Probing of B—Z Junctions in Recombinant Plasmids in Vitro and in the Cell with Different Osmium Tetroxide Complexes

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Abstract. Complexes of OsO₄ with 2,2′-bipyridine (Os,2,2′-bipy), 4,4′-bipyridine (Os,4,4′-bipy), 1,10-phenanthroline (Os,phe), bathophenanthroline disulfonic acid (Os,bpds) and OsO₄, pyridine reagent (Os,py) were used to probe structural distortions at the junctions between right-handed B and left-handed Z DNA in supercoiled plasmids pRW751 and pPK1 (both containing (dC—dG)ₘ and (dC—dG)ₖ₆ segments). With all five complexes the site-specific modification at the B—Z junctions was detected in vitro but only Os, 2,2′-bipy and Os,bpds produced strong site specific modification at submillimolar concentrations. In addition to the B—Z junctions, Os,phe also reacted at other sites. With the exception of Os,2,2′-bipy no one of the tested OsO₄ complexes has proved to be suitable for probing structural distortions at the B—Z junctions in E. coli cells.

Key words: B—Z junctions — Supercoiled recombinant plasmids — Osmium tetroxide ligands — DNA structure chemical probing — DNA structure in the cell

Introduction

Osmium tetroxide, pyridine (Os,py) was introduced as a probe of the DNA structure in the beginning of the 80s (Paleček et al. 1981; Lukášová et al. 1982; Paleček and Hung 1983; Glikin et al. 1984; Lukášová et al. 1984). In recent years it has become one of the most frequently used chemical probes of the DNA structure (Glikin et al. 1984; Lilley and Hallam 1984; Lilley and Paleček 1984; Greaves et al. 1985; Johnston and Rich 1985; Lilley et al. 1985; Nejedlý et al. 1985; Vojtíšková et al. 1985; Galazka et al. 1986; McClellan et al. 1986; Galazka et al. 1987; Hanvey et al. 1987; Johnston and Rich 1987; Paleček et al. 1987a,b; Vojtíšková and Paleček 1987; Johnston 1988; Paleček et al. 1988a). It has been applied mainly to studies of local DNA structures in supercoiled plasmids, including cruciforms (Glikin et al. 1984; Lilley and Hallam 1984;

Even more recently we could show (Paleček et al. 1987c, d, 1988b) that osmium tetroxide with 2,2'-bipyridine (Os,2,2'-bipy) can be applied to studies of the DNA structure in bacterial cells. Using this probe we detected structural distortions at the B—Z junction and offered direct evidence of the existence of left-handed DNA in E. coli cells (Paleček et al. 1987c, d, 1988b). In addition we demonstrated the presence of an unusual structure in (dA—dT)_16 segments of a pAT32 plasmid in the cells (Paleček et al. 1988b).

Our results (Paleček et al. 1987a, c; Vojtíšková and Paleček 1987; Paleček et al. 1988b) have thus shown that the possibility of application of osmium tetroxide as a probe of DNA structure can be significantly extended by using a ligand other than pyridine. It is known that a number of different tertiary amines can be used as ligands to obtain osmium (VI) esters (Schröder 1980). We therefore decided to test some of them differing in size, stereochemistry, electrical charge, etc.

In this paper we used, in addition to pyridine (py) and 2,2'-bipyridine (2,2'-bipy), the following osmium tetroxide ligands (Fig. 1c): 4,4'-bipyridine (4,4'-bipy), 1,10-phenanthroline (phe) and bathophenanthroline disulfonic acid (bpds) and tested the ability of the resulting osmium complexes to recognize structural distortions at the B—Z junctions of supercoiled plasmids in vitro and in situ. Our results show that osmium tetroxide complexes with phe and bpds are able to recognize B—Z junctions in vitro. Application of the phe is, however, limited to a narrow range of reaction conditions, as its osmium complex binds under reaction conditions exceeding the defined range. No one of the newly tested osmium complexes was able to recognize the B—Z junction in situ.

Materials and Methods

Plasmid DNA. Plasmids pRW751 (Fig. 1a) and pK1 (Fig. 1b) were isolated from E. coli cells, strain JRS 856, by the method described by Holmes and Quigley (1981); the samples were twice deproteinated using chloroform; the final purification step in the case of pRW751 was centrifugation.

