Antibodies to DNAs Chemically Modified with Osmium Structural Probes

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Abstract. It has previously been shown that osmium tetroxide, pyridine (Os,py) and osmium tetroxide, 2,2′-bipyridine (Os,bipy) are powerful probes of the DNA structure. To increase the possibilities of the detection of osmium-modified DNAs polyclonal antibodies against DNA modified with Os,py and Os,-bipy were elicited in rabbits. Specificity of these sera or purified IgG was tested by ELISA and retardation of the DNA electrophoretic mobility in agarose gels. Antibodies against DNA-Os,py (anti-DNA-Os,py) reacted with single-stranded and double-stranded DNA-Os,py but they did not react with unmodified DNA; with DNA-Os,bipy only a weak reaction was observed. The specificity of the anti-DNA-Os,bipy was similar. Competition experiments with anti-DNA-Os,py showed a weak reaction with RNA-Os,py but no reaction with osmium-modified proteins and unmodified proteins and RNA. The results suggest that anti-DNA-Os,py may become an important tool in studies of DNA structure *in situ*.

Key words: Immunoassay of osmium-modified DNA — Immunochemical probing of DNA structure — Antibodies against modified DNA — Recombinant supercoiled plasmids — DNA gel retardation

Introduction

Osmium tetroxide has been used as a probe of DNA structure since the beginning of 80s (Paleček et al. 1981; Lukášová et al. 1982, 1984; Glikin et al. 1984). In the presence of a suitable ligand such as pyridine, OsO_4 binds covalently to pyrimidine bases through the addition across the 5,6 double bond (Fig. 1) reacting preferentially with single-stranded and double-stranded distorted regions in DNA (Lukášová et al. 1982, 1984). Osmium tetroxide, pyridine (Os,py) has been applied in the studies of cruciform structures (Lilley and Paleček 1984;

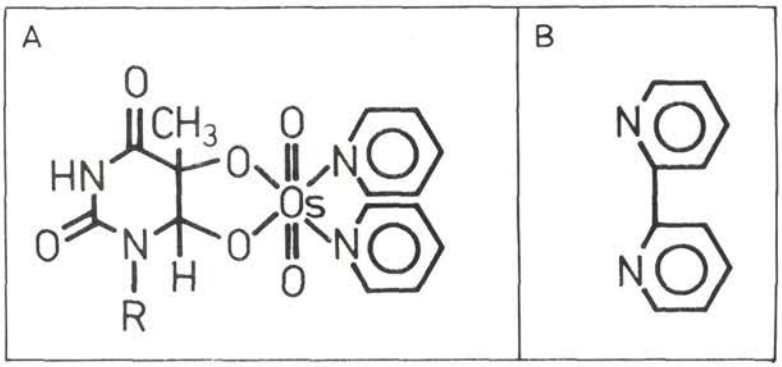

Fig. 1. *A* — adduct between thymine and osmium tetroxide, pyridine complex; *B* — 2,2′-bipyridine which can replace pyridine in the complex.

McClellan and Lilley 1987), B—Z junctions (Nejedlý et al. 1985; Johnston and Rich 1985; Gałazka et al. 1986; Paleček et al. 1987a, b; Wells 1988), unusual structures in homopurine.homopyridine stretches (Vojtíšková and Paleček 1987; Vojtíšková et al. 1988; Collier et al. 1988; Hanvey et al. 1988) and AT rich sequences (Paleček et al. 1988a) in supercoiled plasmids and unusual structure of (A—T)*n* sequences in linearized plasmids (McClellan et al. 1986).

Recently we have shown that OsO_4 complex with 2,2′-bipyridine (Os,bipy) can be used as a probe of DNA structure in the cell (Paleček et al. 1987b, 1988b) producing evidence of the presence of left-handed Z DNA in the cell. Cells containing a recombinant plasmid were treated with Os,bipy and the DNA site-specific modification determined after plasmid isolation. This approach opened up new possibilities in the studies of DNA structure in bacterial cells but its direct application for studies of structural changes in eukaryotic chromosomes is difficult. Immunological techniques have recently shown their great value in the studies of non B DNA structures (for review see Stollar 1986, 1988; Eilat 1986; Leng 1987) and especially the left-handed Z DNA (e.g. Jovin et al. 1983; Nordheim et al. 1982). These techniques have also been applied for the studies of chemically modified DNAs (for review see Munns and Liszewski 1980; Strickland and Boyle 1984; Leng 1985), no antibody has, however, been reported against DNA modified with a chemical agent which is a probe of the DNA structure. In this work we attempted to raise antibodies against osmium-modified DNAs to extend the possibilities of the detection of these DNAs in eukaryotic and prokaryotic cells and to obtain an immunological method of studying properties of osmium-modified DNAs.

