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Uranyl photoprobing of a four-way DNA junction: evidence for specific metal ion binding

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Metal ions are very important in mediating the folding of nucleic acids, as exemplified by the folding of the four-way DNA junction into the stacked X-conformation. Uranyl ion-mediated photocleavage provides a method for the localization of high-affinity ion binding sites in nucleic acids, and we have applied this to the four-way DNA junction. We have made the following observations. (i) Uranyl ions (UO_2^{2+}) suppressed the reactivity of junction thymine bases against attack by osmium tetroxide, indicating that the uranyl ion induces folding of the junction into a stacked conformation. (ii) DNA located immediately at the point of strand exchange on the two exchanging strands was hypersensitive to uranyl photocleavage. The relative hypersensitivity was considerably accentuated when the photocleavage was carried out in the presence of citrate ions. This suggests the presence of a tight binding site for the uranyl ion in the junction. (iii) The same positions were significantly protected from uranyl cleavage by the presence of hexamminecobalt (III) or spermidine. These ions are known to induce the folded conformation of the four-way junction with high efficiency, suggesting a direct competition between the ions. By contrast, magnesium ions failed to generate a similar protection against photocleavage. These results suggest that the uranyl, hexamminecobalt (III) and spermidine ions compete for the same high affinity binding site on the junction. This site is located at the centre of the junction, at the point where the exchanging strands pass between the stacked helices. We believe that we have observed the first known example of a metal ion 'footprint' on a folded nucleic acid structure.

Key words: DNA structure/footprinting/Holliday junction/metal ion interactions/recombination

Introduction

Three-dimensional folding of nucleic acids is of major importance in biological function. This is true both for most RNA species, exemplified by catalytic ribozymes (Celander and Cech, 1991), and for DNA in particular situations such as recombination (Lilley and Clegg, 1993). Metal ions have a critical role to play in the folding processes of these charged species, and in many cases the correct tertiary structure cannot be achieved in their absence (Karpel *et al.*, 1975). In addition, metal ions can play an active part in the function of nucleic acids. Magnesium is essential to the catalytic activity of ribozymes (Uhlenbeck, 1987; Piccirilli *et al.*, 1993), where it is assumed to be held in the folded RNA structure such that it is precisely juxtaposed to the reaction centre so as to participate directly in the reaction. A well-studied example of tertiary folding in DNA that is promoted by metal ions is provided by the four-way DNA junction, believed to be the central intermediate in many recombination events (Holliday, 1964; Meselson and Radding, 1975; Hoess *et al.*, 1987; Kitts and Nash, 1987; Nunes-Düby *et al.*, 1987; Jayaram *et al.*, 1988). In the presence of ions such as magnesium, the junction undergoes a precise folding process (Gough and Lilley, 1985; Cooper and Hagerman, 1987, 1989; Churchill *et al.*, 1988; Duckett *et al.*, 1988; Murchie *et al.*, 1989; von Kitzing *et al.*, 1990; Clegg *et al.*, 1992), while in the absence of added ions the folding is prevented, and the junction remains extended (Duckett *et al.*, 1988, 1990; Clegg *et al.*, 1993). This suggests that the folding might generate an electronegative cleft with high ion occupancy, that reduces electrostatic repulsion such that folding becomes energetically favourable.

We have proposed (Duckett *et al.*, 1988) that in the presence of metal cations the four-way junction adopts a folded conformation by pairwise coaxial stacking of helical arms, to generate an antiparallel, right-handed X-shaped structure (reviewed in Duckett *et al.*, 1992; Lilley and Clegg, 1993). Two isomers of the structure are possible, which differ in the selection of stacking partners. This structure has two non-equivalent types of strand (see Figure 1), termed the continuous strands and the exchanging strands. This structure is completely consistent with all available experimental data, including gel electrophoresis (Gough and Lilley, 1985; Cooper and Hagerman, 1987; Duckett *et al.*, 1988), fluorescence resonance energy transfer (Murchie *et al.*, 1989; Clegg *et al.*, 1992) and probing experiments (Churchill *et al.*, 1988; Murchie *et al.*, 1990, 1991).

Electrostatics clearly play an extremely important role in the formation of the folded stacked X-structure of the four-way junction, since it remains an extended structure in which the four arms are directed towards the corners of a square in the absence of sufficient concentrations of metal ions (Duckett *et al.*, 1988, 1990; Murchie *et al.*, 1989). Presumably the folding of the junction brings about close approximation of charged phosphate groups, and unless these are partially screened by interactions with ions, electrostatic repulsion prevents the folding. The formation of the folded state is likely to be driven principally by the increase in stacking interactions across the junction, but for folding to occur the increase in negative free energy obtained by these means must be greater than the unfavourable change in electrostatic free energy. We have shown that the structure may be perturbed by changes in the nature of the phosphate

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groups at the point of strand exchange (Duckett *et al.*, 1990), and in the character of the basepairing (Duckett and Lilley, 1991).

