiated DNA resistant to the indicated restrictase cleavage on the concentration of ethanol in the presence of which the DNA was UV irradiated. The solution conditions were the same as in the course of the CD spectroscopic measurements.

restriction endonuclease target sites at the 3' end of the pUC19 polylinker, but there was a sharp decrease of about 3.5% between the midpoints of neighboring BamHI and Eco88I sites (Table 2). The midpoint of the B-A transition of the next 5'-site (KpnI) significantly increased, which continued through the SacI to EcoRI site. The midpoint ethanol concentration values correlated with the (G+C) content of the target sites (see below).

Effect of cesium cations on the B-A transition

It is known from previous studies that cesium cations shift the B-A transition to higher ethanol concentrations (7). Figure 3 documents the effect of cesium cations on the B-A transition detected by the ScaI, SacI and XbaI restriction endonucleases. Indeed, in line with CD studies, we observed a clear cesium

cation-induced shift to higher ethanol concentrations in all three cases. The B-A transition started at much lower ethanol concentrations in the absence of cesium cations than at the 'low' (0.09 mM) and 'high' (0.3 mM) cesium chloride concentrations. The ethanol-induced changes, observed with the SspI reflecting the B-B' transition, are shown (Figure 3) in the absence and presence of the cesium cations for a comparison. Even in this case, the transition is shifted to higher ethanol concentrations in the presence of cesium ions.

Dependence of the B-A transition midpoint on the (G+C) content

It is known (24) that the B-A transition midpoint shifts to lower ethanol concentrations with the increasing (G+C) content. We tried to find out whether our results reflect any correlation

Table 1. Parameters of structural B-A transition as detected by restriction cleavage resistance upon UV irradiation of pUC19 plasmid

Restriction endonuclease (recognition site)	Uncleaved DNA ^a		Relative decrease of uncleaved DNA fraction after B-A transition (%) ^c	Structural B-A transition (%EtOH) ^a	
	in B-form (%) ^b	in A-form (%) ^b		Beginning	Midpoint ^d
SspI (AAT*ATT)	36.0 ± 1.9	41.7 ± 1.9 ^c	-16.2 ± 4.7 ^c	60.8 ± 1.4 ^e	64.4 ± 0.6 ^e
EcoRI (G*AATTC)	33.4 ± 2.9	25.0 ± 3.0	27.1 ± 4.6	70.0 ± 0.0	73.7 ± 1.3
XmnI (GAAAA*CGTTC)	32.1 ± 2.3	25.7 ± 3.0	20.1 ± 3.7	73.8 ± 2.5	77.1 ± 0.7
ScaI (AGT*ACT)	31.1 ± 0.4	2.7 ± 1.1	91.4 ± 3.6	66.7 ± 2.9	72.2 ± 0.6
XbaI (T*CTAGA)	25.9 ± 0.8	4.7 ± 0.6	82.0 ± 1.6	70.0 ± 0.0	72.8 ± 0.4
SacI (GAGCT*C)	22.3 ± 4.8	3.8 ± 0.1	83.3 ± 2.9	65.0 ± 2.9	70.8 ± 1.9
HindIII (A*AGCTT)	22.0 ± 0.1	12.9 ± 2.1	41.5 ± 9.5	70.0 ± 0.0	72.8 ± 0.4
NarI (GG*CGCC)	21.8 ± 0.6	8.2 ± 1.2	62.7 ± 3.8	68.3 ± 2.9	72.5 ± 0.2
Eco88I (C*CCGGG)	19.2 ± 3.0	0.4 ± 0.4	98.2 ± 4.6	53.3 ± 5.8	67.0 ± 2.2
Ecl136II (GAG*CTC)	19.1 ± 0.4	1.8 ± 3.5	95.2 ± 12.8	61.7 ± 2.9	71.3 ± 1.6
NdeI (CA*TATG)	13.8 ± 1.9	1.5 ± 0.7	89.0 ± 3.9	61.2 ± 2.5	68.1 ± 0.9
BamHI (G*GATCC)	13.8 ± 1.1	1.9 ± 1.2	86.5 ± 8.0	65.0 ± 5.0	70.6 ± 2.1
KpnI (GGTAC*C)	13.2 ± 1.8	-1.0 ± 0.9	107.2 ± 6.3	61.0 ± 2.2	68.6 ± 1.4
EheI (GGC*GCC)	6.6 ± 0.1	0.6 ± 0.2	94.5 ± 11.8	61.7 ± 2.9	71.2 ± 1.1
SalI (G*TCGAC)	6.2 ± 1.2	1.0 ± 0.7	82.4 ± 9.2	66.2 ± 2.5	71.2 ± 1.6
PstI (CTGCA*G)	4.5 ± 1.7	0.5 ± 1.8	88.3 ± 22.4	66.7 ± 5.0	72.1 ± 0.5
PaeI (GCATG*C)	4.3 ± 0.5	0.5 ± 1.1	92.2 ± 4.6	70.0 ± 0.0	72.5 ± 0.0