Material and Methods

Calf thymus DNA and plasmid pColIR215 DNA were isolated and characterized as described (Lukášová et al. 1982; Nejedlý et al. 1988). pColIR215 (which is a clone of the inverted repeat-containing region of ColE1 in pBR322) (Lilley 1980; Lilley and Paleček 1984) was kindly donated by Dr. D. M. J. Lilley while pAT32 (a derivative of pUC19 containing $(dA—dT)_{16}$ insert) (Panyutin et al. 1985; Paleček et al. 1988b) was a gift from Dr. S. Mirkin. Bovine serum albumin (BSA) and yeast RNA (highly polymerized, A grade) were from Calbiochem. Other chemicals were of analytical grade.

Chemical modification. a. DNA used for immunization: Denatured DNA at a concentration of 160 μg/ml in 0.06 mol/l sodium phosphate pH 7 was modified with 2.2 mmol/l OsO_4, 10 % pyridine (DNA-Os,py) or with 2.2 mmol/l OsO_4, 2.2 mmol/l 2,2′-bipyridine (DNA-Os,bipy) for 24 hours at 26 °C. Osmium modified DNA samples were thoroughly dialysed and the extent of modification was checked by UV absorption spectroscopy and electrochemical measurements (Lukášová 1982, 1984; Paleček and Hung 1983). b. Biomacromolecules used in gel electrophoresis and ELISA experiments: Thermally denatured (100 °C, 6 min in SSC/20) calf thymus DNA at a concentration of 290 μg/ml and the RNA and BSA at a concentration of 900 μg/ml were modified with 2 mmol/l OsO_4, 4 % pyridine in SSC/20 for 16 hours at 26 °C. The samples were then dialysed and treated in the same way as DNA samples used for immunization. If not stated otherwise pColIR215 DNA was modified with 2 mmol/l OsO_4, 2 % pyridine in 10 mmol/l TE for 30 min at 26 °C. The osmium-modified DNA samples were purified by ethanol precipitation.

Immunizations. Six male rabbits (obtained from Stolbovaj AMS USSR) were inoculated at multiple intradermal sites with osmium-modified DNAs mixed with methylated bovine serum albumin (mBSA). For the first injections complexes of DNA-Os,py or DNA-Os,bipy (60 μg) and mBSA (90 μg) were mixed with complete Freund's adjuvant. Total volume of modified DNA-mBSA complex and adjuvant was 1.0 ml. Subsequent injections of complexes in such doses were given at intervals of 3 and 4 weeks with incomplete Freund's adjuvant. Boosting was repeated from time to time. Rabbits were bled by the marginal ear vein 7 days after immunization.

Immunoglobulin Gs (IgG) were purified from the antisera by a combination of ammonium sulphate precipitation and DEAE-cellulose chromatography (Jaton et al. 1979). For titrations, the IgG concentrations were adjusted to approximately 20 mg/ml, assuming that 1 A_{280} unit is 1.4 mg/ml of IgG.

Enzyme-linked immunosorbent assay (ELISA) was carried out as described by Kiseleva and Poverennyi (1987). Polystyrene plates (Koh-i-Noor) were pretreated with DEAE-dextran solution (30 μg/ml) in 0.05 mol/l sodium carbonate buffer (pH 9.6) for 16—18 hours at room temperature. Then aliquots of 100 μl of modified DNA (1 μg/ml) in 0.05 mol/l sodium carbonate buffer were added into each well and the plates were stored overnight at 4 °C. Wells were then washed three times with 100 μl PBS (0.14 mol/l NaCl, 0.01 mol/l KH_2PO_4, Na_2HPO_4) containing 0.05 % Tween 20.

Each well was postcoated with 1 % bovine serum albumin (BSA) in 0.05 mol/l sodium carbonate buffer for 2 hours at room temperature. After 3 washes (with PBS, 0.1 % BSA, 0.05 % Tween 20) the wells were drained and 100 μl of the mixture of equal volumes of the competitor antigen and appropriate antisera (in PBS containing 0.1 % BSA and 0.05 % Tween 20, preincubated at 37 °C for 1 hour) was added to each well. The following dilutions of the antisera against modified DNA were used: DNA-Os,bipy (1 : 1000), DNA-Os,py (1 : 2000). The plates were washed as before and 100 μl of conjugate of protein A – horseradish peroxidase (Amersham, England) (in PBS with 0.05 % Tween 20) was added to the wells. The plates were incubated at room temperature for 1 hour, washed as before, and 100 μl of the enzyme substrate added, consisting of 0.5 mg/ml ortho-phenyldiamine in 0.004 mol/l Na_2HPO_4, 0.002 mol/l citric acid and 0.01 % H_2O_2. The reaction was stopped by addition of 100 μl 2 mol/l H_2SO_4 and optical density at 492 nm monitored with a Titertek Multican Plate Reader (Flow Laboratories, England).

