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Osmium tetroxide, *N,N,N′,N′*-tetramethylethylendiamine

A new probe of DNA structure in the cell

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It was shown that the complex of osmium tetroxide with *N,N,N′,N′*-tetramethylethylendiamine can be applied as a probe of DNA structure in the cell. This probe site-specifically recognized structural distortions at the B-Z junctions in plasmids pRW751 and pPK1 (containing $(dC\text{-}dG)_n$ segments) in *E. coli* cells.

Osmium tetroxide; *N,N,N′,N′*-Tetramethylethylendiamine; DNA structure chemical probing; Z DNA, cellular

1. INTRODUCTION

It was shown at the beginning of the 80s [1–3] that osmium tetroxide pyridine (Os,py) reacts preferentially with single-stranded and distorted double-stranded regions in DNA. Recently Os,py has become one of the most frequently used probes of DNA structure (for review, see [4,5]). In 1987 we showed [6] that replacing pyridine in Os,py by 2,2′-bipyridine (bipy) makes it possible to work at lower probe concentrations and use Os,bipy to probe DNA structure in *E. coli* cells [7]. By means of this probe direct evidence of the existence of left-handed DNA in the cell was obtained [7,8] and the relations between the presence of the left-handed DNA in *E. coli* cells, local DNA superhelical density and transcription were demonstrated [9]. This probe was also successfully applied to studies of the DNA cruciform ([8], McClellan, Boublíková, Paleček, Lilley, unpublished results) and protonated triplex DNA structures [5] in a bacterial cell.

Quite recently we studied the effect of ligand replacement in osmium tetroxide complexes on the ability of the complexes to probe DNA structure in vitro [10,11] and in the cell [11]. We have been able to show that some osmium tetroxide complexes yield in vitro results comparable to those obtained with Os,py and Os,bipy. On the other hand from four complexes tested none was suitable for probing DNA structure in *E. coli* cells.

Osmium tetroxide complexes and their esters with nucleic acid components were studied by Midden et al. [12]. These authors have shown that in addition to pyridine and phenanthroline derivatives other tertiary amines form complexes with osmium tetroxide. From among them we have chosen *N,N,N′,N′*-tetramethylethylendiamine (TMEN), which forms a very stable complex with osmium tetroxide and greatly differs in its properties from the osmium tetroxide ligands so far tested in the DNA structure probing [10]. Here we show that Os,TMEN is a powerful probe of DNA structure which, similarly to Os,bipy, can be applied both in vitro and in situ.

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Abbreviations: Os,py, osmium tetroxide pyridine; Os,bipy, osmium tetroxide 2,2′-bipyridine; Os,TMEN, osmium tetroxide *N,N,N′,N′*-tetramethylethylendiamine; TE, Tris-Cl, EDTA buffer (pH 7.8); bp, base pair

2. MATERIALS AND METHODS

2.1. *Plasmid DNA*

The construction of plasmids pRW751 and pPK1 (Fig. 1) is described in [13] and [7]. The cultivation of plasmid-carrying bacteria is described in detail in [7].

2.2. *Modification of plasmid DNA in vitro*

pRW751 and pPK1 were incubated at a concentration of ~100 μg DNA/ml in 25 mM TE plus 200 mM NaCl at 37°C in the presence of 2 mM Os,bipy or 2 mM Os,TMEN. The reaction was terminated by ethanol precipitation; the pelleted DNA was after vacuum drying dissolved in distilled water.

2.3. *Modification of plasmid DNA in bacterial cells (in situ)*

E. coli cells (strain JRS 856) carrying plasmid pRW751 or pPK1 were incubated in a medium containing 0.5 M potassium phosphate (pH 7.4), 2 mM Os,bipy or 2 mM Os,TMEN for 30 min at 37°C, the concentration of cells in the incubation medium corresponding to ~2 mg dry weight of cells per ml. The reaction was halted by dilution of the suspension with a tenfold volume of 0.1 M phosphate buffer,

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the cells were centrifuged and rinsed in the same buffer. The plasmids were isolated by the boiling method [14] supplement by double deproteination of the isolated material by means of chloroform.

2.4. *Enzyme reactions*

Restriction endonucleases were purchased from the Institute of Sera and Vaccines, Prague. Modified DNA was cleaved with restriction endonucleases in medium salt buffer [15].

3. RESULTS

3.1. *Site-specific modification of the B-Z junction in vitro*

Superhelical plasmids pRW751 and pPK1 (at native superhelical density) were incubated for 30 min at 37°C in the presence of 2 mM Os,bipy or 2 mM Os,TMEN in a buffer containing 25 mM TE and 200 mM NaCl. After purification pRW751 was linearised with *PstI*, pPK1 was cleaved with *BglI*, and in both cases there was subsequent cleavage of the material of restriction endonuclease *Bam*HI. The restriction sequences of *Bam*HI are in the region of the B-Z junctions on the outer ends of the $(dG\text{-}dC)_n$ segments (Fig. 1).