Abbreviations: py — pyridine; 2,2'-bipy — 2,2'-bipyridine; 4,4'-bipy — 4,4'-bipyridine; phe — phenanthroline (1,10-phenanthroline); bpds — bathophenanthroline disulfonic acid, disodium salt; sc-DNA — supercoiled DNA; lin-DNA — linear DNA; rel-DNA — relaxed DNA; EtBr — ethidiumbromide; σ — superhelical density
Probing of DNA Structure with OsO₄ Complexes

Fig. 1. a, b. Map of plasmids (a) pRW751 and (b) pPK1. Plasmids were constructed (Kłóysik et al. 1982; Paleček et al. 1987c) by cloning the 157 bp insert into the BamHI site of pBR322 and pUC19, respectively; this was done in such a way that the BamHI recognition sequence GGATCC was regenerated at both ends of the insert. Thus BamHI recognition sites in pRW751 and pPK1 lie on the boundary between the (dC—dG)ₙ blocks and the pBR322 or pUC19 sequence (the first guanine of the recognition sequence being the last guanine of the (dC—dG)ₙ block and the first cytosine of the (dC—dG)ₙ block being the last cytosine of the recognition sequence). The segments (dC—dG)₁₆ and (dC—dG)₁₃ at the ends of the insert may (given sufficiently negative superhelical density of the plasmids (Singleton et al. 1983; Azorin et al. 1984)) be in the form of left-handed Z-DNA; the plasmids then contain four B—Z junctions, the two “outer” of which reach the area of the restriction sequences B and A of restriction endonuclease BamHI. For more detailed map of 157 bp insert in pRW751 and pPK1 see Klóysik et al. (1982), Nejedlý et al. (1985), Paleček et al. (1987c). c) The Os.py complex with thymine and some ligands (I = 4,4′-bipyridine, II = 2,2′-bipyridine, III = 1,10-phenanthroline, IV = bathophenanthroline disulfonic acid) which can replace pyridine in the osmium complexes. The Os.py and Os,2,2′-bipy complexes react with thymine and cytosine residues in DNA (Chang et al. 1977); in the complexes Os,4,4′-bipy, Os,phe and Os,bpds the specificity of the reaction towards bases is not yet known.
in a CsCl EtBr gradient. The methods of cultivation of E. coli cells and amplification of plasmids were described in our previous work (Paleček et al. 1987c).

Chemicals. OsO₄ was obtained from Fisher Scientific Co., pyridine from Merck, other ligands (2,2'-bipy, 4,4'-bipy, phe, bpds; Fig. 1c) from Laehema (CSSR), all of p.a. purity. Other chemicals were of analytical grade. Restriction endonucleases were obtained from the Institute of Sera and Vaccines (Prague), nuclease S1 was isolated from "Takadiastase" (Vogt 1973).

Modification of plasmid in vitro. The typical reaction mixture contained DNA at a concentration of 40-80 µg/ml in a medium of 25 mmol/l Tris-Cl (pH 7.5), 2.5 mmol/l EDTA, 200 mmol/l NaCl, a maximum of 2 mmol/l OsO₄ plus 2% or 2 mmol/l concentration of other ligand (Fig. 1c); pRW751 was modified for 30 min at 26°C, pPK1 for 20 min at 37°C. DNA was precipitated with ethanol, extracted with ether and after vacuum drying dissolved in a defined volume of distilled water (Nejedlý et al. 1985; Galazka et al. 1986, 1987; Paleček et al. 1987a, b, c, 1988b).

Modification of plasmid pPK1 in situ. Plasmid-bearing E. coli cells were incubated in 2 mmol/l OsO₄ plus 2% py (or at 2 mmol/l concentration of other ligand (Fig. 1c)) and 0.5 mol/l potassium phosphate (pH 7.4) for 20 min at 37°C. The cell concentration in the reaction mixture was about 2 mg dry weight of cells per ml of medium. Modification was stopped by dilution of the sample with a tenfold volume of cooled phosphate buffer (0.1 mol/l, pH 7.4), the cells were centrifuged, rinsed in the same buffer, and kept maximally until the following day. when the modified plasmid was isolated from them (Paleček et al. 1987c, 1988b).

Enzyme cleavage. The modified DNA was cleaved after purification by restriction endonucleases (concentration of restriction endonucleases 1-2 U/µg DNA modified in vitro, at least 10 U/µg DNA modified in situ). DNA was usually further cleaved with restriction endonuclease BamHI (at least 10 U/µg DNA modified in vitro, 20 U/µg DNA modified in situ) (pRW751, pPK1), or was precipitated with ethanol and cleaved with S1 nuclease (pRW751).