Ions may interact with polyelectrolytes in two extreme ways. Most interaction between polynucleotides and cations is probably best characterized as atmospheric binding, in which a cloud of highly mobile ions screens the phosphodiester backbone to some degree (Pörschke, 1976). Alternatively, folding of the nucleic acid may generate an electronegative cleft that can site-bind certain ions, leading to high occupancy and slow exchange of the ions. We may therefore ask which of these modes of binding best characterizes the interaction between the folded conformation of the four-way DNA junction and cations. We have found that a number of different ions may fold the junction, with differing efficiencies. Magnesium and other group II cations fold the junction into the stacked X-structure at a critical concentration of ~100 μM (Duckett *et al.*, 1990); this brings about the gel electrophoretic pattern of species with two long and two short arms that is characteristic of the X-shaped structure, and the protection of junction thymine bases against attack by osmium tetroxide which is indicative of helical stacking. Group I metal cations, such as sodium and potassium, appear to bring about a similar folding. Thus a similar pattern of gel mobilities was observed in the presence of these ions (Duckett *et al.*, 1990), and the end-to-end fluorescent energy transfer efficiencies across the small angle of the junction at saturating ion concentrations were similar for sodium and magnesium (Clegg *et al.*, 1992). If group I metals can achieve a folding of the junction then it can be argued that site-binding of ions is unlikely. On the other hand certain aspects of the ion interactions are not completely in accord with this point of view. The concentrations of group I metals required to generate apparently complete folding are extremely high (>0.5 M), the electrophoretic mobility patterns are less symmetrical than those observed in other ions, and junction thymine bases remain reactive to osmium tetroxide (Duckett *et al.*, 1990). It seems that the group I metal cations produce a folded conformation that is very similar to the stacked X-structure in its global geometry, but which remains more open around the point of strand exchange. There is a third class of cation that produces high efficiency folding of the junction. Hexamminecobalt (III) (i.e. the cation $[Co(NH_3)_6]^{3+}$) is the most efficient ion for the stabilization of the four-way junction that we have identified (Duckett *et al.*, 1990), generating a symmetrical gel electrophoretic pattern indicative of the stacked X-structure, and conferring protection to junction thymine bases at concentrations of <2 μM. Polyamines were also found to be relatively efficient in promoting the folding process.

The uranyl ion (UO_2^{2+}) complexes with DNA phosphates, and upon irradiation with light of 420 nM wavelength it oxidizes the two proximal deoxyribose rings to an approximately equal extent (Nielsen *et al.*, 1988), generating a break in the DNA backbone. Although the detailed mechanism has not been elucidated, experiments have indicated that the oxidation most probably occurs via a direct electron transfer mechanism, resulting in 3′ and 5′ termini of the cleaved DNA, a liberated nucleic acid base and as yet unidentified oxidation products of the deoxyribose moiety (Nielsen *et al.*, 1992). Thus, uranyl ion-mediated photocleavage of DNA reflects the accessibility and affinity of phosphates towards ion binding. We asked if the uranyl ion might exhibit elevated affinity for the four-way junction, and whether or not such binding might be affected by competition with other metal cations. Our photocleavage results appear to reflect the presence of a high affinity site for uranyl ions at the point of strand exchange in the junction, and strong competition for binding at this site by hexamminecobalt (III) or spermidine. We conclude that the binding of these large cations is probably best described in terms of site-specific binding.

