# Osmium Tetroxide Recognizes Structural Distortions at Junctions between Right- and Left-Handed DNA in a Bacterial Cell

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**Abstract.** It was shown for the first time that the structural distortions at the junctions between contiguous right-handed and left-handed Z-DNA segments can be recognized in bacterial cells. *E. coli* containing recombinant plasmid pPK1 (a derivative of pUC19 containing $(dC\text{-}dG)_{13}$ and $(dC\text{-}dG)_{16}$ blocks) were treated with osmium tetroxide, 2.2'-bipyridine (Os,bipy); after this treatment pPK1 DNA was isolated by the boiling method. pPK1 DNA was then cleaved with BglI, and inhibition of BamHI (with its recognition sequence GGATCC lying on the boundary between the $(dC\text{-}dG)_n$ segments and the pUC19 nucleotide sequence) cleavage was tested. Treatment of cells with 2 mmol/l Os,bipy resulted in a strong inhibition of BamHI cleavage at both restriction sites showing a site-specific osmium modification at the B—Z junction. About the same inhibition of BamHI cleavage was observed after treatment of isolated pPK1 DNA with 0.2 mmol/l Os,bipy.

**Key words:** DNA structure in situ — Supercoiled plasmid — Right-handed B and left-handed Z DNA — Structural distortions at the B—Z junction — Chemical probing of DNA structure — Inhibition of restriction cleavage

## Introduction

The original idea of a regular DNA double helix structure irrespective of the nucleotide sequence has in the last decade gradually been replaced (Paleček 1976; Klug 1983; Rich 1983) by the conception of a polymorphic double helix. An important contribution to this turnaround was the discovery of left-handed DNA (Wang et al. 1979). In recent years many works dealing with left-handed Z-DNA have been published (for review see Rich et al. 1984), but the question of its biological function has yet to be resolved. Most publications deal with the structure and properties of left-handed DNA in vitro. Research into left-handed

DNA in vivo or in situ has been confined mainly to the use of immunological methods (Rich et al. 1984).

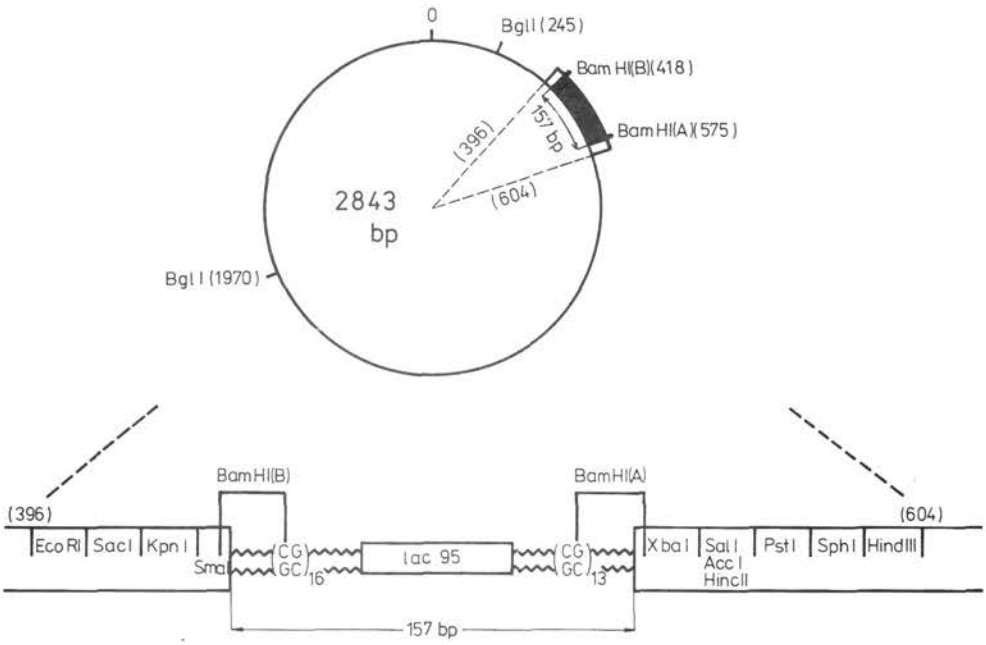
It has recently been shown that suitable tools in research into left-handed DNA in superhelical plasmids are osmium tetroxide (Nejedlý et al. 1985; Johnston and Rich 1985; Galazka et al. 1986, 1987; Paleček 1986; Paleček et al. 1987), diethyl pyrocarbonate (Herr 1985; Johnston and Rich 1985; Runkel and Nordheim 1986; Galazka et al. 1987) and other chemical probes (Johnston and Rich 1985; Barton 1986; Paleček 1986; Paleček et al. 1987). Osmium tetroxide modifies site-specifically structural distortions in the junction region between right-handed B and left-handed Z-DNA in the supercoiled plasmids. This site-specific modification can be detected in various ways (Paleček 1986), including the use of nucleotide sequencing techniques which make it possible to determine the osmium binding sites at the level of nucleotide resolution. Of the chemical probes so far used for the detection of structural distortions in the B—Z junction region osmium tetroxide is used at the lowest concentration (Paleček et al. 1987), and thus seems to be a suitable candidate for work in situ or in vivo.

Haniford and Pulleyblank (1983) showed on the basis of an analysis of topoisomer distribution that $(dC\text{-}dG)_n$ segments within an E. coli plasmid may adopt a Z conformation in vivo under conditions of blocked protein synthesis. We tried in this work to find out whether under these conditions structural distortions at the B—Z junction can be detected by means of osmium tetroxide directly in the bacterial cell. Our results show that osmium tetroxide in combination with 2,2-bipyridine (Os,bipy) is suitable for this purpose.

