## Structural perturbation in supercoiled DNA: hypersensitivity to modification by a single-strand-selective chemical reagent conferred by inverted repeat sequences

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ABSTRACT

Bromoacetaldehyde, a reagent which modifies unpaired adenine residues, selectively modifies supercoiled DNA in the region of inverted repeats which are known targets for single-strand-specific nucleases. The reaction is dependent upon the topological state of the molecule, and the absolute importance of the inverted repeat has been demonstrated. Finer mapping of the distribution of the modification pattern reveals significant and interesting differences from the S1 nuclease target positions. Bromoacetaldehyde modification is distributed over a wider region covering the whole inverted repeat, with greatest extent of reaction in the regions which flank the inverted repeat. It is suggested that an altered conformation may be propagated into these sequences. These results further support the contention that inverted repeats adopt an altered conformation when negatively supercoiled, for which the principal suggestion remains the cruciform structure.

INTRODUCTION

Many natural DNA molecules exist as torsionally underwound, or negatively supercoiled, species, and this has both structural and functional consequences. The free energy of supercoiling will drive processes which effectively reverse the underwinding, i.e., a local twist reduction may partially compensate for the linkage deficiency, and allow the molecule to adopt a more relaxed overall conformation. Perhaps the simplest example of this behaviour is the pre-melting of supercoiled DNA (1), and the high transcriptional efficiency of supercoiled templates (2,3) is another consequence. Even greater twist change occurs when a B to Z transition is undergone, and the stabilisation of local sections of Z-DNA by underwinding has been demonstrated (4,5). Thus there is both a strong theoretical case and considerable experimental support for supercoil-driven perturbation of DNA structure. Sequence-dependent structural polymorphism in DNA has become a subject of renewed interest with recent crystallographic (6,7) and solution (4,5,8-10) studies.

Negatively supercoiled circular DNA molecules possess topologically-generated single-stranded-like properties and are cleaved by single-strand-

specific nucleases such as S1 nuclease (11). Examination of preferred sites of cleavage by these enzymes reveal that great site selectivity occurs (12-14) and mapping of these sites has shown each of them to possess inverted repeat sequence symmetry. Inverted repeats, or palindromes, have a formal conformational alternative, that of the intra-strand bonded hairpin loop or cruciform structure (15,16). This has been the most seriously considered explanation for the nuclease hypersensitivity data (12-14,17-20) for several reasons. Firstly, it provides a single-stranded loop region at the extreme of the stem-loop structure which might be expected to be susceptible to nucleolysis by a single-strand-specific nuclease, and such a view is supported by fine mapping of cleavages in the region (13). Secondly, cruciform structures are expected to be stabilised by negative supercoiling (2,12,14) since they provide a positive twist equivalent to melting the entire region, but at a fraction of the free energetic cost. Experimental observation and theory are once again in accord in terms of dependence upon both twist changes imparted (13) and linking deficiency (21,22, Otter, R., Lilley, D.M.J. and Cozzarelli, N., unpublished data). There remains, however, a serious reservation concerning the interpretation of data obtained by the use of single-strand-specific nucleases. In order to recognise single-stranded DNA, these enzymes must be capable of binding to it and thus they must possess single-strand stabilising protein-like activity. There exists a theoretical possibility, therefore, that in the absence of the enzyme the inverted repeats remain entirely in a regular helical conformation. Long inverted repeats produced in vitro have been shown to adopt cruciform geometry (23) when sufficiently negatively supercoiled, although for the particular sequences studied it appears that the activation energy involved in the interconversion process is relatively high (24). The question remains, therefore, whether or not the S1 hypersensitive sites previously demonstrated in viable plasmids reflect cruciform structure which pre-exist in the torsionally stressed DNA. To attempt to answer this question a non-enzymic probe was sought, i.e. a single-strand-selective modification reagent. Such a species would have to be a small molecule for which there is no question of conformational stabilisation of the DNA. There are several documented examples of single-strand-specific chemical reactions (25-27) but the reagent chosen for the present investigation was bromoacetaldehyde, which has been developed as a probe of single-stranded DNA character by Kohwi-Shigematsu and Weintraub (28). The haloacetaldehydes (29) form an adduct with adenine residues at the hydrogen bonding nitrogen atoms and are thus ideally single-strand-selective. In this study it emerges that

inverted repeats confer considerable local hypersensitivity to modification by this reagent, but that the distribution of modification sites is different from the S1 cleavage loci. These results clearly provide further evidence for a pronounced sequence-dependent structural perturbation in DNA, and one which is dependent upon the topological state of the molecule.