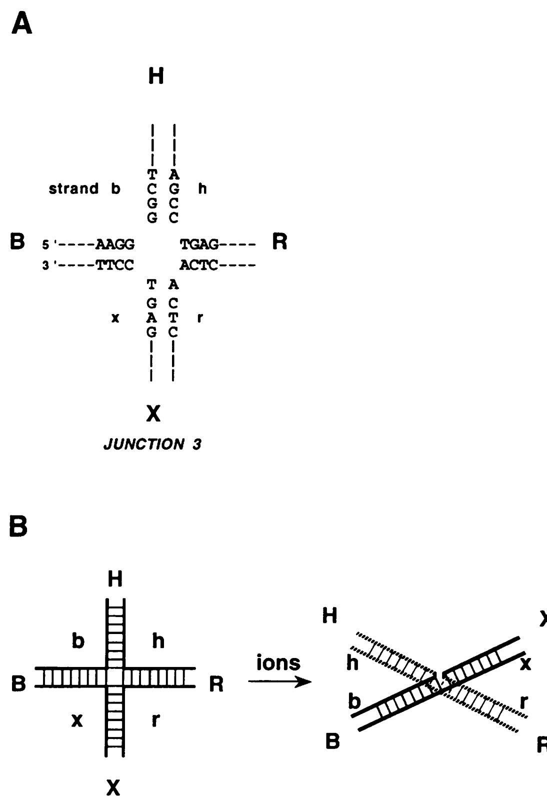

Fig. 1. Junction 3 used in these studies. The junction comprised four strands (b, h, r and x) each of 50 nucleotides in length. The arms are each 25 bp in length and are labelled B, H, R and X. (**A**) The central sequence of the junction used in these investigations. This is the sequence of the junction 3 characterized by gel electrophoresis (Duckett *et al.*, 1988) and fluorescence resonance energy transfer (Clegg *et al.*, 1992) experiments. (**B**) In the presence of added cations the extended form of the four-way junction undergoes folding into the stacked X-structure. In the predominant stacking isomer for junction 3 arms B and X are coaxial, as are arms H and R. Thus strands h and x are continuous strands, while strands b and r are exchanging strands.

Results

We chose to analyse a four-way junction with the central sequence of our extensively characterized junction 3 (Duckett *et al.*, 1988), constructed by the hybridization of four 50 base oligonucleotides (Figure 1). The point of strand exchange was centrally located, giving four arms each of 25 bp. For the studies reported here, the junction was assembled from one oligonucleotide radioactively labelled at the 5′ with ^{32}P, and three unlabelled strands. Corresponding duplex species were also constructed by hybridization of the same radioactive strands to their perfect complementary sequences. We asked whether or not the uranyl (VI) ion might exhibit elevated affinity for the four-

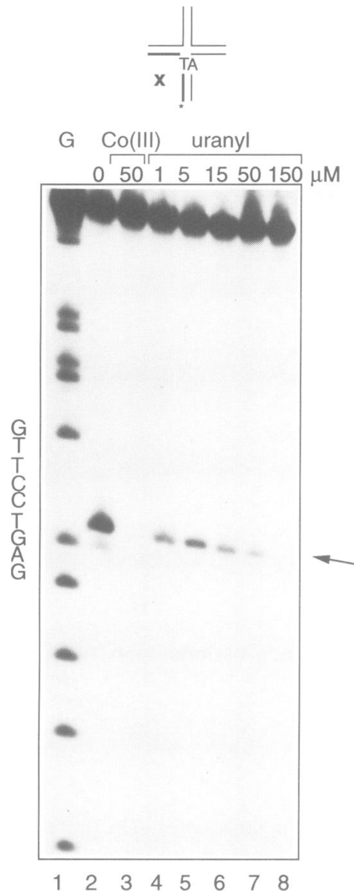

Fig. 2. Osmium tetroxide modification of junction 3. Junction 3 was prepared radioactively 5′-^{32}P-labelled on the x strand (i.e. one of the two strands that have thymine bases at the point of strand exchange—refer to Figure 1). This was reacted with 1 mM osmium tetroxide, 1% pyridine at 0°C for 4 min. The DNA was then subjected to cleavage in 1 M piperidine at 90°C for 30 min, sequencing gel electrophoresis and autoradiography. The junction was reacted with osmium tetroxide under a number of sets of conditions: track 2, 50 mM Tris−HCl (pH 7.2); track 3, 50 mM Tris−HCl (pH 7.2) plus 50 μM hexamminecobalt (III); tracks 4−8, 50 mM Tris−HCl (pH 7.2) plus 1, 5, 15, 50 or 150 μM uranyl nitrate respectively. Track 1 contains a G sequencing marker derived from the x strand by chemical degradation using dimethylsulfoxide (Maxam and Gilbert, 1980). The sequence around the centre of the x strand is indicated on the left. Note the modification of the junction thymine base (arrowed on right) by osmium tetroxide in the absence of added cations, and the suppression of reactivity by both uranyl and hexamminecobalt (III) ions. Exactly equivalent results were also obtained by radioactive labelling of the h strand.

way junction, which might be revealed as sites of elevated sensitivity to cleavage when irradiated at 420 nm, and whether such binding might be affected by competition with other metal cations.