## Materials and Methods

*Construction of pPK1 plasmid.* pPK1 was constructed by cloning the 157 bp insert (Klysik et al. 1982) into the BamHI site in the polylinker of pUC19; this was done in such a way that the BamHI recognition sequence GGATCC was regenerated at both ends of the insert similarly as in pRW751 DNA from which the insert was cut out. Thus the BamHI recognition sites in pPK1 lie on the boundary between the $(dC\text{-}dG)_n$ blocks and the pUC19 sequences (the first guanine of the recognition sequence being the last quanine of the $(dC\text{-}dG)_{13}$ block (Fig. 1*a*) and the first cytosine of the $(dC\text{-}dG)_{16}$ being the last cytosine of the recognition sequence; the nucleotide sequence was determined by Maxam-Gilbert technique (R. Matyášek, K. Nejedlý and E. Paleček, unpublished). The orientation of the insert in the pPK1 plasmid is the same as that in pRW751 plasmid (Klysik et al. 1982). More details concerning pPK1 plasmid will be published elsewhere.

*Cultivation of bacteria and amplification of plasmid DNA.* The stock culture of the *E. coli* JRS 856 strain carrying the recombinant plasmid pPK1 was kept on a solid nutrient substrate containing ampicillin (50 μg/ml). The inoculum was cultivated without aeration for 20 h at 37 °C in Luria-Bertani medium (Maniatis et al. 1982) with the addition of ampicillin (100 μg/ml). The actual cultivation took place with intensive aeration in M9CA medium (Maniatis et al. 1982) with an increased content of casamino acids (5 g/l) and NaCl (5 g/l) and the addition of thiamine (10 mg/l)




| DNA treated with | BamHI | Os BamHI | Os BamHI | BglI | BglI / BamHI | Os BglI / BamHI | Os BglI / BamHI | Os BglI / BamHI |
|---|---|---|---|---|---|---|---|---|
| bp | | | | | | | | |
| LIN 2843 / 2686 | — | — | | | | | | |
| SC (2843) | | | — | | | | | |
| 1725 | | | | — | | | | — |
| 1552 | | | | | | | — | |
| 1395 | | | | | — | — | | |
| 1118 | | | | — | — | — | — | — |
| BamHI cleavage inhibition in site | – | A or B | A plus B | | – | B[a] | A | A plus B |
| fragments shorter than 1000 bp[b] | 157 bp | – | – | – | 157 bp 173 bp | 330 bp | 173 bp | – |

**Fig. 1.** *a*) Map of the plasmid pPK1 (a recombinant 2843 bp plasmid derived from pUC19 that contains a 157-bp insert at the BamHI site) showing the relative positions of cleavage sites of restriction endonucleases used in this study, and detailed diagram of the polylinker with incorporated 157-bp insert. A and B denote the BamHI sites, ■ designates the region of the polylinker and the insert, including the $(dC\text{-}dG)_n$ segments. *b*) Schematic presentation of the results of agarose gel electrophoresis of DNA fragments obtained after digestion of untreated or osmium-modified (Os) pPK1 DNA samples with restriction endonucleases (BglI and/or BamHI). The results indicate site-specific modification of the B—Z junction in sites A and/or B (Fig. 1*a*). [a] inhibition in site B cannot be recognized on the ground of agarose gel electrophoresis; [b] these short fragments do not appear at the agarose gel electrophoresis shown in this paper.

and uridine (10 mg/l). Following the growth of the culture into the start of the exponential phase ($OD_{550} = 0.8$, or around 0.3 mg dry weight of cells per ml) chloramphenicol (150 mg/l) was added to the medium and the culture was further aerated at 37 °C for 16 h. The cells were separated from the medium by centrifugation and rinsed with 0.1 mmol/l potassium phosphate (pH 7.4).

*Modification of the plasmid in bacterial cells.* Cells were resuspended in 0.1 mol/l potassium phosphate (pH 7.4) and incubated in medium containing 0.5 mol/l potassium phosphate (pH 7.4), osmium tetroxide and 2,2-bipyridine (Os,bipy). Unless stated otherwise, modification was performed in the presence of 2 mmol/l Os,bipy for 60 min at temperature of 37 °C, the concentration of cells in the incubation medium corresponding to ca. 2 mg dry weight of cells per ml medium. The reaction was halted by dilution of the medium with a tenfold volume of chilled phosphate buffer; the cells were centrifuged, rinsed in 10 ml of the same buffer and stored at −20 °C until the next day at the longest, when the plasmid was isolated from them.

*Plasmid isolation.* The plasmid was isolated from cells by the boiling method (Holmes and Quigley 1981) supplemented by double deproteination of the isolated material by means of chloroform. RNA was removed only from material used for modification in vitro (for the procedure and conditions of this see Paleček et al. 1987; Nejedlý et al. 1985; Galazka et al. 1986).

*Mapping of osmium binding sites.* Modified DNA was cleaved with excess (at least 20 U/μg DNA) restriction endonuclease (180 min at 37 °C) (Institute of Sera and Vaccines, Prague), the fragments were subjected to electrophoresis in agarose (1.2%) gel in a 40 mmol/l Tris-acetate (pH 8.0) and 2 mmol/l EDTA medium at laboratory temperature for 16 h with a potential drop of 2 V/cm. The gel was stained with ethidiumbromide and samples visualized in UV radiation.