*Site of restriction endonuclease cleavage.

^aAverage ± SD of 3–5 independent experiments, each performed at ethanol concentrations within 50 and 85%.

^b0% stands for cleavage of control, non-irradiated samples.

^cDifference between uncleaved DNA in B- and A-form, divided by B-form uncleaved DNA value.

^dB-A transition midpoint values expressed as concentration of EtOH necessary to be present at UV irradiation, when one half of DNA is present in A-form, as detected with the respective restriction endonucleases' cleavage inhibition.

^eSupposed B'-form and the B-B' transition.

Samples of linearized DNA were irradiated with 15 kJ m⁻² dose of UV light in the presence of EtOH and consequently cleaved with the respective single-cut restriction endonucleases.

in this respect. We plotted the midpoints obtained for 16 restriction endonucleases (i.e. without SspI) as a function of the (G+C) content of the restriction endonuclease target site, the site +1 upstream nucleotide preceding the site, +2 upstream nucleotides, +3, etc., up to +20 upstream nucleotides. The same was carried out for both downstream nucleotides and for the upstream and downstream regions of the same length. An example of the dependences is shown in Figure 4 demonstrating that an increasing (G+C) content in the recognition site plus 4 nt on both sides leads to a transition midpoint decrease [about 6.5% EtOH between 40 and 80% (G+C), i. e. within the experimental range]. Results for other possibilities of the neighborhood length demonstrate that the midpoints generally decrease with the increasing (G+C) content irrespective of how wide neighborhood is taken into consideration. The dependence is steepest if 6 nt neighborhood is taken into account on both sides of the recognition sequence (data not shown). Nevertheless, the correlation coefficient is the highest with the 4 nt neighborhood (0.72). This corresponds well with the cooperative length of the B-A transition determined by CD spectroscopy [12–18 bp (33)].

DISCUSSION

We believe that DNA conformation *in vivo* is dynamic rather than static, involving conformational isomerizations among various conformers. The absolute conformation is difficult to assess but conformational isomerizations can be detected even *in vivo* using agents causing conformation-dependent changes in DNA covalent structure that can be determined

upon DNA isolation. In this respect, the most useful agent is probably the UV light because it damages DNA in a conformation-dependent way, penetrates various media including cells and can easily be detected (24,31,34–39). Here, we have introduced a relatively simple method that can be used to map the B-A conformational transition along plasmid DNA.

A-form is a constitutive conformation of double-stranded regions in RNA. This A-form is stabilized by ribose whose preferred puckering is C3'-endo occurring in the A-form (2). DNA contains deoxyribose instead of ribose, and deoxyribose is more stable in C2'-endo puckering in the aqueous solution to promote the B-form (40). However, DNA can undergo the B-A transition (6,7,32) that is induced by a number of agents, including spermine (16) and other multivalent cations (17,18), methanol (8) and, most importantly, proteins such as the catabolite activator protein (12) and numerous polymerases (9,10,13,41). The most common inducer of the A-form is ethanol (6,7,42) that was used to induce the B-A transition in the present study.