Uranyl ion protects the junction against osmium tetroxide modification

In order to interpret the results of uranyl photocleavage, we first needed to know if the uranyl ion could itself induce a folding of the four-way junction. One of the characteristics of the folded conformation is that thymine bases present at the point of strand exchange become protected against osmium tetroxide addition (Duckett *et al.*, 1988, 1990). We therefore examined the reactivity to osmium tetroxide of the thymine bases located at the point of strand exchange in junction 3, which are found on strands h and x (see Figure 1). Junction 3, 5′-^{32}P-labelled on the x strand, was modified with 1 mM osmium tetroxide as a function of added cations. Following the osmium tetroxide reactions, the DNA was incubated with 1 M piperidine at 90°C to cleave the phosphodiester backbone at the position of osmium *cis*-diester adducts, and the DNA examined by gel electrophoresis and autoradiography. The results are shown in Figure 2. In the absence of added ions, the thymine base at the point of strand exchange was strongly modified by osmium tetroxide (unlike all the remaining thymine bases of the strand, which are stacked into helical stems and therefore protected irrespective of conditions); this reactivity could be suppressed by the addition of hexamminecobalt chloride, as we have shown previously (Duckett *et al.*, 1990). Addition of uranyl nitrate induced strong suppression of reactivity to osmium tetroxide on both strands, at concentrations of ≥100 μM. Equivalent uranyl-induced protection was observed on the h strand (data not shown). These results indicate that uranyl (VI) brings about a folding of the four-way DNA junction, with an efficiency that is similar to that of the magnesium ion.

The four-way junction is hypersensitive to uranyl photocleavage at the point of strand exchange

Junction 3 that was 5′-^{32}P-labelled on the b strand, and the corresponding perfect duplex species, were subjected initially to photocleavage by 1 mM uranyl ion in buffer without additional ions. The autoradiograph is shown in Figure 3A, and densitometric scans in Figure 3B (top left). The photocleavage of the b strand duplex is very even, with no evidence for hyperreactivity at any point. By contrast, the same strand incorporated into the four-way junction exhibited a significant increase in uranyl photocleavage at the GGGG sequence that lies at the point of strand exchange; this is seen most clearly in the scans shown in Figure 3B. When the photocleavage was repeated in the presence of 0.75 mM citrate [which complexes the uranyl and suppresses photocleavage of normal DNA (Jeppersen and Nielsen, 1989; Nielsen *et al.*, 1992)], a very marked hyperreactivity was evident at this position in the junction. Under these conditions, virtually the only photocleavage observed was confined to the central GGGG sequence, with no background cleavage elsewhere in the strand. However, upon performing the photocleavage in the presence of hexamminecobalt (III) [the most efficient ion for folding the four-way junction (Duckett *et al.*, 1990)], we observed the opposite effect, i.e. a protection was now evident at the central GG sequence in the junction. This is most apparent in the scans shown in Figure 3B. Thus the uranyl photocleavage generated a ‘footprint’ of the hexamminecobalt ion on the junction, thereby revealing the position of a high affinity site. This protection induced by the hexamminecobalt ion was specific to the junction, and was not observed on the equivalent duplex species, showing that it resulted from the DNA structure, rather than its sequence.

These experiments were repeated for junction 3 separately labelled in each of the remaining strands; the densitometric scans are compared in Figure 3B. Strands b and r exhibited hypersensitivity both with and without citrate ions, with the most sensitive linkages being located around the point of strand exchange. Hexamminecobalt (III) conferred protection against photocleavage in these same regions. These results