## Results

We have recently shown (Nejedlý et al. 1985; Paleček et al. 1987) that in superhelical plasmid pRW751 containing $(dC\text{-}dG)_{13}$ and $(dC\text{-}dG)_{16}$ segments osmium tetroxide site-specifically modifies all the four B—Z junctions (cf. Fig. 1*a*). Site-specific modification of two B—Z junctions was detected on the basis of inhibition of BamHI cleavage (in pRW751 the BamHI recognition sequence GGATCC lies on the border between the $(dC\text{-}dG)_n$ segments and the pBR322 sequences; Fig. 1*a*). The method of detection of site-specific modification on the basis of the inhibition of restriction cleavage is very simple and sensitive; we therefore considered it a suitable method for the detection of site-specific modification of the B—Z junction in the *E. coli* cells.

The pPK1 plasmid used in this work contains similar insert as does pRW751, but the former is more suitable for studying specific modifications of the B—Z junctions since it offers the possibility of observing the inhibition of several restriction endonucleases in the polylinker which has recognition sequences at various distances from the ends of the $(dC\text{-}dG)_n$ segments (see Fig. 1*a*). In this work, however, we confined ourselves to study of the inhibition of BamHI cleavage whose recognition sequence GGATCC is very close to both ends of the insert (Fig. 1*a*). In Fig. 1*b* schematic presentation of the results of the gel electrophoresis is given. In most experiments osmium-modified DNA was first cleaved by BglI and then tested for inhibition of BamHI cleavage.

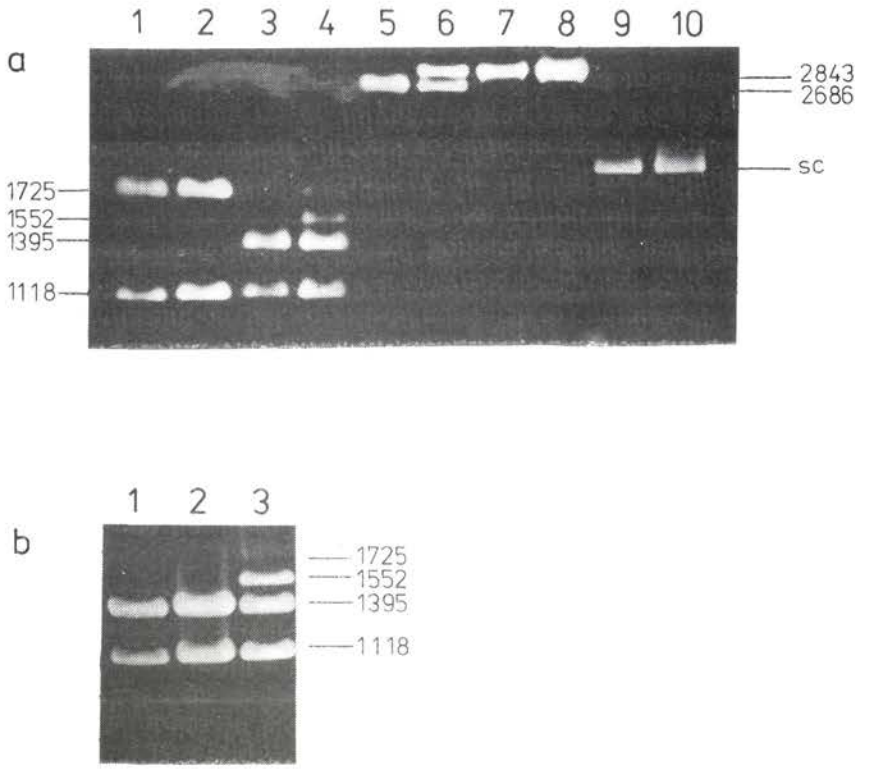



**Fig. 2.** *a*) Cleavage of the pPK1 plasmid by various restrictases. The isolated plasmid was modified in vitro (1 mmol/l Os,bipy, 60 min, 37 °C). The modified sample (lanes 2, 4, 6, 8) and unmodified DNA (lanes 1, 3, 5, 7) were cleaved with restriction endonucleases BglI (lanes 1, 2) BglI followed by BamHI (lanes 3, 4), BamHI (lanes 5, 6) and EcoRI (lanes 7, 8). The last runs contain uncleaved unmodified (lane 9) and modified (lane 10) supercoiled DNAs. The lengths of various fragments are given in bp, sc denotes uncleaved supercoiled DNA. *b*) BamHI cleavage of pPK1 DNA modified in linear or supercoiled form in vitro. Supercoiled DNA (lane 3) and DNA cleaved with BglI (lane 2) were modified (2 mmol/l Os,bipy, 60 min, 37 °C) and cleaved with BglI followed by BamHI, respectively. Lane 1 contains unmodified superhelical DNA cleaved with BglI followed by BamHI.

### *Site-specific modification of the pPK1 DNA B—Z junctions in vitro*

First, we tried to find out whether pPK1 DNA would be modified in vitro at "native" superhelical density. We used 2,2-bipyridine as an $OsO_4$ ligand; it can be applied at concentrations equimolar to $OsO_4$ (Paleček et al. 1987), unlike pyridine used previously, which had to be applied at substantially higher concentrations (1% or more) (Lukášová et al. 1984; Glikin et al. 1984). We considered lower concentration of the ligand to be advantageous for the modification of plasmid in the bacterial cell. We isolated the plasmid from osmium-

untreated cells by the same method as was intended to be used for plasmid isolation from Os,bipy treated cells.