DNA gel retardation technique (Pohl et al. 1982; Zarling et al. 1984). The DNA was incubated with purified anti-DNA-Os,py IgG 45 min at 37 °C followed by 15 min incubation at 0 °C; in competition experiments DNA was mixed with a competitor prior to addition of IgG. Following incubation the mixture was analysed by electrophoresis in 1.0 or 0.8 % agarose gel in TAE buffer and stained with ethidium bromide. Samples were visualized in UV light.

Results

Specificity of antibodies against osmium modified DNAs as detected by ELISA

The specificity of anti-DNA-Os,bipy was measured by competition between calf thymus DNA-Os,bipy and varying amounts of DNA-Os,bipy, DNA-Os,py and unmodified thermally denatured DNA from the same source using the ELISA method. DNA-Os,bipy was specifically and completely competed with DNA-Os,bipy (Fig. 2*B*). DNA-Os,py only partially competed, suggesting structural similarities in these two modified DNAs. There was no significant competition with unmodified denatured DNA. Testing of the specificity of anti-DNA-Os,py (Fig. 2*A*) gave similar results, i.e. no significant competition with unmodified DNA, weak inhibition with DNA-Os,bipy and complete inhibition with DNA-Os,py.

Binding of anti-DNA-Os,py to supercoiled pColIR215 causes DNA gel retardation

Osmium probing of DNA unusual structures in supercoiled and linearized plasmids has mostly been done with Os,py (Paleček 1989). We therefore did further experiments mainly with anti-DNA-Os,py. We attempted to find out whether interaction of anti-DNA-Os,py with osmium modified plasmid will result in DNA gel retardation, as is the case with, e.g., antibodies against left-handed Z DNA (Jovin et al. 1983; Pohl 1987; Zarling et al. 1984; Nordheim

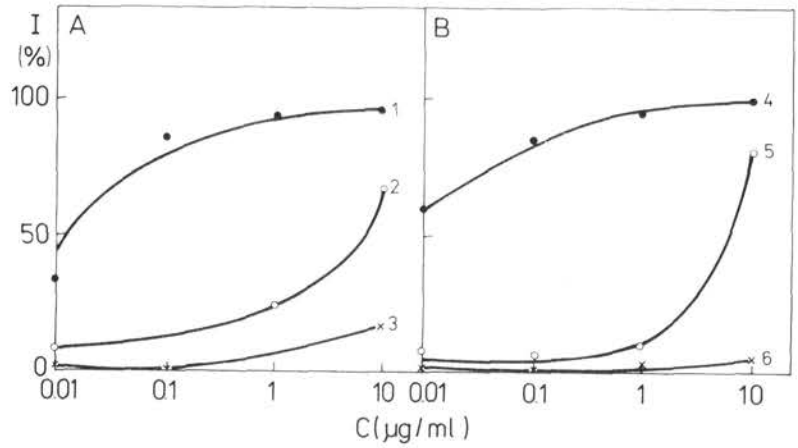

Fig. 2. Competitive binding assay of anti-DNA-Os,py and anti-DNA-Os,bipy by ELISA method. Dependence of binding inhibition (*I*) on the competitor concentration (*C*). *A* — DNA-Os,py (0.1 μg) as an antigen on the plate surface; anti-DNA-Os,py (2 μg) in the second layer. Competitors: 1, DNA-Os,py; 2, DNA-Os,bipy; 3, unmodified DNA. *B* — DNA-Os,bipy as an antigen on the plate; anti-DNA-Os,bipy in the second layer. Competitors: 4, DNA-Os,bipy; 5, DNA-Os,py; 6, unmodified DNA. The mixture of the antibody and a competitor (modified or unmodified thermally denatured calf thymus DNA) was preincubated (60 min at 37 °C) before the application on the plate.