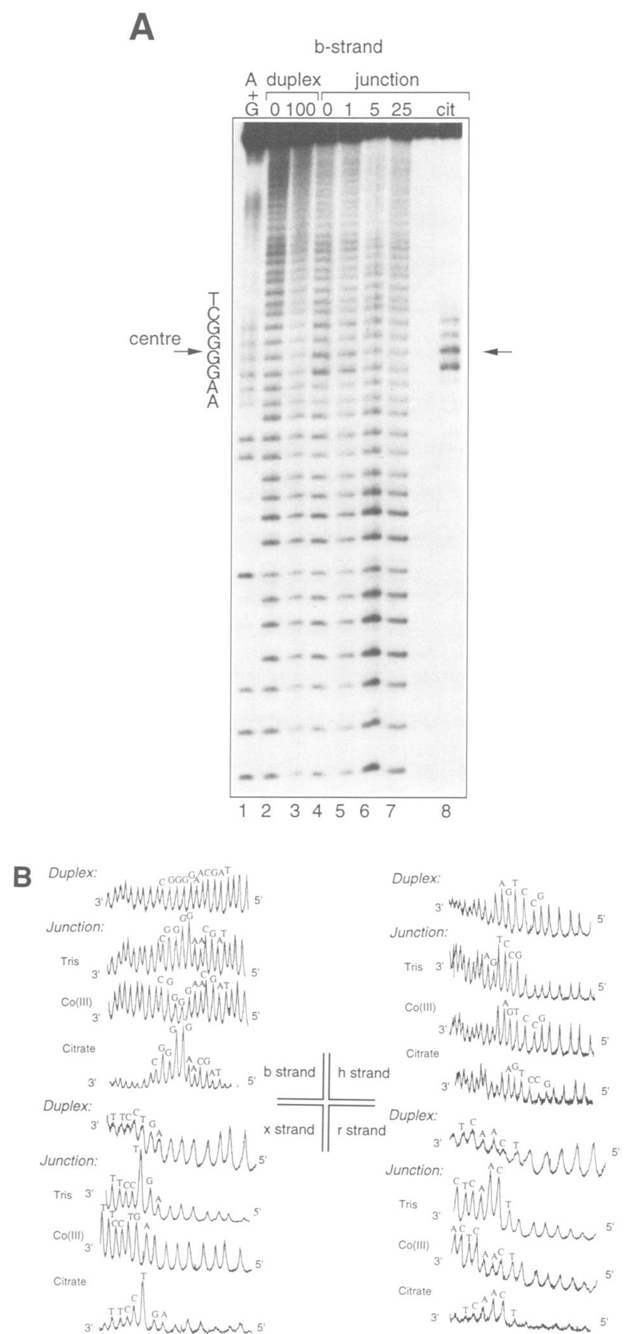

Fig. 3. Results of uranyl photocleavage of junction 3, and the corresponding duplex species. Four forms of the junction (and corresponding duplex molecules) were prepared in which one strand was radioactively 5′-^{32}P-labelled. The different species were subjected to uranyl photocleavage under the conditions indicated below, followed by analysis by electrophoresis on a sequencing gel. This was subjected to autoradiography, and the film scanned by laser densitometry. (**A**) Autoradiograph of sequencing gel containing the analysis of uranyl photocleavage of junction 3 5′-^{32}P-labelled in the b strand. Track 1, A+G sequencing reaction; track 2, duplex photoreacted in 10 mM Tris−HCl (pH 7.2); track 3, duplex photoreacted in 10 mM Tris−HCl (pH 7.2) plus 100 μM hexamminecobalt chloride; tracks 4−7, junction 3 photoreacted in 10 mM Tris−HCl (pH 7.2) plus 0, 1, 5 or 25 μM hexamminecobalt chloride respectively; track 8, junction photoreacted in 50 mM Tris−HCl (pH 7.2), 0.75 mM citrate. The sequence at the centre of strand b is indicated on the left, and the arrows indicate the point of strand exchange. Note the pronounced photocleavage observed around the point of strand exchange in the presence of citrate ion (track 8). (**B**) Densitometric scans of the equivalent autoradiographs from experiments of junction 3 individually 5′-^{32}P-labelled on each of the four strands.

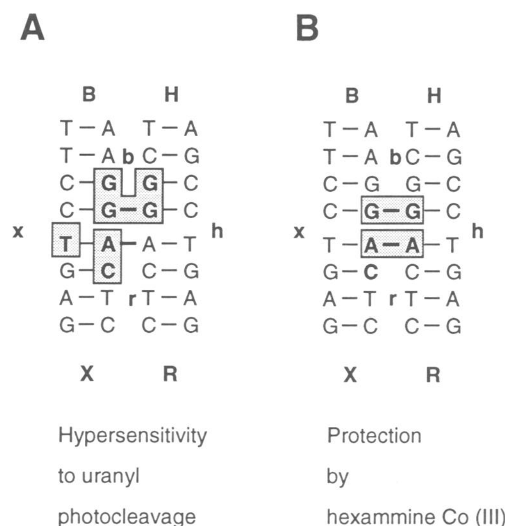

Fig. 4. Summary of major sites of elevated reactivity and protection in the four-way DNA junction. (**A**) Positions of hypersensitivity to uranyl photocleavage, and (**B**) positions of protection conferred by hexamminecobalt (III) ion against photocleavage. Note that the predominant hypersensitivities and protections are found on the exchanging strands, around the point of strand exchange.

are summarized in Figure 4. In the folded stacked X-structure for junction 3 (see Figure 1) there is coaxial stacking of arms B and X (Duckett *et al.*, 1988; Murchie *et al.*, 1989; Clegg *et al.*, 1992), and strands b and r are the exchanging strands in this conformation. Thus the nucleotides defining the point of strand exchange are hypersensitive to photocleavage by the uranyl ion, and the location of the cobalt ion footprint. In addition, a single hypersensitive nucleotide was observed at the centre of strand x, which is a continuous strand in this stacking isomer of the stacked X-structure. This site was not protected by hexamminecobalt (III) relative to the corresponding duplex, although the presence of the ion did abolish the hyperreactivity. These results suggest a specific interaction of the uranyl ion with the region of strand exchange in the folded conformation of the junction.