Supercoiled pPK1 DNA was modified in 200 mmol/l NaCl, 25 mmol/l Tris-HCl (pH 7.8), 2.5 mmol/l EDTA, i.e. under conditions close to those used previously for the modification of pRW751 (Nejedlý et al. 1985; Paleček et al. 1987) and pRW777 DNAs (Galazka et al. 1986). Osmium-treated pPK1 was purified by ethanol precipitation and extraction of the pellet with ether. The purified DNA was treated with restriction endonuclease BglI or EcoRI and no sign of inhibition of its cleavage with these nucleases was observed (Fig. 2*a*, lanes 2 and 8). On the other hand the restriction cleavage with BamHI endonuclease was strongly inhibited (Fig. 2*a*, lane 6). If the digestion of osmium-treated pPK1 DNA with BglI was followed by BamHI treatment, agarose gel electrophoresis showed, in addition to bands corresponding to 1118 bp and 1395 bp (formed due to complete BamHI digestion, Fig. 1*b*) further bands corresponding to ca. 1550 bp and 1720 bp (Fig. 2*a*, lane 4). The presence of the ca. 1550 bp fragment suggests that BamHI cleavage was inhibited at site A (Fig. 1*b*), while the longer fragment indicates inhibition of BamHI cleavage at both restriction sites.

Inhibition of BamHI was not observed when the DNA of pPK1 was first cleaved with BglI, then modified with Os,bipy, purified and cleaved with BamHI (Fig. 2*b*, lane 2). These results suggest that the "native" superhelical density of the isolated pPK1 plasmid is sufficiently negative to induce left-handed structure in the $(dC\text{-}dG)_n$ segments of the plasmid.

*Dependence on Os,bipy concentration and modification temperature.* At a 37 °C we tested the effect of Os,bipy concentration in a range from 0.1 mmol/l to 2 mmol/l on the inhibition of BamHI cleavage of pPK1 DNA. The first sign of inhibition at site A was observed at 0.2 mmol/l Os,bipy (Fig. 3*a*, lane 3); at 1 mmol/l and 2 mmol/l concentrations (Fig. 3*a*, lanes 5 and 6) in addition to the fragments corresponding to the inhibition of BamHI at site A a fragment appeared corresponding to BamHI cleavage inhibition at site A and B.

DNA was modified with 2 mmol/l Os,bipy for 40 min at the following temperatures: 0, 11, 26, 37 and 42 °C. In the temperature range from 11 to 42 °C major inhibition of BamHI cleavage took place (Fig. 4*a*, lanes 3—6). Modification at 0 °C induced less marked but clear BamHI inhibition (Fig. 4*a*, lane 2).

These results show that site-specific modification of the B—Z junctions in pPK1 DNA occurs over quite a wide range of temperatures and concentrations of Os,bipy.

*Osmium modification of pPK1 DNA in bacterial cells*

*Treatment of bacterial cells with Os,bipy.* After plasmid amplification with chloramphenicol (see Materials and Methods) cells were incubated with