The occurrence in RNA and induction in DNA by proteins make the A-form a biologically relevant conformation. We are therefore interested in regions within human genomic molecules of DNA where the A-form or the B-A transition plays a physiological role. Besides studies in oriented fibers, long molecules of DNA have so far only been studied by circular dichroism spectroscopy (6,7), infrared, Raman and NMR spectroscopy in solution (21,23,27,31,43). These methods are, however, unsuitable to B-A transition mapping along kilobase or even megabase molecules of genomic DNA because CD spectroscopy is an integral method providing the signal from the whole molecule of DNA. The other spectroscopies provide

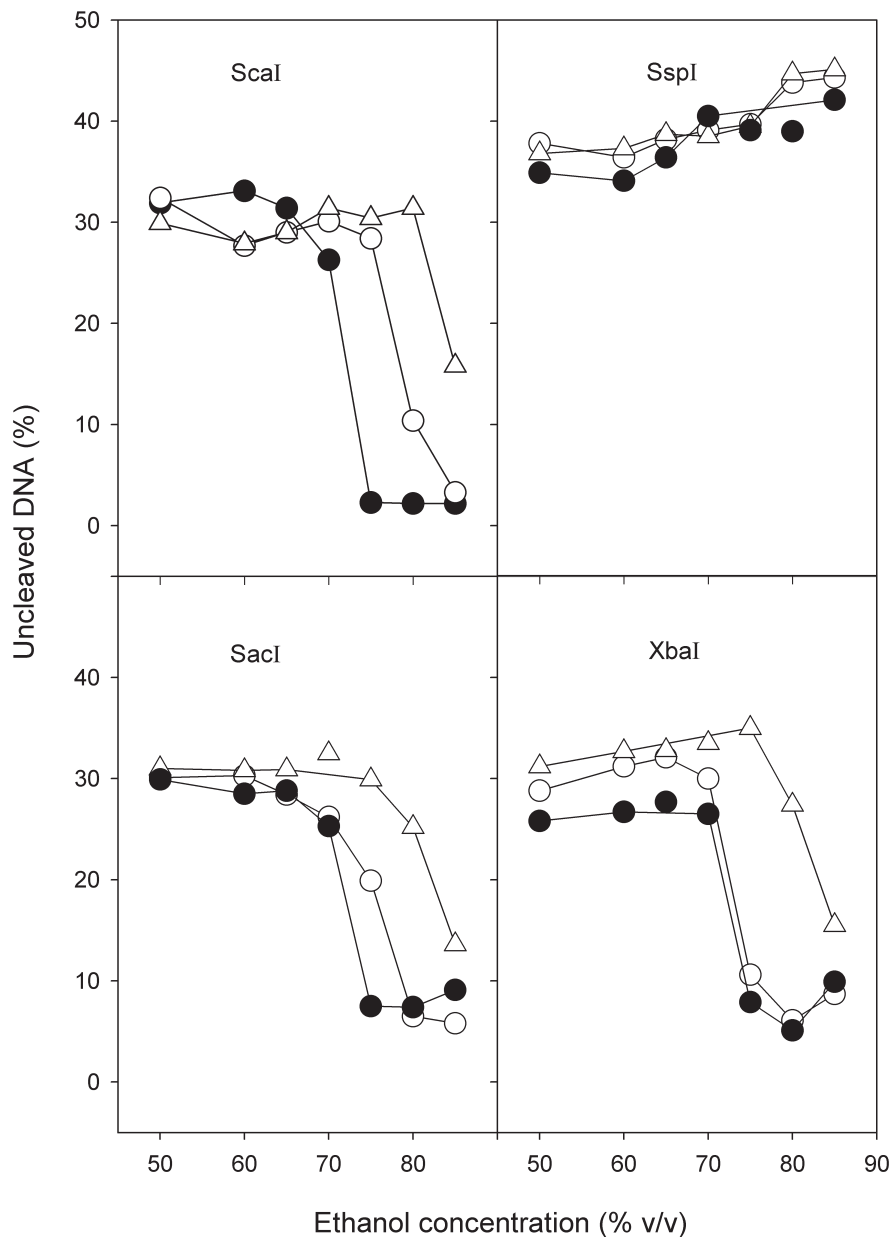


Figure 3. Dependences of the percentage of UV-irradiated DNA resistant to ScaI, SspI, SacI and XbaI restriction endonuclease cleavage on the concentration of ethanol in the presence of no (closed circles), 0.09 mM (open circles) and 0.3 mM (open triangles) CsCl.