Electrophoresis. Agarose (Serva) gel (1.5%) electrophoresis was performed using 40 mmol/l Tris-acetate buffer (pH 8.0), 2 mmol/l EDTA at ambient temperature. Gels were stained in ethidium bromide and photographed in midrange UV light (302 nm).

Results

We showed in our previous works (Nejedlý et al. 1985; Paleček et al. 1987a, b) that Os,py and Os,2,2'-bipy specifically modify distorted regions of the B—Z junctions in topoisomeric samples of pRW751. In this work we used pRW751 with native superhelical density (σ), and we obtained results similar to those reported in our previous works (Paleček et al. 1987a, b) with σ more negative than about −0.05, i.e. site-selective modification of all four B—Z junctions of two (dC—dG), segments at the ends of the 157 bp insert (Fig. 1a; Table 1). The modification of "inner" junctions was detected by nuclease S1 cleavage and that of the "outer" junctions by inhibition of BamHI cleavage (not shown) (BamHI restriction sequences are on the "outer" junctions of pRW751 (Fig. 1a)). To
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Table 1. Fragments arising after osmium modification and cleavage of plasmids containing B–Z junctions (cf. Fig. 1)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Modification</th>
<th>Cleavage</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRW75I</td>
<td>-</td>
<td>BamHI</td>
<td>4362 bp, 157 bp</td>
</tr>
<tr>
<td>RS B</td>
<td>-</td>
<td>-</td>
<td>lin-DNA (4519 bp)</td>
</tr>
<tr>
<td>RS A</td>
<td>-</td>
<td>-</td>
<td>lin-DNA (4519 bp)</td>
</tr>
<tr>
<td>RS B + A</td>
<td>-</td>
<td>-</td>
<td>sc-DNA</td>
</tr>
<tr>
<td>RS B</td>
<td>BamHI</td>
<td>-</td>
<td>3233 bp, 1129 bp, 157 bp</td>
</tr>
<tr>
<td>RS A</td>
<td>BamHI</td>
<td>-</td>
<td>3233 bp, 1286 bp</td>
</tr>
<tr>
<td>RS B + A</td>
<td>BamHI</td>
<td>-</td>
<td>3390 bp, 1129 bp</td>
</tr>
<tr>
<td>B–Z in B</td>
<td>PstI/BamHI</td>
<td>-</td>
<td>lin-DNA (4519 bp)</td>
</tr>
<tr>
<td>B–Z in A</td>
<td>PstI/BamHI</td>
<td>-</td>
<td>lin-DNA (4519 bp)</td>
</tr>
<tr>
<td>B–Z in B + A</td>
<td>PstI/BamHI</td>
<td>-</td>
<td>3259 bp, 1260 bp</td>
</tr>
<tr>
<td>B–Z out B</td>
<td>PstI/S1</td>
<td>-</td>
<td>3259 bp, 1161 bp, 99 bp</td>
</tr>
<tr>
<td>B–Z out A</td>
<td>PstI/S1</td>
<td>-</td>
<td>3233 bp, 1286 bp</td>
</tr>
<tr>
<td>B–Z out B + A</td>
<td>PstI/S1</td>
<td>-</td>
<td>3390 bp, 1129 bp</td>
</tr>
</tbody>
</table>

| pPK1      | -            | BglI/BamHI        | 1118 bp, 1395 bp, 173 bp, 157 bp |
| RS B      | -            | BglI/BamHI        | 1118 bp, 1395 bp, 330 bp       |
| RS A      | -            | BglI/BamHI        | 1118 bp, 1552 bp, 173 bp       |
| RS B + A  | -            | BglI/BamHI        | 1118 bp, 1725 bp              |

Notes:
— length of fragments arising from cleavage with S1 nuclease is stated only approximately
— short fragments (<700 bp) were not observed under the selected conditions of electrophoresis (on agarose gels)