Site-specific modification of B-Z junctions can be detected on the basis of inhibition of *Bam*HI cleavage [6–8,10,11,16,17]. This method is very simple and sensitive [16]. Fragments of 1286 bp, 3390 bp and 4519 bp (linear DNA) in the case of pRW751 (Fig. 2A, lanes 3, 4) or 1552 bp and 1725 bp in the case of pPK1 (Fig. 2B, lanes 3, 4) indicated inhibition of *Bam*HI in both restriction sequences A and B (Fig. 1) following modification of plasmids in vitro.

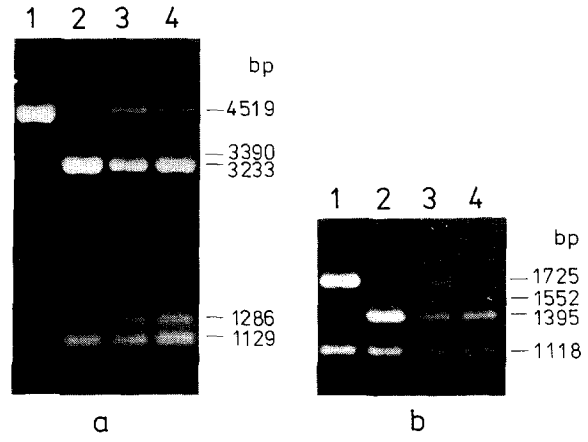

Fig. 2. Modification of (a) pRW751 and (b) pPK1 DNA by Os,TMEN or Os,bipy in vitro. Supercoiled DNA was reacted (30 min, 37°C) with 2 mM Os,bipy (lanes 3) or 2 mM Os,TMEN (lanes 4), after purification cleaved with *PstI* (pRW751; a, lanes 3, 4) or *BglI* (pPK1; b, lanes 3, 4) followed by *Bam*HI cleavage. For comparison, lanes 1 and 2 contain unmodified pRW751 and/or pPK1 cleaved with *PstI* and/or *BglI* (lanes 1), *PstI* plus *Bam*HI and/or *BglI* plus *Bam*HI (lanes 2). Positions of fragments are denoted.

3.2. *DNA structure in* E. coli *cells*

E. coli cells carrying plasmid pRW751 or pPK1 were incubated for 30 min at 37°C in 0.5 M phosphate buf-

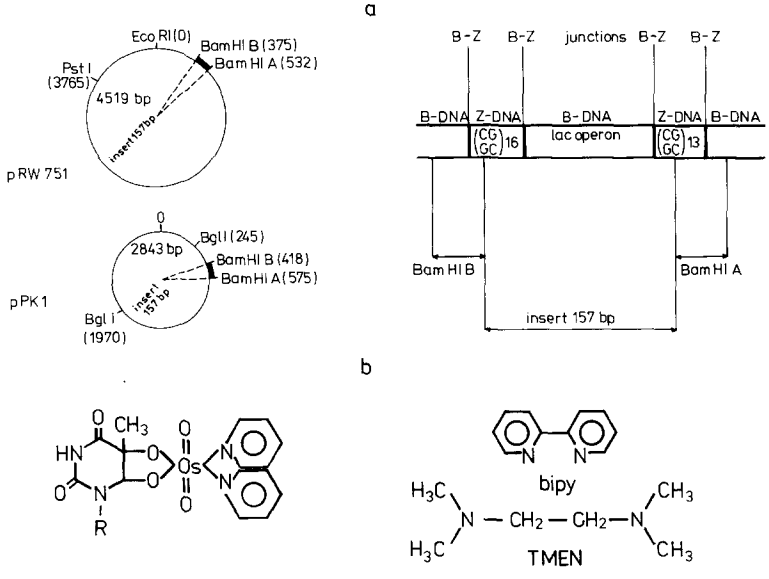

Fig. 1. (a) Maps of plasmids pRW751 and pPK1. Plasmids pRW751 [13] and pPK1 [7] were constructed by cloning the 157 bp insert into the *Bam*HI site of pBR322 and pUC19, respectively; this was done in such a way that the *Bam*HI recognition sequence GGATCC was regenerated at both ends of the insert. Thus *Bam*HI recognition sites in pRW751 and pPK1 lie on the boundary between the $(dG\text{-}dC)_n$ blocks and the pBR322 or pUC19 sequences (the first guanine of the recognition sequence being the last guanine of the $(dG\text{-}dC)_{13}$ block and the first cytosine of the $(dG\text{-}dC)_{16}$ block being the last cytosine of the recognition sequence). The segments $(dG\text{-}dC)_{13}$ and $(dG\text{-}dC)_{16}$ at the ends of the insert may (given sufficiently negative superhelical density of the plasmids [28,29]) be in the form of left-handed 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 *Bam*HI. In the plasmid pPK1 the insert was cloned into the polylinker of pUC19 containing restriction sites of 12 restrictases including *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I [7].
(b) The Os,py complex with thymine and ligands which can replace pyridine in the osmium complex used in this work.