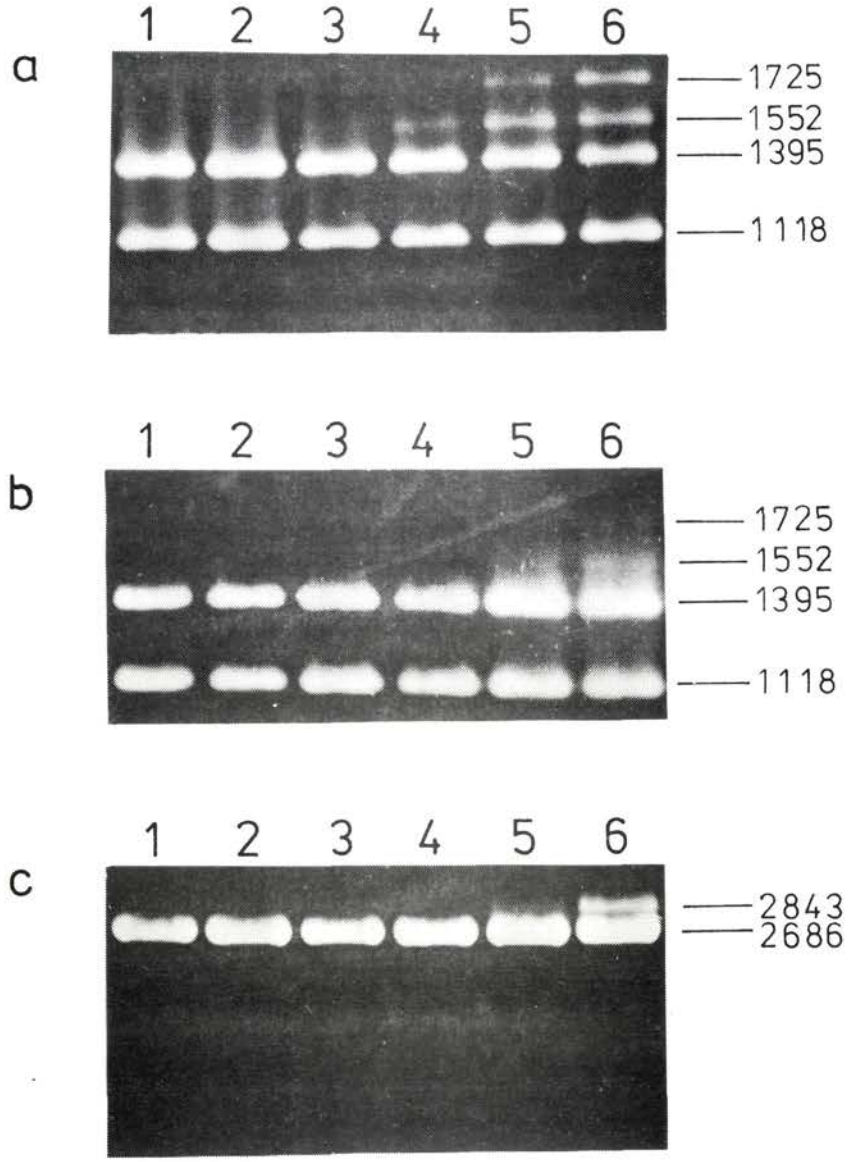



**Fig. 3.** Inhibition of BamHI cleavage of pPK1 DNA modified at various concentrations of Os,bipy. Isolated DNA (*a*) or *E. coli* cells carrying the plasmid pPK1 (*b, c*) were incubated in an environment containing 0.1 (lane 2), 0.2 (lane 3), 0.5 (lane 4), 1 (lane 5) and 2 (lane 6) mmol/l Os,bipy or without Os,bipy (lane 1) for 60 min at 37 °C. pPK1 DNA was isolated from the Os,bipy treated cells in the same way as from untreated cells (see Materials and Methods); isolated DNA samples were first cleaved with restriction endonuclease BglI followed by BamHI (*a, b*) or only with BamHI (*c*)

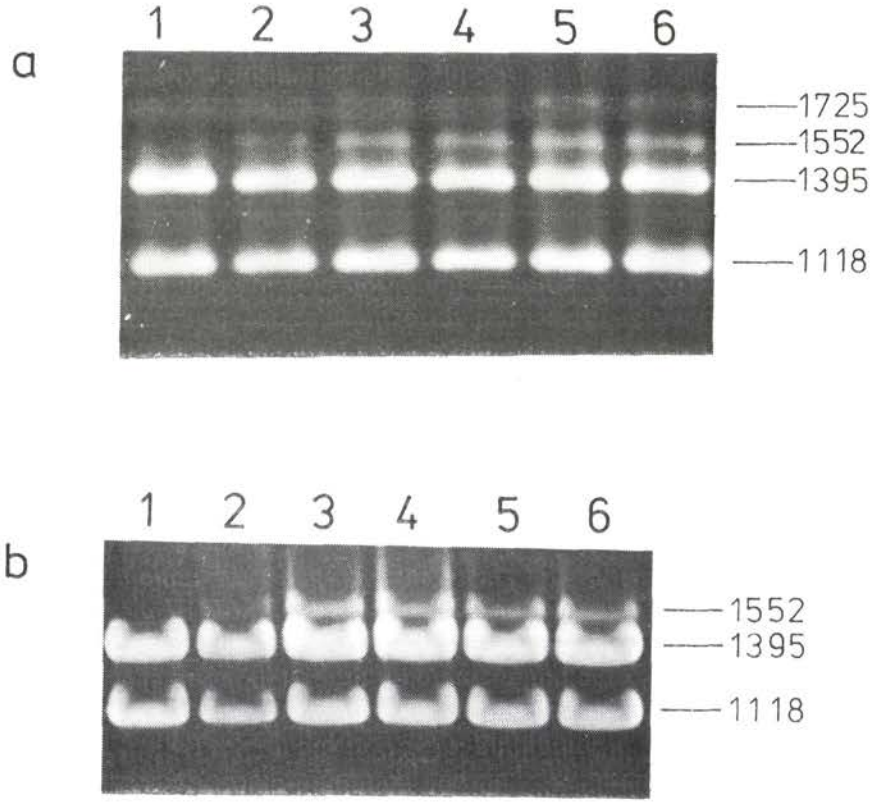



**Fig. 4.** Inhibition of BamHI cleavage of pPK1 DNA modified with Os,bipy at various temperatures. The isolated pPK1 DNA (*a*) or cells carrying the plasmid pPK1 (*b*) were incubated in a medium containing 2 mmol/l Os,bipy for 40 min at 0 (lane 2), 10 (lane 3), 26 (lane 4), 37 (lane 5) or 42 (lane 6) °C. Lane 1 contains the unmodified sample. The plasmid samples were first cleaved with BglI and then with BamHI. Other conditions as in Fig. 3.

2 mmol/l Os,bipy at 26 °C for various periods (0 to 60 min). Following incubations of 5 min or longer cells showed a greater tendency to aggregate; no cell lysis was observed even after 60-min treatment. Observation of cells under a microscope (using phase contrast) revealed integrity of the cells following 60-min treatment with Os,bipy (Fig. 5).

It was not until about 2 hour's incubation that clear cell lysis was observed. Further details of the effect of osmium tetroxide on bacterial cells will be published elsewhere.

*Isolation of chemically modified DNA*. The boiling method (Holmes and Quigley 1981) used for the isolation of pPK1 plasmid includes a short-term (60 s)