RS . . . . . . . . . . BamHI restriction sequence (cf. Fig. 1)
B . . . . . . . . . . RS adjacent to (dC–dG)$_6$ segment
A . . . . . . . . . . RS adjacent to (dC–dG)$_3$ segment
B–Z in . . . . . . . “inner” B–Z junction (cf. Fig. 1)
B–Z out . . . . . . . “outer” B–Z junction (cf. Fig. 1)
B–Z in B . . . . . . . “inner” B–Z junction proximal to RS B
   (segment (dC–dG)$_6$)
B–Z in A . . . . . . . “inner” B–Z junction proximal to RS A
   (segment (dC–dG)$_3$)
B–Z out B . . . . . . . “outer” B–Z junction involving RS B
   (segment (dC–dG)$_6$)
B–Z out A . . . . . . . “outer” B–Z junction involving RS A
   (segment (dC–dG)$_3$)
obtain this modification it was necessary to use Os,py at least at a concentration of 1 mmol/l OsO₄ with 1 % py (30 min at 26 °C) while only 0.5 mmol/l OsO₄ with 0.5 mmol/l 2,2'-bipy was sufficient to obtain approximately the same effect.

**Os,bpds site-specifically modify B—Z junctions in vitro**

Chang et al. (1977) showed that Os,bpds (Fig. 1c) reacts with DNA similarly as does Os,py. We were interested whether Os,bpds (with a negative charge and a substantially larger molecule than Os,py) can be used as a probe of DNA structure and selectively modify B—Z junctions in pRW751 at native σ. Treatment of this plasmid with 2 mmol/l Os,bpds resulted in a partial inhibition of BamHI cleavage (Fig. 2, lane 2) while the unmodified control was completely cleaved by BamHI (Fig. 2, lane 1). Restriction endonucleases EcoRI, HindIII and PstI (with a single restriction sequence per molecule distant from the B—Z junction) completely cleaved the modified sample (Fig. 2, lanes 3—5), as did BglII (3 restriction sequences) and BspRI (22 restriction sequences per molecule) (not shown). Treatment of PstI linearized pRW751 with 2 mmol/l Os,bpds (not shown) did not result in any inhibition of BamHI (in agreement with absence of Z DNA in the linearized plasmid).

Site-specific modification of superhelical pRW751 with 2 mmol/l Os,bpds was also recognized by site-specific nuclease S1 cleavage (after linearization with PstI) resulting in an appearance of pairs of fragments (about 3259 bp plus 3358 bp and 1161 plus 1260 bp; Fig. 3a, lane 3), corresponding to cleavage at one or both “inner” B—Z junctions. Treatment of the same sample with BamHI
Fig. 3. The effect of Os.phe and Os.bpds modification of pRW751 in vitro on cleavage with restriction endonuclease BamHI and nuclease S1. Supercoiled DNA was reacted (30 min at 26°C) with 2 mmol/l Os.phe (lanes 2) or 2 mmol/l Os.bpds (lanes 3), linearized with PstI and cleaved by a) nuclease S1 or b) BamHI. For comparison, lane 1 contain unmodified DNA cleaved with S1 and/or BamHI. Position of linear DNA (L, 4519 bp) and fragment lengths are denoted.

Fig. 4. The dependence of modification of pRW751 in vitro on Os.bpds concentration. Supercoiled DNA was reacted (30 min at 26°C) with 0.2 mmol/l (lanes 3, 8), 0.5 mmol/l (lanes 4, 9), 1.0 mmol/l (lanes 5, 10) or 2.0 mmol/l (lanes 6, 11) Os.bpds, linearized with PstI and cleaved with either BamHI (lanes 3—6) or nuclease S1 (lanes 8—11). For comparison, lanes 1, 2, 7 contain unmodified DNA cleaved with PstI, PstI plus BamHI and PstI plus S1, respectively. Position of linear DNA (L, 4519 bp) and fragment lengths are denoted.

produced in addition to fragments corresponding to complete cleavage (3233 bp and 1129 bp), also 3390 bp and 1286 bp fragments, indicating inhibition of BamHI at the “outer” B—Z junctions (Fig. 3b, lane 3).

0.5 mmol/l Os.bpds was sufficient to induce site-specific modification at the
Fig. 5. The dependence of modification of pRW751 in vitro on Os.phe concentration. Supercoiled DNA was reacted (30 min at 26°C) with 0.2 mmol/l (lane 2), 0.5 mmol/l (lane 3), 1 mmol/l (lane 4) or 2 mmol/l (lane 5) Os.phe, linearized with PstI and cleaved with nuclease S1. For comparison, lane 1 contains unmodified DNA cleaved with PstI plus S1. Position of linear DNA (L, 4519 bp) and fragment lengths are denoted.