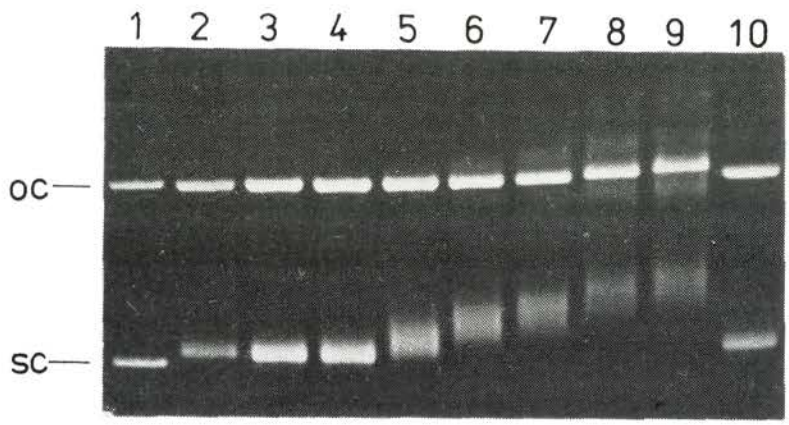

Fig. 3. Electrophoretic retardation of pColIR215 DNA-Os,py by anti-DNA-Os,py; dependence on IgG concentration. Lane 1, unmodified pColIR215 DNA; lanes 2, 10; pColIR215 DNA-Os,py (2 mmol/l OsO_4, 2 pyridine, 10 mmol/l TE pH 7.6, 26 °C, 30 min) without the antibody, 3—9, pColIR215 DNA-Os,py after incubation (in 0.08 mol/l NaCl, 45 min at 37 °C followed by 15 min at 0 °C) with 0.02 (lane 3), 0.2 (lane 4), 2 (lane 5), 6 (lane 6), 10 (lane 7), 20 (lane 8) and 30 (lane 9) μg of the IgG in a volume of 20 μl. 0.8 % agarose gel. sc, supercoiled DNA; oc, relaxed DNA.

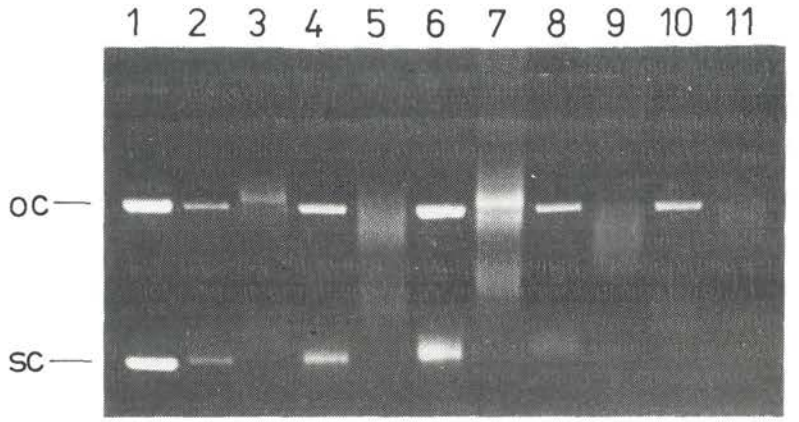

Fig. 4. Gel retardation of pColIR215 DNA-Os,py by anti-DNA-Os,py; dependence on pyridine concentration in the reaction mixture. pColIR215 DNA was modified by 2 mmol/l OsO_4 with 1 % (lanes 2, 3), 2 % (lanes 4, 5), 3 % (lanes 6, 7), 4 % (lanes 8, 9) and 5 % (lanes 10, 11) pyridine and incubated with anti-DNA-Os,py (1 mg/ml) (lanes 3, 5, 7, 9, 11); modified DNAs without antibody (lanes 2, 4, 6, 8, 10), unmodified DNA (lane 1). Other conditions as in Fig. 3.

et al. 1982). Supercoiled pColIR215 DNA was modified with 2 mmol/l OsO_4, 2 % pyridine in 10 mmol/l TE for 30 min at 26 °C. Purified DNA-Os,py was then incubated with various concentrations of anti-DNA-Os,py and DNA-protein mixtures were analyzed by agarose gel electrophoresis. With increasing anti-DNA-Os,py concentration the supercoiled form was retarded in mobility (Fig. 3, lanes 3—9). Retardation of relaxed form was observed at higher anti--DNA-Os,py concentrations.

Extent of DNA modification

We have shown previously (Glikin et al. 1984; Lilley and Paleček 1984) that at low ionic strengths and high pyridine concentration osmium modification may not be limited to the region of the local structural deviation but in can propagate along the strands forming a "denaturation bubble"; such a process can result in relaxation of negative supercoils and even in formation of positively supercoiled molecules (Vojtíšková et al. 1985). We were interested whether the extent of modification would be related to the extent of DNA gel retardation.

Supercoiled pColIR215 DNA was modified by 2 mmol/l OsO_4 with pyridine concentration ranging from 1 to 5 % in 10 mmol/l TE pH 8 for 30 min at 26 °C. Purified DNA-Os,py samples were divided into two parts, one of them was incubated with anti-DNA-Os,py; all samples were then electrophoresed.