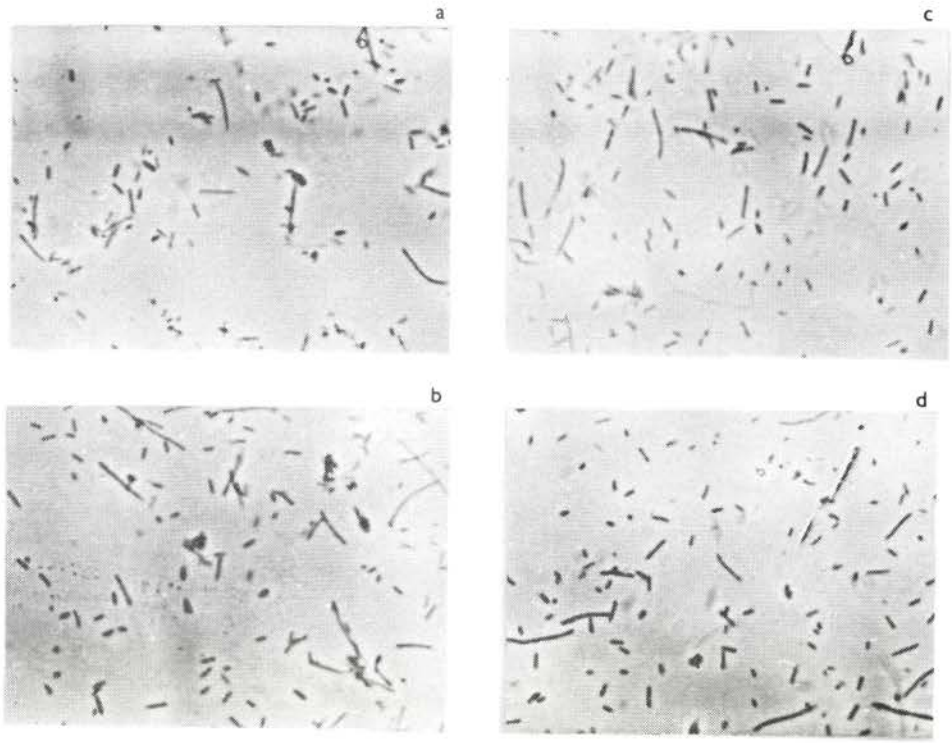



**Fig. 5.** Microscopic observation of *E. coli* cells incubated in the presence of Os,bipy. *a*) untreated cells, *b*) cells incubated in a medium of 2 mmol/l Os,bipy for 5 min, *c*) 10 min, *d*) 60 min. Filaments in *a*) resulted from chloramphenicol treatment (see Materials and Methods).

exposure of the DNA to 100 °C. We first tried to ascertain whether the binding of the Os,bipy complex to DNA is sufficiently stable under these conditions. DNA was modified in vitro with Os,bipy, and the sample was split into two parts, one of them being subjected for 60 s to a temperature of 100 °C. Following cooling DNA was purified and BamHI cleavage was tested by the method as above. Short heating of the sample did not affect BamHI inhibition (not shown). Thus from the point of view of thermal stability of the Os,bipy bond, the method of DNA isolation used can be considered suitable.

We further tried to establish whether some secondary modification of the plasmid can occur during its isolation, since a priori the possibility cannot be ruled out that Os,bipy binds, during incubation, to various cell components and gets subsequently released from them in the course of DNA isolation; the free Os,bipy might become a source of a secondary site-specific modification of the DNA released from cells. Following experiment was made: *E. coli* cells containing no plasmid were incubated under the usual conditions in an Os,bipy

medium, centrifuged and rinsed. They were then mixed with cells containing pPK1 plasmid which had not been exposed to Os,bipy. The plasmid was isolated from the mixture of these cells and tested for BamHI cleavage. No inhibition of BamHI restriction cleavage was observed (Fig. 6, lane 3). A further source of secondary site-specific modification of the B—Z junction in pPK1 might be traces of free Os,bipy not removed by rinsing. This free Os,bipy could in the course of cell lysis during the short exposure to a temperature of 100 °C react

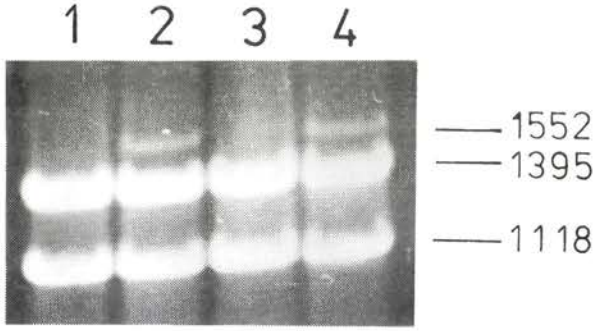



**Fig. 6.** Testing of the occurrence of site-specific modification during isolation of pPK1 DNA. pPK1 DNA was isolated from 1:1 mixtures of *E. coli* cells (dry weight of cells): 1) control cells containing plasmid pPK1 untreated with Os,bipy ($p^+$,un) and cells not containing plasmid ($p^-$,un); 2) $p^+$,un and $p^+$ treated with Os,bipy ($p^+$,Os); 3) $p^-$,Os and $p^+$,un; 4) $p^-$,Os and $p^+$,Os. pPK1 DNA isolated from the cell mixture was cleaved with BglI and than treated with BamHI. Other conditions as in Fig. 3.

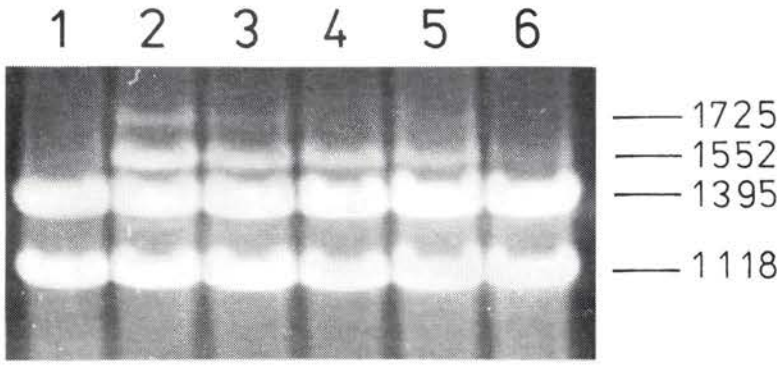



**Fig. 7.** The effect of cell concentration on site-selective modification of pPK1 plasmid in situ. Cells carrying the plasmid pPK1 were incubated in a medium containing 2 mmol/l Os,bipy for 60 min at 37 °C. The medium contained 1.0 (lane 2), 1.8 (lane 3), 2.0 (lane 4), 2.2 (lane 5) or 4.0 (lane 6) mg dry weight of cells per ml. The pPK1 DNA was isolated from 10 mg dry weight of cells and cleaved first with BglI and then with BamHI. Lane 1 contains unmodified DNA cleaved with BglI followed by BamHI. Other conditions as in Fig. 3.

with plasmid released from cells. Heating of isolated pPK1 DNA with 0.01 mmol/l Os,bipy (i.e. the concentration attained following dilution of the incubation medium and the rinsing of cells provided no Os,bipy was absorbed by cells, cf. Materials and Methods) for 60 s at 100 °C combined with longer incubation of the sample at laboratory temperature did not reveal any BamHI cleavage inhibition (not shown). It may thus be concluded that under the given conditions there is no detectable secondary site-specific modification of the BamHI restriction sites of pPK1 DNA.