B—Z junction detectable by nuclease S1 (Fig. 4, lane 9) and inhibition of BamHI cleavage (Fig. 4, lane 4); with growing concentration of Os.phe, intensity of bands corresponding to the specific fragments increased (Fig. 4, lanes 5, 6, 10, 11).

Os.phe reacts at B—Z junctions and at other sites

To our knowledge, Os.phe (Fig. 1c) has not been used so far in reaction with DNA. In samples modified with 2 mmol/l Os.phe (30 min at 26°C) in addition to BamHI (Fig. 3b, lane 2), also EcoRI, HindIII, PstI, BglI and BspRI were inhibited (not shown). In agreement with these results cleavage of the osmium modified sample with nuclease S1 (after linearisation with PstI) gave only a number of short fragments (Fig. 3a, lane 2) suggesting that the chemical modification occurred at multiple sites. As shown in Fig. 5, with falling Os.phe concentration in the reaction mixture the number of short fragments decreased.
and longer fragments appeared. At lower Os.phe concentrations (0.2 to 1.0 mmol/l) very weak bands whose length corresponded to those obtained with other osmium complexes (due to the cleavage at the B—Z junctions) were observed (Fig. 5, lanes 2—4). These results suggest that unlike Os.py, Os,2,2'-bipy and Os.bpds, Os.phe may show some selectivity for the structural distortions at the B—Z junctions only under the conditions inducing small extent of DNA modification while at higher degree of Os.phe modification binding to other sites prevails. In contrast to the results obtained so far with other osmium modified DNAs the electrophoretic mobility of Os.phe modified restriction fragments was altered (Fig. 3b, lane 2; Table 1). The mobility changes depended on the extent of modification and on the number of bp contained in the fragments. More details concerning Os.phe reactions with DNA will be published elsewhere.

Os,2,2'-bipy is suited to probe DNA structure in the cell, but Os.phe and Os.bpds are not.

We recently showed (Paleček et al. 1987c, d. 1988b) that Os,2,2'-bipy binds at the B—Z junctions of plasmids contained in E. coli cells. Here we tried to ascertain whether other OsO₄ complexes behave similarly. We chose a strain of E. coli carrying the plasmid pPK1, which we had previously (Paleček et al. 1987c) used in experiments to detect Z DNA in the cell by means of Os,2,2'-bipy. The isolated pPK1 DNA and pPK1-carrying E. coli cells were incubated under similar conditions (20 min at 37°C) in the presence of 2 mmol/l OsO₄ and 2 mmol/l ligands, except for py, which was at 2% concentration.

pPK1 modified in vitro by Os.py, Os,2,2'-bipy and Os.bpds gave on cleavage with BgII/BamHI, apart from 1118 bp and 1396 bp fragments (corresponding to complete BgII/BamHI cleavage (Fig. 6a, lane 2)), 1552 bp or 1725 fragments (Fig. 6a, lanes 3, 4, 7). These fragments indicate inhibition of BamHI in the restriction sequence A or in both restriction sequences (Fig. 1b, Table 1). The sample modified with Os,4,4'-bipy gave only very poor inhibition (BamHI — restriction sequence A) fragment 1552 bp (Fig. 6a, lane 5). The sample modified with Os.phe broke into a total of five fragments with electrophoretic mobilities differing from those of the fragments of the other samples (Fig. 6a, lane 6).

Plasmid isolated from cells treated with Os.py or Os,2,2'-bipy gave on cleavage with BgII/BamHI a fragment of 1552 bp indicating inhibition of BamHI in the restriction sequence A (Fig. 6b, lanes 3, 4). In the case of modification with Os.py cell lysis was observed after only 10 min incubation. Apparently BamHI inhibition also occurred in plasmid isolated from cells treated with Os.phe (Fig. 6b, lane 6), similarly as in the case of modification in vitro (Fig. 6a, lane 6); however, a larger number of fragments was observed (Fig. 6b, lane 6).
than in the other modified samples (Fig. 6b, lanes 3, 4). Treatment of *E. coli* cells with Os,4,4'-bipy and Os,bpds did not result in inhibition of BamHI (Fig. 6b, lanes 5, 7). Our results thus suggest that Os,py, Os,4,4'-bipy, Os,phe and Os,bpds are unsuitable for detection of the structural distortions at the B—Z junctions of pPK1 plasmid in *E. coli* cells.