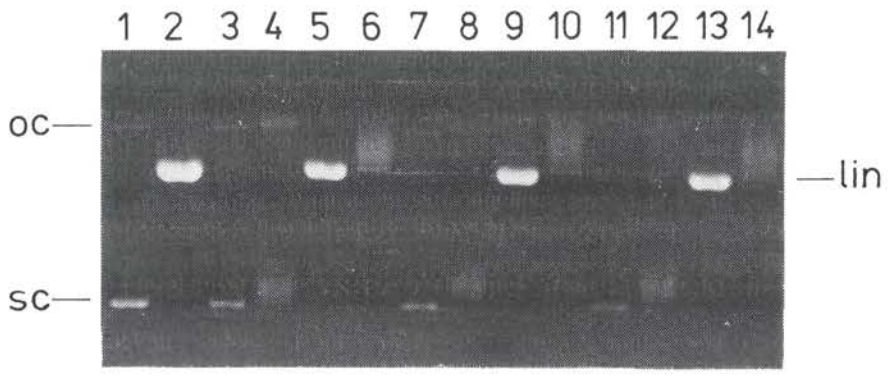

Fig. 5. Gel retardation of supercoiled and linearized pColIR215 DNA-Os,py by anti-DNA-Os,py; dependence on modification time. Lanes 1, 2, unmodified pColIR215 DNA without antibody; 1, supercoiled and 2, linearized DNA. DNA was modified with 1 mmol/l OsO_4, 1 % pyridine at 26 °C for 30 min (lanes 3—6), 60 min (7—10) and 90 min (11—14). Supercoiled DNA without (lanes 3, 7, 11) and with antibody (4, 8, 12); (lanes 3, 7, 11) and with antibody (4, 8, 12); EcoRI linearized (lin) DNA without (lanes 5, 9, 13) and with antibody (1 mg/ml) (6, 10, 14); 1 % agarose gel. Other conditions as in Fig. 3.

Osmium modification resulted in markedly decreased mobilities of samples modified in the presence of 3—5 % pyridine (Fig. 4, lanes 6, 8, 10). In these DNA samples interaction with anti-DNA-Os,py induced the largest DNA retardation (Fig. 4, lane 11).

Further, we attempted to investigate changes in DNA gel retardation at different modification times under the conditions of low extent of osmium-modification which does not itself induce any observable changes in the electrophoretic mobility. We were also interested in whether the DNA gel retardation (resulting from the interaction of DNA-Os,py with IgG) is also observable after DNA linearization. pColIR215 DNA was modified with 1 mmol/l OsO_4, 1 % pyridine in 10 mmol/l TE at 26 °C for 30, 60 and 90 min, respectively. Purified DNA-Os,py samples were divided into two parts; one of them remained in the supercoiled form while the other one was linearized with EcoRI. All samples were then incubated with the anti-DNA-Os,py and electrophoresed. Interaction of anti-DNA-Os,py with osmium modified DNA samples resulted in mobility retardation in both supercoiled and linear DNAs (Fig. 5, lanes 4, 8, 12 and 6, 10, 14, respectively). The strongest effect was observed with DNA modified for the longest time (Fig. 5, lanes 12, 14).

McClellan and Lilley (1987) have recently shown that the $(dA—dT)_{16}$ insert in a supercoiled plasmid may assume two different structures in dependence on ionic strength and other conditions. In the presence of NaCl a cruciform