Effects of other cationic species on uranyl-mediated photocleavage of the junction

Uranyl photocleavage was carried out in the presence of other cations (Figure 5). In the presence of magnesium ions the hyperreactivity to uranyl-induced photocleavage was reduced, but no distinct ion footprint was observed. Calcium ions had no effect on photocleavage. Sodium ions (or potassium ions) produced very little change in the pattern of photocleavage, even at 150 mM. By contrast, 50 μM spermidine generated a localized footprint, similar to that observed with hexamminecobalt (III). It is significant that, after hexamminecobalt (III), the polyamines were the most efficient species observed to promote the folding of the four-way junction (Duckett *et al.*, 1990).

Discussion

Studies of a four-way DNA junction using the uranyl ion-induced photocleavage has revealed the following. (i) Osmium tetroxide probing indicates that uranyl ion efficiently induces folding of the junction into a stacked conformation. (ii) Above-background uranyl photocleavage

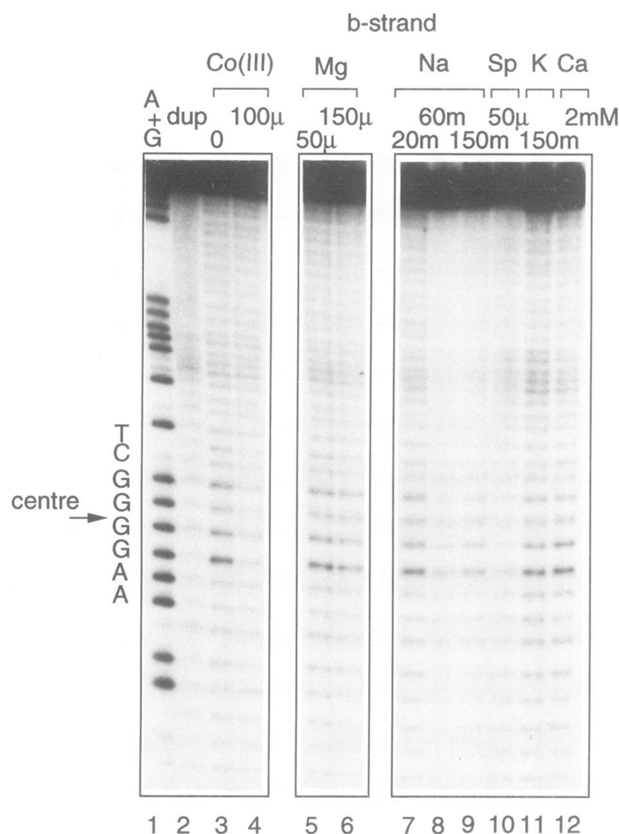

Fig. 5. Effects of difference cations on the photocleavage of the b strand of junction 3. Autoradiograph of sequencing gel containing the analysis of uranyl photocleavage in the presence of the indicated ions of junction 3 5'-^{32}P-labelled in the b strand. Ion concentrations given above tracks are in mM (indicated by m) or μM (indicated by μ). Track 1, A+G sequencing reaction; track 2, duplex photoreacted in 10 mM Tris–HCl (pH 7.2); track 3, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2); track 4, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 100 μM hexamminecobalt chloride; tracks 5 and 6, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 50 and 150 μM magnesium chloride respectively; tracks 7–9, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 20, 60 and 150 mM sodium chloride respectively; track 10, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 50 μM spermidine; track 11, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 150 mM potassium chloride; track 12, junction 3 photoreacted in 10 mM Tris–HCl (pH 7.2) plus 2 mM calcium chloride. The sequence at the centre of strand b is indicated on the left, and the arrow indicates the point of strand exchange.

was observed immediately at the point of strand exchange on the two exchanging strands. These were enhanced when the photocleavage was carried out in the presence of citrate ions. (iii) The same positions were protected from uranyl photocleavage in the presence of hexamminecobalt (III) ions or spermidine. This suggests a direct competition between the ions. (iv) One continuous strand was also hypersensitive to uranyl photocleavage. However, this position did not exhibit hexamminecobalt (III) protection relative to duplex, although hexamminecobalt (III) ions did abolish the hypersensitivity.