Table 2. B-A transition midpoint values of restriction sites of pUC19 polylinker, expressed as EtOH concentrations (% v/v), which correspond to transition of one half of DNA to the A-form, as detected via the respective restriction endonuclease cleavage inhibition of pUC19 plasmid, UV irradiated in the presence of ethanol

EcoRI	SacI (Ecl136II)	KpnI	Eco88I	BamHI	XbaI	SalI	PstI	PaeI	HindIII
73.7	70.8 (71.3)	68.6	67.0	70.6	72.8	71.2	72.1	72.5	72.8

Ecl136II is a neoschizomer of SacI; Eco88I and PaeI are isoschizomers of AvaI and SphI, respectively.

signals originating from the atomic nuclei or molecular bond vibrations that are too many in the kilobase fragments of DNA to be resolved.

The B-A transition was mapped along the 5S ribosomal RNA gene by a UV footprinting technique (24) whose

advantage is a single nucleotide resolution of the acquired data, but this technique is limited to short genomic fragments that can be analyzed on sequencing gels. Here, we propose another UV footprinting method that is based on sensitivity of restriction endonucleases to damage caused in DNA by UV

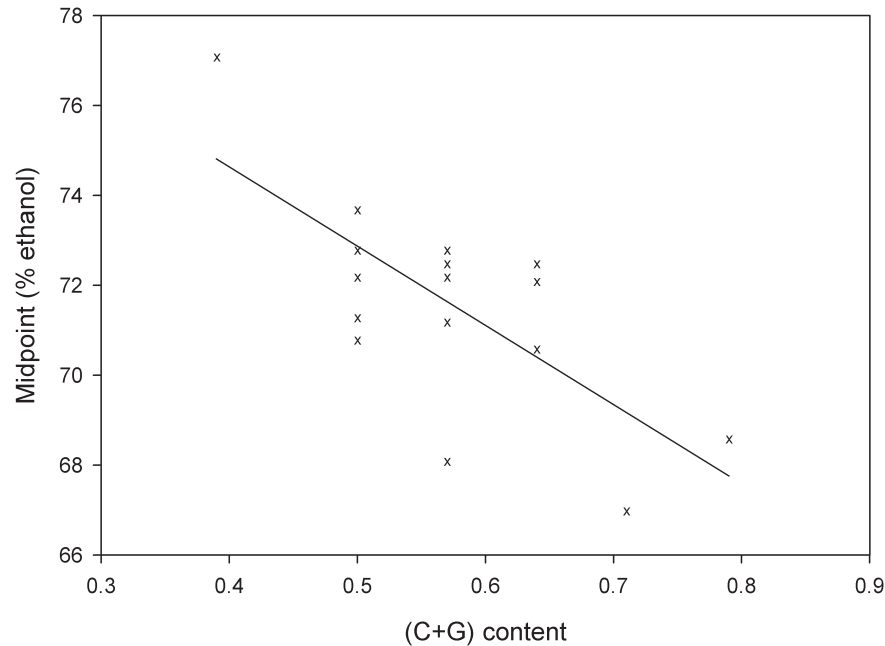


Figure 4. Dependences of the B-A transition midpoints (as determined with 16 restriction endonuclease target sites in the linearized pUC19 DNA) on the (G+C) content of these target sites plus their neighborhood (4 bp on both sides).

light irradiation. Our method does not provide data at nucleotide resolution, but it can be used to analyze genomic fragments as long as 50 kb that can routinely be separated on agarose gels. The restriction endonuclease and sequencing methods can be combined (K. Nejedlý, unpublished data) to provide the B-A transition maps on both global and local scales along the genomic DNA.

The results reported above suggest that the present method can be used to study the B-A transition in PCR fragments of the human genome *in vitro*. The situation is complicated by proteins bound to genomic DNA *in vivo* (34–36,44). This will require a use of internal controls, i.e. such restriction endonuclease target sites (e.g. SspI described above) in the genomic DNA whose photoreactivity does not significantly change in the course of the B-A transition.

We do not know whether the present approach will work with DNA conformational transitions other than the B-A one. However, the photochemical studies of triplexes (37,38,45) are promising in this respect because triplex formation is accompanied by changes in UV damage. The B-Z transition is also sensitive to UV light irradiation (46). The transition can be probed by HhaI methylase and restriction endonuclease (47). We will test whether the B-Z transition is also reflected by changes in the UV damage detected by restriction endonucleases.

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