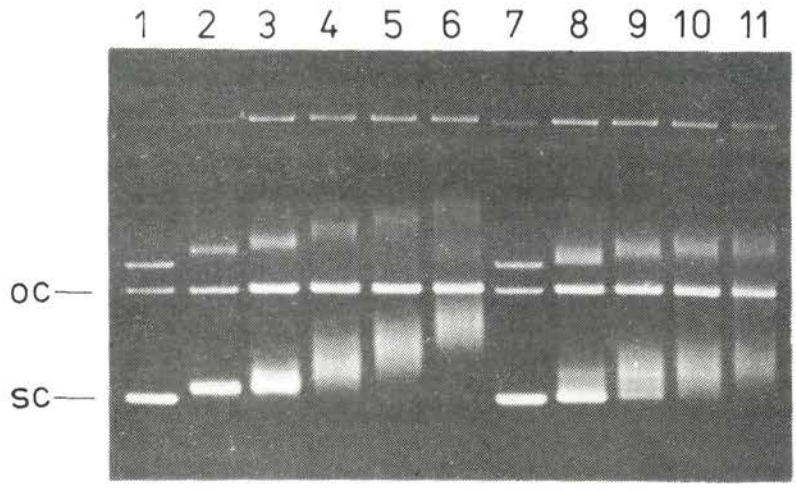

Fig. 6. Gel retardation of pAT32 DNA-Os.py (containing two different local structures modified with Os.py) by anti-DNA-Os.py; DNA was modified with 1 mmol/l OsO_4, 3 % pyridine, 20 min at 20 °C in 5 mmol/l TE pH 7.6 in absence of NaCl (lanes 2—6) and in 50 mmol/l NaCl (lanes 7—11). Osmium-modified DNA samples were incubated with 0 (lanes 2, 7), 2 (lanes 3, 8), 6 (lanes 4, 9), 10 (lanes 5, 10) and 20 (lanes 6, 11) μg of the anti-DNA-Os.py in a volume of 20 μl; unmodified pAT32 DNA (lane 1); 1 % agarose gel. Other conditions as in Fig. 3.

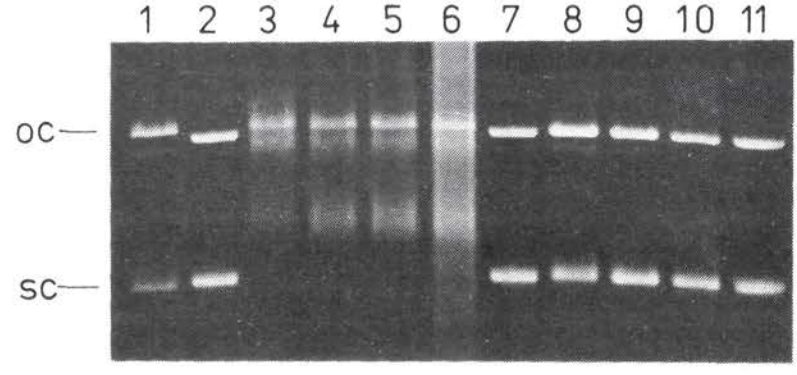

Fig. 7. Gel retardation of pColIR215 DNA-Os.py (2 mmol/l OsO_4, 2 % py, 10 mmol/l TE pH 7.6, 30 min, 26 °C) by anti-DNA-Os.py (1 mg ml); testing of competition of unmodified and osmium modified thermally denatured calf thymus DNA. Unmodified pColIR215 DNA with (lane 1) and pColIR215 DNA-Os.py without the IgG (lanes 2, 7, 11), pColIR215 DNA-Os.py and the IgG without (lane 3) and with competitors (lanes 4—6 and 8—10). Competitors: thermally denatured unmodified calf thymus DNA at concentrations of 8, 32, and 206 μg/ml respectively (lanes 4—6) DNA-Os.py at concentrations of 6, 23 and 74 μg ml resp. (lanes 7—10). Other conditions as in Fig. 3.

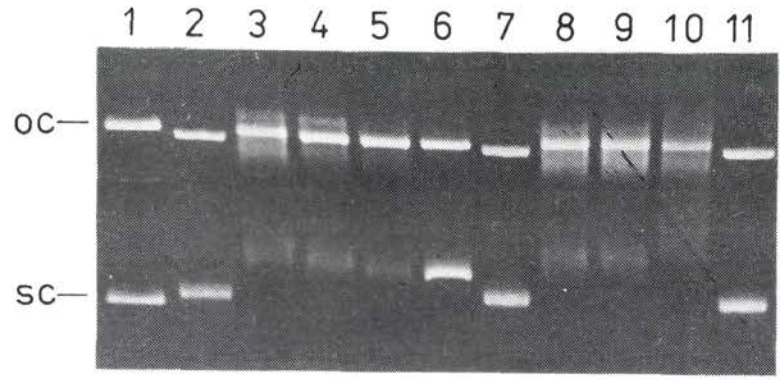

Fig. 8. Gel retardation of pColIR215 DNA-Os,py by anti-DNA-Os,py; testing of competition of osmium-modified RNA and BSA. Unmodified pColIR215 DNA with (lane 1) and pColIR215 DNA-Os,py without the IgG (lanes 2, 7, 11), pColIR215 DNA-Os,py and the IgG without (lane 3) and with competitors (lanes 4—6 and 8—10). Competitors: RNA-Os,py at concentrations of 5, 15 and 194 μg/ml, respectively (lanes 4—6) and Os,py-modified BSA at concentrations of 4, 13 and 168 μg/ml resp. (lanes 8—10). Other conditions as in Fig. 3 and 7.

structure is formed, which is modified by Os,py only in its loop, while in the absence of salt even modification of all thymines in the $(dA—dT)_{16}$ insert is observed (resembling the modification pattern obtained with linearized plasmid, McClellan et al. 1986). We modified supercoiled plasmid pAT32 (containing the $(dA—dT)_{16}$ insert) under the conditions applied by McClellan and Lilley (1987) to learn whether such a small extent of modification would be detectable by anti-DNA-Os,py (Fig. 6). We observed a DNA gel retardation (Fig. 6) together with a marked difference in the retardation of the samples modified at different ionic strengths. DNA modified in the presence of salt (Fig. 6, lanes 8—11) was substantially less retarded than that modified in the absence of NaCl (Fig. 6, lanes 3—6). Addition of unmodified DNA in a fourfold excess did not influence the gel retardation of pAT32 DNA-Os,py-IgG complexes (not shown).