Thus these results suggest the presence of a defined and tight binding site for ions such as hexamminecobalt (III) and UO_2^{2+}, around the point of strand exchange in the four-way junction (Figure 6). The phosphate groups at this position are those that have been previously identified as important sources of electrostatic interactions by replacement with electrically neutral methyl phosphonate groups (Duckett

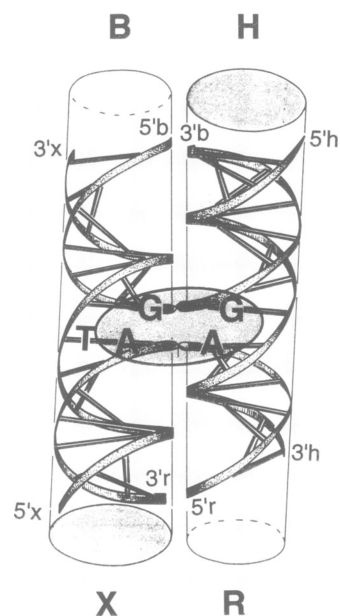

Fig. 6. Area of hypersensitivity and hexamminecobalt (III) protection on the stacked X-structure of the four-way junction. Schematic shows a ribbon model of the minor groove side view of the junction (Murchie *et al.*, 1989), with the region of hypersensitivity protection shown stippled. The principal area of the junction to be affected is the region of strand exchange.

et al., 1990). The hypersensitivities to photocleavage were reduced by magnesium ions, but little protection was apparent with sodium or potassium concentrations up to 150 mM. The degree of protection conferred by different ions (hexamminecobalt (III) $\geq$ spermidine > magnesium >> sodium) correlates well with their efficiencies in folding the four-way junction (Duckett *et al.*, 1990). The differences could simply reflect a higher affinity for the more complex ions, or they might indicate binding of a different character. This could stem from a number of characteristics of the different ions. Hexamminecobalt (III) is extremely efficient in promoting a number of structural transitions in DNA, such as the formation of left-handed Z-DNA (Behe and Felsenfeld, 1981) and the stabilization of four-way junctions, and this is likely to be a function of charge, size and the ability to donate hydrogen bonds. Uranyl lacks the ammine ligands that are such effective hydrogen bond donors, and its localized binding in the junction therefore suggests the probable importance of ionic radius. This might mean, for example, that the larger ions can locate between the phosphate groups on the two exchanging strands of the stacked X-structure (see Figure 6). The failure to observe a pronounced ion footprint (relative to duplex) by magnesium ions might indicate that the smaller ions neutralize these phosphates by binding with rapid exchange in and out of the site, or by atmospheric binding, although a degree of site-binding by magnesium seems probable. The manner of ion interaction is therefore likely to be a function of the nature both of the ion and its binding site, and this is probably true for the majority of folded nucleic acids.

Materials and methods

Oligonucleotide synthesis

Oligonucleotides were synthesized by β-cyanoethylphosphoramidite chemistry (Beaucage and Caruthers, 1981; Sinha *et al.*, 1984) implemented

on an Applied Biosystems 394 DNA synthesizer. After purification by gel electrophoresis, they were radioactively labelled using T4 polynucleotide kinase and [γ-^{32}P]ATP (Maxam and Gilbert, 1980).

Construction of DNA junctions and duplexes

Junctions and duplexes were constructed by annealing stoichiometric quantities of the appropriate oligonucleotides (one of which carried a 5′-^{32}P radioactive label) for 2 h at 65°C in 450 mM NaCl, 45 mM sodium citrate, 1 mM $MgCl_2$ followed by slow cooling. Junctions and duplexes were purified by gel electrophoresis in 5 or 10% polyacrylamide, excision of bands, electroelution and ethanol precipitation.

Uranyl photocleavage of DNA

Radioactive DNA samples (5 pmol) were irradiated in 100 μl volumes for 30 min in 50 mM Tris−HCl (pH 7.2), 1 mM uranyl nitrate, with or without the additional components indicated in the text (Nielsen *et al.*, 1988). The light source was a Phillips Tl 40 W/03 fluorescent tube emitting light at a wavelength of 420 nM. Following irradiation, DNA was recovered by ethanol precipitation in the presence of 0.2 M sodium acetate (pH 4.5), and analysed by gel electrophoresis.

Osmium tetroxide reactions

Labelled DNA was incubated with 1 mM osmium tetroxide (Duckett *et al.*, 1988), 1% pyridine in 50 mM Tris−HCl (pH 7.2), together with the additions indicated in the text at 0°C for 4 min. DNA was recovered by precipitation with ethanol and cleaved with 1 M piperidine at 90°C for 30 min, followed by extensive lyophilization.

Sequencing of DNA

Radioactively labelled oligonucleotides were sequenced by chemical degradation (Maxam and Gilbert, 1980). DNA was reacted with dimethylsulfoxide (G specific) or formic acid (A+G specific), and cleaved with 1 M piperidine at 90°C for 30 min, before extensive lyophilization.