**Discussion**

*Probing the DNA structure in vitro with various OsO₄ complexes*

Due to negative superhelicity local “unusual” structures are stabilized in DNA (Lilley 1984; Gellert and Menzel 1987), and may play an important role in the regulation of gene expression and the realization of further functions of DNA. The use of physical methods to study these local structures is highly limited. On the other hand, chemical probes preferentially reacting with the structurally changed places are highly suitable for this purpose, provided the site of their reaction in the polynucleotide chain can be precisely determined. The first such probes were bromoacetaldehyde and Os,py (Kohwi-Shigematsu et al. 1983; Lilley 1983; Vojtíšková et al. 1983; Paleček 1986). A combination of probes of
known specificity can offer more detailed information on the local structure studied (Johnston and Rich 1985; Paleček 1986; Paleček et al. 1987a, b; Vojtíš­ková and Paleček 1987; Johnston 1988). New chemical probes of DNA struc­ture have thus been sought; e.g. diethyl pyrocarbonate (Herr 1985; Johnston and Rich 1985; Furlong and Lilley 1986; Runkel and Nordheim 1986; Scholten and Nordheim 1986; Johnston 1988) and glyoxal (Lilley et al. 1985; Gough 1988; Lilley 1986; Paleček et al. 1987b; Vojtíšková and Paleček 1987) have already proved their worth. OsO₄ can be combined with many ligands affecting the properties of the osmium complexes, which may be of importance for probing of DNA structure.

In this work we investigated three osmium complexes not as yet used as DNA structure probes, Os,phe, Os,bpds and Os,4,4′-bipy (Fig. 1c). Os,4,4′-bipy (with 4,4′-bipy at millimolar concentrations) modified the B—Z junctions in vitro only very slightly. Similarly as in the case of py, two molecules of 4,4′-bipy form the complex with OsO₄; in view of low solubility of 4,4′-bipy in water it is not possible to attain a concentration comparable to that of py (1—3 %) to obtain sufficient concentration of Os,4,4′-bipy in the modification medium. On the other hand, bidentate Os,bpds offered similar results as did Os,2,2′-bipy when working with DNA in vitro. Os,bpds is thus a new probe of DNA structure, usable in submillimolar concentrations (Fig. 4), whose binding site in polynucleotide chain is detectable using SI nuclease and/or inhibition of restric­tion cleavage. In experiments in vitro Os,bpds can yield the same results as Os,2,2′-bipy, except that the negatively charged bulky complex might appear more selective with tiny conformational changes and in DNA-protein interaction studies.

The results obtained with Os,phe in this work show that the properties of this complex differ greatly from those of osmium probes known to date. Apart from the weak reaction at the B—Z junctions (Fig. 5, lane 2—4), there is also a binding of Os,phe at other sites in the molecule. This binding might be probably connected with intercalation of Os,phe into DNA analogous to the binding of the complex with platinum (Lippard et al. 1976). Further work is required to draw definitive conclusions on the nature of Os,phe binding to DNA. The very fact that Os,phe binds covalently to DNA is interesting, and offers the prospect of making use of this complex in DNA research. phe has in recent years been used in a reaction mixture with other substances to generate OH⁺-radicals, inducing site-specific cleavage of the polynucleotide chain (Barton 1986; Sigman 1986; Chen and Sigman 1987). The combination of findings obtained with this system and with Os,phe could open up new possibilities for the use of phe in studying DNA structure.
The results obtained so far by testing the B—Z junctions in supercoiled plasmids with chemical probes (Johnston and Rich 1985; Nejedlý et al. 1985; Galazka et al. 1986, 1987; Paleček et al. 1987a, b; Johnston 1988) suggest that the B—Z junction is a narrow structurally distorted region whose detailed spatial arrangement may vary in dependence on flanking sequences, superhelical density and other factors. We have shown that the B—Z junctions in pRW751 are site-specifically modified by chloroacetaldehyde (Paleček et al. 1987a) and glyoxal (Paleček et al. 1987b), i.e. agents requiring non-paired bases for the reaction to occur. Similar results have recently been obtained by other authors with bromoacetaldehyde (McLean et al. 1987) and chloroacetaldehyde (Kohwi-Shigematsu et al. 1987). Pörschke et al. (1987) demonstrated that the hydrodynamic dimensions of the 153-bp fragment (containing two (dC—dG)n segments) are not affected by the presence of two B—Z junctions. Does this mean that hydrogen bonds are broken only in one base pair, without much influence on base stacking? Such a change would not be expected to alter local flexibility of the molecule, nor to induce a strong curvature of the molecule. On the other hand, the results of probing of the B—Z junctions in supercoiled plasmids with Os,py (Johnston and Rich 1985; Nejedlý et al. 1985; Galazka et al. 1986, 1987; Paleček et al. 1987a, b; Johnston 1988), Os,2,2′-bipy (Paleček et al. 1987a) and glyoxal (Paleček et al. 1987b) suggest that at least one AT and one GC pair are chemically modifiable. The results obtained with bulky Os,bpds (Fig. 4) might also be interpreted in terms of changes involving more than one base pair. For better interpretation of this result model building will, however, be necessary. On the other hand, it cannot be excluded that the B—Z junction in a linear fragment (Z DNA induced by [Co(NH3)6]3+) (Pörschke et al. 1987) may structurally differ from that in a supercoiled plasmid. This assumption is supported by the results of chemical probing of the B—Z junctions (Nejedlý et al. 1985; Paleček et al. 1987a, b) at superhelical densities more negative than those necessary for B—Z transition. Experiments to test these assumption are in progress.