Competition of other Os,py-modified and unmodified biomacromolecules

If Os,py is used for studies of DNA structure *in situ* not only DNA but also other species can be modified; Modification of RNAs (Lukášová et al. 1982) and proteins (Deetz and Behrman 1981) may represent the most probable source of interference in probing DNA structure by Os,py in eukaryotic chromosomes. We therefore attempted to find out whether Os,py-modified proteins and RNAs would react with anti-DNA-Os,py.

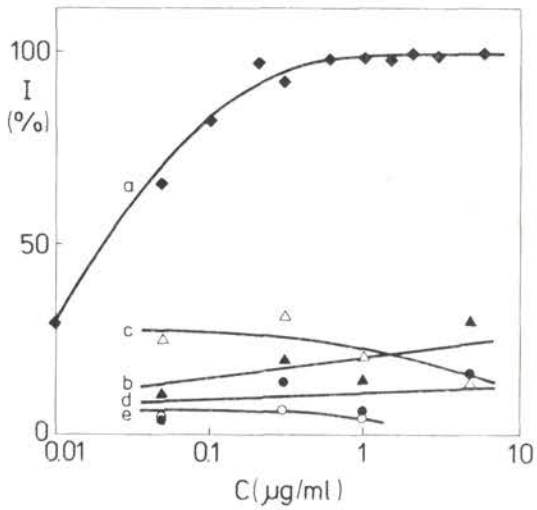

Fig. 9. Competitive binding assay of anti-DNA-Os,py by ELISA method. Dependence of binding inhibition (*I*) on the competitor concentration (*C*). Calf thymus DNA-Os,py (0.5 μg) as an antigen of the plate. Anti-DNA-Os,py (3 μg) in the second layer. Competitors: *a*, thermally denatured calf thymus DNA-Os,py, *b*, RNA-Os,py, *c*, unmodified RNA, *d*, BSA-Os,py, *e*, unmodified BSA. Other conditions as in Fig. 2.

The specificity of anti-DNA-Os,py was measured by competition between pColIR215 DNA-Os,py and varying amounts of calf thymus DNA-Os,py, yeast RNA-Os,py, Os,py-modified BSA and lysozyme. pColIR215 DNA-Os,py was specifically and completely competed with calf thymus DNA-Os,py as measured by DNA gel retardation (Fig. 7, lanes 8—10). No sign of competition was observed with Os,py-modified lysozyme (not shown) and BSA (Fig. 8, lanes 8—10). RNA-Os,py competed weakly with pColIR215 DNA-Os,py (Fig. 8, lanes 4—6); weak competition of RNA-Os,py and practically no competition of Os,py-modified BSA with calf thymus DNA-Os,py was also observed by the ELISA technique (Fig. 9).

In agreement with the results obtained by ELISA technique (Fig. 9) unmodified thermally denatured calf thymus DNA induced no changes in gel retardation of the pColIR215 DNA complex with anti-DNA-Os,py (Fig. 7, lanes 4—6). Nor was competition observed with unmodified yeast RNA and BSA (not shown). These results suggest that anti-DNA-Os,py may be suitable for testing local changes in DNA structure in eukaryotic chromosomes.

Discussion

Since their discovery in the late 50s antibodies to DNAs have been used for various purposes, including DNA structure studies (for review see Eilat 1986; Stollar 1986, 1988). Recently antibodies against DNAs modified with some carcinogenic agents have also been raised (Sage et al. 1979; Munns and Liszewski 1980; Strickland and Boyle 1984; Leng 1985, 1987; Stollar 1988). Radioimmunoassay was used to detect thymine glycols in OsO_4-treated DNA (OsO_4 was used in the absence of any ligand and no stable DNA-Os complex was formed) (West et al. 1982). To our knowledge our results represent the first attempt to elicit antibodies against DNAs modified with chemical probes of the DNA structure. Such antibodies may significantly extend the possibilities of the determination of the probe reactions in the DNA molecules and may be especially valuable in the studies of the DNA structure in prokaryotic and eukaryotic cells. For this purpose it is, however, necessary to produce antibodies with a high specificity for the probe-modified DNAs.