Electrophoresis of DNA

DNA photocleavage and osmium modification were analysed by electrophoresis in 15% polyacrylamide gels in 90 mM Tris−borate (pH 8.3), 0.1 mM EDTA containing 7 M urea. Radioactivity was visualized by autoradiography and quantified by laser densitometry (Molecular Dynamics).

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References

Beaucage,S.L. and Caruthers,M.H. (1981) *Tetrahedron Lett.*, **22**, 1859−1862.
Behe,M. and Felsenfeld,G. (1981) *Proc. Natl Acad. Sci. USA*, **78**, 1619−1623.
Celander,D.W. and Cech,T.R. (1991) *Science*, **251**, 401−407.
Churchill,M.E., Tullius,T.D., Kallenbach,N.R. and Seeman,N.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 4653−4656.
Clegg,R.M., Murchie,A.I.H., Zechel,A., Carlberg,C., Diekmann,S. and Lilley,D.M.J. (1992) *Biochemistry*, **31**, 4846−4856.
Clegg,R.M., Murchie,A.I.H., Zechel,A. and Lilley,D.M.J. (1993) *Biophys. J.*, **66**, 99−109.
Cooper,J.P. and Hagerman,P.J. (1987) *J. Mol. Biol.*, **198**, 711−719.
Cooper,J.P. and Hagerman,P.J. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 7336−7340.
Duckett,D.R. and Lilley,D.M.J. (1991) *J. Mol. Biol.*, **221**, 147−161.
Duckett,D.R., Murchie,A.I.H., Diekmann,S., von Kitzing,E., Kemper,B. and Lilley,D.M.J. (1988) *Cell*, **55**, 79−89.
Duckett,D.R., Murchie,A.I.H. and Lilley,D.M.J. (1990) *EMBO J.*, **9**, 583−590.
Duckett,D.R., Murchie,A.I.H., Bhattacharyya,A., Clegg,R.M., Diekmann,S., von Kitzing,E. and Lilley,D.M.J. (1992) *Eur. J. Biochem.*, **207**, 285−295.
Gough,G.W. and Lilley,D.M.J. (1985) *Nature*, **313**, 154−156.
Hoess,R., Wierzbicki,A. and Abremski,K. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 6840−6844.
Holliday,R. (1964) *Genet. Res.*, **5**, 282−304.
Jayaram,M., Crain,K.L., Parsons,R.L. and Harshey,R.M. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 7902−7906.
Jeppersen,C. and Nielsen,P.E. (1989) *Nucleic Acids Res.*, **17**, 4947−4956.
Karpel,R.L., Miller,N.S., Lesk,A.M. and Fresco,J.R. (1975) *J. Mol. Biol.*, **97**, 519−532.
Kitts,P.A. and Nash,H.A. (1987) *Nature*, **329**, 346−348.
Lilley,D.M.J. and Clegg,R.M. (1993) *Annu. Rev. Biophys. Biomol. Struct.*, **22**, 299−328.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499−560.
Meselson,M.S. and Radding,C.M. (1975) *Proc. Natl Acad. Sci. USA*, **72**, 358−361.
Murchie,A.I.H., Clegg,R.M., von Kitzing,E., Duckett,D.R., Diekmann,S. and Lilley,D.M.J. (1989) *Nature*, **341**, 763−766.
Murchie,A.I.H., Carter,W.A., Portugal,J. and Lilley,D.M.J. (1990) *Nucleic Acids Res.*, **18**, 2599−2606.
Murchie,A.I.H., Portugal,J. and Lilley,D.M.J. (1991) *EMBO J.*, **10**, 713−718.
Nielsen,P.E., Jeppersen,C. and Buchardt,O. (1988) *FEBS Lett.*, **235**, 122−124.
Nielsen,P.E., Hiort,C., Sonnichsen,S.H., Buchardt,O., Dahl,O. and Norden,B. (1992) *J. Amer. Chem. Soc.*, **114**, 4967−4975.
Nunes-Düby,S.E., Matsomoto,L. and Landy,A. (1987) *Cell*, **50**, 779−788.
Piccirilli,J.A., Vyle,J.S., Caruthers,M.H. and Cech,T.R. (1993) *Nature*, **361**, 85−88.
Pörschke,D. (1976) *Biophys. Chem.*, **4**, 383−394.
Sinha,N.D., Biernat,J., McManus,J. and Koster,H. (1984) *Nucleic Acids Res.*, **12**, 4539−4557.
Uhlenbeck,U.C. (1987) *Nature*, **328**, 596−600.
von Kitzing,E., Lilley,D.M.J. and Diekmann,S. (1990) *Nucleic Acids Res.*, **18**, 2671−2683.

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