Probing of the DNA structure in the cell

Recently we have shown that Os,2,2′-bipy can be applied for the studies of DNA structure in situ and offered the first direct and unequivocal evidence of the existence of left-handed DNA in the bacterial cell (Paleček et al. 1987c, d, 1988b). Jaworski et al. (1987), using a special molecular genetic technique, have shown independently that left-handed DNA exists and elicits a biological response in E. coli cells. Their evidence lies in the inhibition of methylation of a restriction EcoRI site in the Z DNA segment or at the B—Z junction. Such an
inhibition may be due directly to the presence of left-handed DNA or to the binding of a specific protein which prevents the restriction site in DNA being methylated. The evidence obtained with a chemical probe is more direct, but this technique can hardly avoid perturbation of the intracellular environment, due to the penetration of Os,2,2'-bipy into the cell. Thus the results obtained with the chemical and molecular genetic techniques complement each other, bringing strong evidence of the existence of left-handed DNA in vivo. In contrast to the molecular genetic technique (Jaworski et al. 1987) which was designed especially to study left-handed DNA in the bacterial cell, the chemical probe can be applied to studies of various local structures which contain base accessible to a chemical probe.

In this paper we attempted to find osmium complexes other than Os,2,2'-bipy which may be applied as probes of DNA structure in situ. We obtained positive results with Os,py (Fig. 6b, lane 3); this agent induced, however, lysis of E. coli cells at pyridine concentrations necessary for the reaction. At higher pyridine concentrations lysis proceeded faster. It cannot be excluded that Os,py may serve as a probe of the DNA structure in situ with some other cells which resist the relatively high concentration of pyridine. The results obtained with Os,phe (Fig. 6b, lane 6) suggest that this agent also is able to penetrate into E. coli cells; no cell lysis was observed under the given conditions, but the Os,phe action on DNA is not yet fully understood, and Os,phe cannot thus be considered as a probe of DNA structure at present. The negative result obtained with Os,bpds (Fig. 6b, lane 7) can be explained either by inability of this agent to penetrate the cell membrane, or by a sterical hindrance which prevents the bulky, negatively charged Os,bpds from site-specifically reacting at the the B-Z junction in the cell environment. Even if Os,bpds cannot penetrate into the E. coli cell, it may find an application as a probe of DNA structure in situ with some other cells or viruses. Further work will be necessary to elucidate the potentialities of this probe. Thus, at present Os,2,2'-bipy represents the only chemical probe capable of recognizing "open" DNA structures (Paleček 1976) in the cell. This agent easily penetrates into E. coli cells without attacking their integrity (Paleček et al. 1987c). Other aspects of the influence of this agent on the cell are currently being studied.

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References

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Lilley D. M. J., Paleček E. (1984): The supercoiled-stabilised cruciform of ColEl is hyper-reactive to osmium tetroxide. EMBO J. 3, 1187—1192


McClellan J. A., Paleček E., Lilley D. M. J. (1986): (A—T)n tracts embedded in random sequence DNA — formation of a structure which is chemically reactive and torsionally deformable. Nucl. Acid Res. 14, 9291—9309


Paleček E., Boublíková P., Karlovský P. (1987c): Osmium tetroxide recognized structural distor-
tions at junctions between right- and left-handed DNA in a bacterial cell. Gen. Physiol. Biophys. 6, 593–608

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