*Dependence of BamHI cleavage inhibition on Os,bipy and cell concentrations.* Cells carrying the pPK1 plasmid were treated with various concentrations of Os,bipy (0.1 to 2 mmol/l). As in the case of pPK1 modified in vitro (Fig. 2*a*, lane 2) BglI cleavage of pPK1 DNA isolated from these cells was not inhibited as a result of the treatment with Os,bipy (not shown). In contrast to the results of DNA modification in vitro (Fig. 3*a*) no inhibition of BamHI cleavage resulted from cell treatment with low concentrations of Os,bipy (up to 0.5 mmol/l) (Fig. 3*b*, *c*, lanes 2—4). Treatment of cells with 1 mmol/l Os,bipy produced a weak inhibition while 2 mmol/l Os,bipy induced a pronounced inhibition of BamHI cleavage (Fig. 3*b*, *c*, lanes 5, 6). This inhibition was observed no difference whether BamHI was applied directly to supercoiled (Fig. 3*c*) or BglI digested (Fig. 3*b*) DNA.

In the experiments described the cell concentration in the modification medium corresponded to around 2 mg dry weight of cells per ml. In order to find out whether cell concentration affected the plasmid modification we incubated suspensions with various concentrations of cells (1 to 4 mg dry weight of cells per ml) with Os,bipy under the usual conditions. BamHI restriction cleavage was inhibited more strongly, the lower was the cell concentration in the suspension (Fig. 7, lanes 2—6).

*Dependence of BamHI cleavage inhibition on modification time and temperature.* Cells were treated with 2 mmol/l Os,bipy for various periods in the range 0 to 60 min (immediately after placing the cells in the reaction medium, an aliquot of the suspension was removed and diluted with buffer, and the cells representing the 0 time sample were centrifuged and frozen). The cleavability of isolated DNA was tested similarly as in the previous experiment. BamHI inhibition was observed similarly as in the experiment in vitro, already after 5 min of treatment with Os,bipy (Fig. 8*a*, *b*, lanes 2—6).

Cells were incubated at various temperatures (0 to 42 °C) for 40 min. Under these conditions BamHI inhibition was observed in site A (Fig. 1); this inhibition was only slightly dependent on the modification temperature (Fig. 4*b*, lanes 2—6).

Cells carrying the plasmid pUC19, which contains no $(dC\text{-}dG)_n$ segments, were used as controls. Following two hours' incubation of cells in an environ-

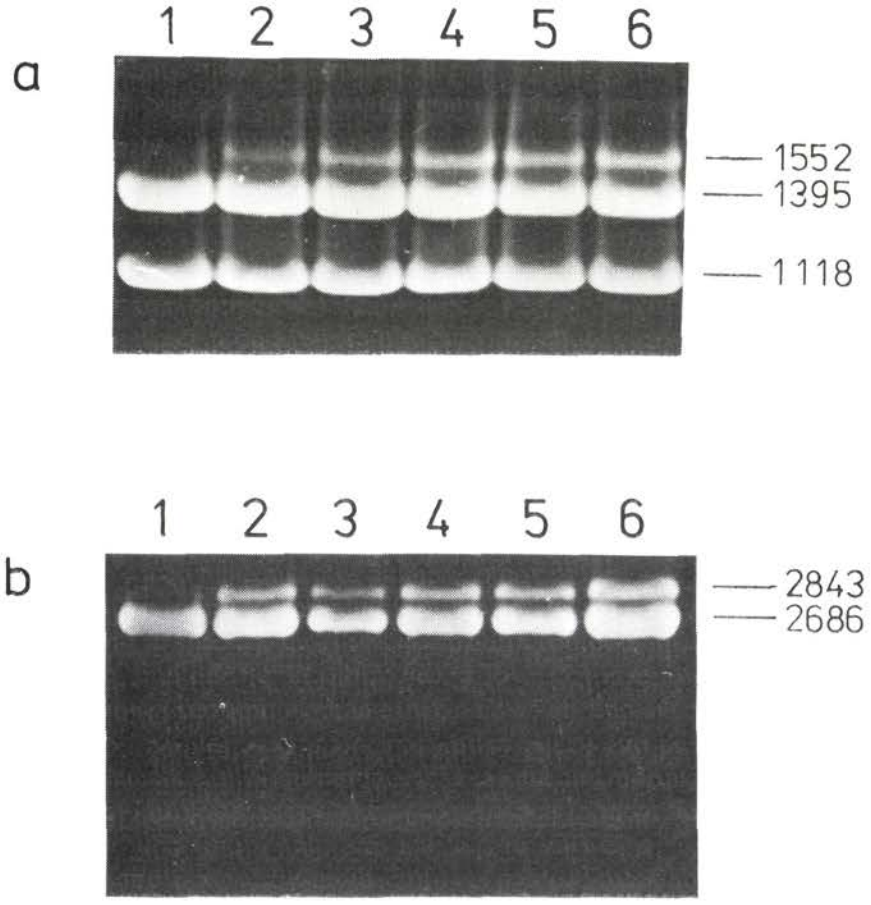



**Fig. 8.** Dependence of inhibition of BamHI cleavage of pPK1 DNA on time of cell treatment with Os,bipy. Cells carrying the pPK1 plasmid were incubated in a medium containing 2 mmol/l Os,bipy at 37 °C for 0 (lane 1), 5 (lane 2), 10 (lane 3), 20 (lane 4), 30 (lane 5) or 60 (lane 6) min. DNA samples were cleaved first with BglI and then with BamHI (*a*) or only with BamHI (*b*). Other conditions as in Fig. 3.

ment containing 2 mmol/l Os,bipy neither BglI nor BamHI restriction cleavage of pUC19 DNA was inhibited (not shown).