The results of the competition experiments presented in this paper (Fig. 7—9) suggest that the polyclonal anti-DNA-Os,py is highly suitable for this kind of work at least in the cases where DNA is isolated and analysed *in vitro* after the treatment of cells, organs or animals with the osmium probe. We have shown that the anti-DNA-Os,py does not cross-react with any tested unmodified DNAs (Fig. 7, lanes 4—6; Fig. 2*A*-3), RNAs and proteins (Fig. 8; Fig. 9*c* and *e*). No cross-reaction was observed even with osmium modified proteins (Fig. 8, lanes 8—10; Fig. 9*d*) and competition of osmium-modified RNA was only very weak (Fig. 8, lanes 4—6; Fig. 9*b*). These results suggest that the antigenic determinant involves not only the osmium, pyridine complex itself but also some parts of DNA.

We observed only weak cross-reactions between the anti-DNA-Os,py and DNA-Os,bipy (Figs. 2*A*-2 and 2*B*-5) in spite of the fact that the difference in the structure between these two complexes (Fig. 1) is very small. A high sensitivity of antibodies for small differences in the DNA adducts was previously also observed with antibodies against *cis*-diamminedichloroplatinum(II)-modified DNA (*cis*-DDP) (Lippard et al. 1983; Leng 1985; Vrána et al. 1988), which showed poor immunoreactivity toward calf thymus DNA modified by a variety of antitumour-inactive platinum compouds including *trans*-DDP, while exhibiting good reactivity toward DNA modified by *cis*-DDP.

If the anti-DNA-Os,py is applied to detect DNA-Os,py directly in the cell, e.g. by immunofluorescence techniques, the problem of cross-reactivity with structurally related antigens becomes more complex (as it is difficult to exclude cross-reaction with an unknown antigen). It has been shown that, e.g., rabbit polyclonal as well as monoclonal antibodies (Guarnieri and Eisner 1974; Lafer

et al. 1981; Schoenfeld et al. 1983) can react with both DNA and the phospholipid cardiolipin. Nevertheless we have been able to apply Os,py to studies of polytene chromosomes of *Chironomus thummi* and *Drosophilla melanogaster* and to obtain specific immunofluorescence staining patterns (M. Robert-Nicoud, T. Jovin, A. Poverennyi and E. Paleček, unpublished) which changed with the development of the insect larvae.

The binding surface of the Fab arm of IgG is approximately 1.5—2.0 nm and would correspond to about three nucleotide pairs along the intact DNA molecule (Stollar 1986). Simultaneous binding of both Fab regions to one DNA molecule requires another binding site about 50 bp away. Such a situation can hardly arise within a single molecule of the supercoiled pColIR215 or pAT32 DNAs, which under mild reaction conditions are site-specifically modified only at the cruciform loop (Lilley and Paleček 1984; McClellan and Lilley 1987). Under stronger reaction conditions a denaturation "bubble" can form, involving 50 or more base pairs. Under these conditions both Fab regions may bind to DNA and more than one antibody molecule can site-specifically interact with one DNA molecule. A larger extent of DNA modification (e.g. Fig. 4, lane 10) may thus result in a greater gel retardation of the DNA-Os,py-IgG complexes (Figs. 4, 5). pAT32 DNA contains 2718 bp and thus the immunodetection of 4—6 modified bases in the cruciform loop of this DNA (Fig. 6, lanes 8—11) in a fourfold excess of unmodified DNA represents the detection of about 0.02 % of all DNA bases. Better sensitivity will probably be obtained with other immunoassay techniques such as ELISA, where much larger excesses of unmodified DNA can be present. Immunoassay in combination with osmium probes thus represents a promising technique for the studies of structural changes in chromosomal DNAs which have so far received little attention.

Recently we have shown (Paleček 1989) that osmium tetroxide can be applied in combination with other ligands, but py or bipy as a probe of DNA structure. These ligands include tetramethylethylendiamine, 1,10-phenantroline and bathophenantroline disulfonic acid and significantly extend the possibilities and usefulness of osmium probes (Boublíková and Paleček, 1989). It appears that eliciting antibodies against DNAs and RNAs modified with the new osmium probes should not be difficult.

Abbreviations used: Os,py — OsO_4,pyridine; Os,bipy — OsO_4,2,2′-bipyridine; DNA-Os,py — DNA modified with Os,py; anti-DNA-Os,py — antibody against DNA-Os,py; IgG — Immunoglobulin G; BSA — bovine serum albumin; mBSA — methylated BSA; PBS — 0.15 mol/l NaCl, 0.01 mol/l KH_2PO_4, Na_2HPO_4, pH 7.4.

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