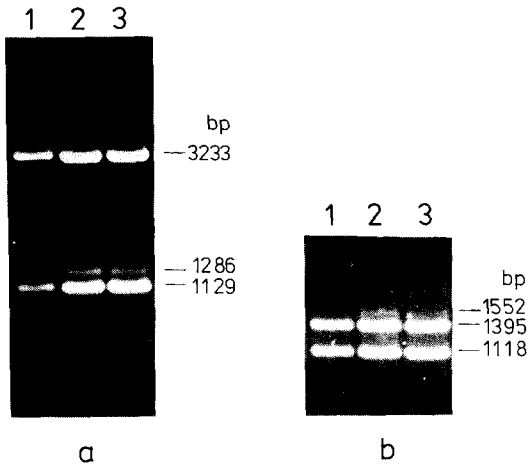

Fig. 3. Modification of pRW751 and pPK1 DNA by Os,TMEN or Os,bipy in situ. *E. coli* cells carrying (a) pRW751 or (b) pPK1 were incubated (30 min, 37°C) in a medium containing 2 mM Os,bipy (lanes 2) or 2 mM Os,TMEN (lanes 3). The purified plasmids were cleaved with *PstI* (pRW751; a, lanes 2, 3) or *BglI* (pPK1; b, lanes 2, 3) followed by *Bam*HI cleavage. For comparison, lanes 1 contain unmodified pRW751 cleaved with *PstI* plus *Bam*HI and/or unmodified pPK1 cleaved with *BglI* plus *Bam*HI. Positions of fragments are denoted.

fer containing 2 mM Os,bipy or 2 mM Os,TMEN. Observation of cells in a microscope (using phase contrast) did not reveal cell lysis or other changes indicating damage to the cell surface (not shown; cf. [7]). Plasmids were isolated from cells [14] and cleaved with restriction endonucleases *PstI* plus *Bam*HI (pRW751) or *BglI* plus *Bam*HI (pPK1). In the case of plasmid pRW751 inhibition of *Bam*HI in the restriction sequence B was observed (Fig. 3a, lanes 2, 3), i.e. in the region of the outer B-Z junction of $(dG\text{-}dC)_{16}$ segment (Fig. 1). In pPK1 there was more marked inhibition of *Bam*HI in the restriction sequence A (Fig. 3b, lanes 2, 3), i.e., in the region of the outer B-Z junction of $(dG\text{-}dC)_{13}$ segment (Fig. 1). The results described are in accordance with our previously published findings [7,8] using Os,bipy.

In order to ascertain whether Os,TMEN like Os,bipy [7] binds site-specifically in the region to the B-Z junctions, we cleaved pPK1 DNA modified in situ with several different restriction endonucleases. Of the tested restrictases with restriction sequences in the region of the polylinker pPK1 (*Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI) only *Bam*HI was inhibited; restriction sequences of the latter restrictase extend into the region of B-Z junctions. The other restrictases were not inhibited (not shown).

4. DISCUSSION

In spite of great progress in research into the polymorphy of the DNA double helix in vitro (for review, see [4]), little is known of the details of the DNA structure in the cells, mainly due to a lack of suitable techniques. Application of chemical probes seems to be a simple and efficient approach solving many questions concerning DNA structure and interactions in the cell. The finding that Os,TMEN is capable of penetrating the bacterial cell wall, recognizing and selectively modifying the DNA B-Z junction in the cell thus appears very significant.

Osmium tetroxide probes of DNA structure have a number of advantages as compared to enzymatic and other chemical probes [4,5,16]; among these is the established chemistry of their interaction with DNA components (for review, see [18]), detection of the osmium binding sites at single nucleotide resolution [5,19–23], availability of antibodies against osmium-modified DNAs [5,24], etc. Os,TMEN represents a second osmium probe which can be applied to probe DNA structure in situ. The differences in structures and properties of Os,TMEN and Os,bipy may come into play in studies of details of local DNA structures in vitro. These differences may become even more important in probing the DNA structure in cells. They may be involved both in the penetration step when working with cells other than *E. coli* and in the recognition step in the complex environment of the cell where DNA interaction with proteins and other cell components can significantly influence the accessibility of the local unusual structures for the given chemical probe.

Quite recently it has been shown [25] that potassium permanganate is another probe which can be applied to studies of DNA structure in situ. $KMnO_4$ oxidizes DNA bases reacting mainly with thymine and to a much lesser extent with cytosine and guanine, i.e., its reactivity towards bases is similar to that of osmium tetroxide itself [18] and to some extent also to that of Os,bipy [5,26]. A combination of chemical probes in the studies of the DNA structure in vitro has been very useful [17,19,22,27] and one can expect that it will be even more important in the studies of the DNA structure in the cell mainly due to the complexity of the environment inside the cell.

It can be expected that further chemical probes suitable for the studies of the DNA structure in cells will be developed soon and that their application will result in a great advance in our knowledge of DNA structural–functional relations in its natural environment.

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