## Discussion

Our results show site-specific modification of the B—Z junction to occur in pPK1 after treatment of this DNA with Os,bipy in vitro as well as after

treatment of a bacterial cell suspension. The lowest concentration of Os,bipy required to induce this site-specific modification was 0.2 mmol/l in experiments in vitro and 1 to 2 mmol/l in treating bacterial cells (at the given cell concentration). In bacterial cells Os,bipy may also react with components other than plasmid DNA (e.g. RNA, chromosomal DNA and proteins), so that the higher concentration of Os,bipy found to be required for the treatment of a bacterial suspension (Fig. 3) and the dependence of the degree of modification on the amount of cells (Fig. 7) are not surprising.

Osmium tetroxide is widely used in electron microscopy as a staining and fixative agent for biological tissues. Staining of biological membranes with osmium tetroxide is thought to proceed via attact of unsaturated entities present in the tissues (Korn 1967). In view of the fact that the Os,bipy concentration used in this work to treatment of bacterial cells is substantially lower than that used for staining and fixation, one may expect that the steric arrangement of the cell will not be destroyed due to the relatively short interaction with Os,bipy.

We have shown that under the given experimental conditions Os,bipy does not have effect to disturb the integrity of *E. coli* cells (Fig. 5), and that the pPK1 plasmid is not secondarily modified after its release from the cells in the course of its isolation. On the basis of these results we may conclude that structural distortions in the B—Z junction region detectable by a chemical probe exist not only in supercoiled plasmids in vitro (Nejedlý et al. 1985; Johnston and Rich 1985; Galazka et al. 1986; Paleček et al. 1987), but also in DNA inside a bacterial cell.

Until recently mainly enzyme structural probes were used to study local structural changes in superhelical plasmids, especially nuclease S1 (Singleton et al. 1983; Lilley 1985). Compared to enzymatic probes chemical probes of the DNA structure have many advantages, some of these have been discussed in our previous papers (Nejedlý et al. 1985; Paleček 1986). Another advantage of the chemical probes shown in the present work is the possibility of using them to study DNA structure in situ.

The use of enzyme probes for this purpose still seems very difficult. Apart from problems associated with the size of the enzyme molecule and its penetration in the cell, serious difficulties would probably occur due to the fact that DNA gets cleaved in the cell. Some of these difficulties would also arise with chemical probes which have nuclease activity, such as copper-phenantroline, EDTA Fe (II), etc. (e.g. Barton 1986; Derwan 1986; Sigman 1986). Thus the choice of probes for investigating the DNA structure in situ is very limited. Nevertheless other agents suitable for this purpose can be expected to be found soon. Osmium tetroxide will probably be usable in combination with other ligands differing in size, stereochemistry, electrical charge, etc.; such ligands might affect the structural specificity of the agent. On the basis of a combination

of suitable structural probes valuable information might be obtained on the detailed arrangement of the structurally changed region, especially in cases where chemical modification can be detected by means of nucleotide sequencing techniques (Johnston and Rich 1985; Galazka et al. 1986; McClellan et al. 1986).

Os,bipy is thus the first chemical agent suitable for studies of structural distortions in the B—Z junction region in situ. In view of the fact that osmium tetroxide has proved useful not only to study B—Z junctions (Nejedlý et al. 1985; Galazka et al. 1986; Paleček et al. 1987), but also to study cruciforms (Lilley and Paleček 1984; Greaves et al. 1985; Lilley and Hallam 1984), structural changes in the region of the polypurine/polypyrimidine sequences in supercoiled and linear DNAs (Vojtíšková et al. unpublished) and structures of the $(dA\text{-}dT)_n$ sequences in linear DNA plasmid molecules (McClellan et al. 1986), one can say that it offers broad possibilities for research into various local changes in DNA structure in situ.

Recently it has been found (e.g. Brahms et al. 1985; Rich et al. 1984; Lilley 1985) that there exists a relation between the superhelical density of DNA, local changes in its structure, and its biological function. Certain signs exist (Kmiec and Holloman 1986) for the B—Z junction to play a role in DNA recombination. The new way of studying local structural changes in DNA in bacterial cell opens up new possibilities in research into the relation between DNA structure and function. Very likely, the application of Os,bipy will not be confined to *E. coli* cells, and this DNA structure probe will also be suitable for other prokaryotic and eukaryotic cells.

Recent works (Greaves et al. 1985; Peck and Wang 1985) indicate that the superhelical density of DNA in the cell may be fifty per cent lower (less negative) than usually found in isolated plasmids. The results obtained in this work show that the superhelical density of pPK1 plasmid in *E. coli* cells is negative enough for left-handed DNA to occur, i.e. that it cannot (at least under conditions of blocked protein synthesis) be less negative than about −0.05 (Nejedlý et al. 1985; Paleček et al. 1987).

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Abbreviations: Os,bipy — $OsO_4$, 2,2′-bipyridine; bp — base pairs

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