## MATERIALS AND METHODS

### Plasmids

pColIR215 is a derivative of pBR322 where the region flanked by EcoRI and BamHI sites has been replaced by a 440bp fragment of ColE1 DNA containing the inverted repeat which confers strong S1 nuclease hypersensitivity both on ColE1 and pColIR215 (13).

pIRbke8 is a derivative of pAT153 containing a synthetic inverted repeat, and will be fully described elsewhere (30). In brief two 13 base oligonucleo-tides synthesised using phosphotriester methodology by Dr. A.F. Markham (Imperial Chemical Industries, Great Britain) were ligated to form a perfectly two-fold-symmetrical dimer, and cloned into the BamHI site of pAT153. The resulting plasmid is identical with pAT153 in every respect except the addit-ional inverted repeat generated, the sequence of which is shown in Figure 2. Both plasmids were propagated in E.coli K12 HB101. Plasmid DNA was prepared by chloramphenicol amplification of log phase cells. After 16h cells were lysed using lysozyme, EDTA and Triton X-100 and supercoiled DNA isolated by isopycnic centrifugation in caesium chloride and ethidium bromide. Plasmid DNA was recovered by side puncture, butan-1-ol extraction and ethanol precipitation.

### Bromoacetaldehyde

Bromoacetaldehyde was prepared according to Secrist et al. (29) and Kohwi-Shigematsu and Weintraub (28). Ten grams of bromoacetaldehyde diethylacetal were refluxed with 50 ml of 50% (w/v) sulphuric acid for 45 min, followed by distillation under reduced pressure. The distillate was adjusted to pH 4.5 using 1M sodium hydroxide and redistilled. The second distillate was approx-imately 1M bromoacetaldehyde.

Supercoiled DNA was reacted with bromoacetaldehyde in a buffer containing 50mM sodium acetate pH 4.5. Typically 2 µg plasmid was incubated for 30 min in a 50 µl total volume containing 100mM bromoacetaldehyde. Unreacted reagent was then removed by extraction into an organic phase, either buffered phenol or chloroform, isoamyl alcohol (24:1) mixture, followed by ethanol precipi-tation. Modified DNA was restriction cleaved under standard conditions.

After ethanol precipitation the now linearised DNA was digested with 8 units of S1 nuclease in 30mM sodium acetate pH 4.6, 50mM sodium chloride and 1mM zinc chloride for 30 min at 37⁰.

S1 nuclease

    S1 nuclease from aspergillus oryzae was obtained from Bethesda Research Laboratories, and gave results which were indistinguishable with those obtained using S1 nuclease purified locally using the method of Vogt (31).

Restriction Enzymes

    Restriction enzymes were obtained from either New England Biolabs or Bethesda Research Laboratories, and used as directed by the manufacturer.

[32P] Kinase Labelling

    DNA to be radioactively labelled with $^{32}$P at 5'-termini was dephosphory-lated by incubation with 15u of bacterial alkaline phosphatase (Bethesda Research Laboratories) at 65⁰C for 60 min in 20mM Tris pH 7.5, 0.1% SDS, followed by removal of enzyme using 20 μg/ml of proteinase K (Bethesda Research Labs) at 37⁰C for 30 min followed by phenol extraction and ethanol precipitation. Dephosphorylated DNA was [32P] labelled using 25u T4 poly-nucleotide kinase (New England Biolabs) with 10 to 50 μCi 2000 Ci/mMol [γ-$^{32}$P]ATP (Amersham) in 50mM Tris pH 7.6, 10mM $MgCl_2$, 0.1mM EDTA and 5mM DTT. Alternatively, some marker fragments were exchange labelled (32).

Electrophoresis

    1.3% agarose gels and 5-10% polyacrylamide gels were performed vertically using 90mM Tris pH 8.3, 90mM borate, 10mM EDTA for 16h. For autoradiography, polyacrylamide gels were dried on 3MM paper (Whatman) and exposed to Kodak X-omat H film at -70⁰C using an Ilford fast tungstate intensifier screen.


RESULTS

    Bromoacetaldehyde, like its chloro-analogue (29) may react at pH 4.5 with the nitrogen atoms of adenine bases to form a cyclic etheno derivative, shown in Figure 1, with recognised fluorescent properties. Since these two nitrogen atoms form the donor and acceptor atoms in the hydrogen bonded adenine-thymine base pair, they are only available for modification when the base is unpaired, and thus the reagent is single-strand-selective and may be used as a probe for 'melted' regions in DNA. In most of the experiments described here super-coiled DNA has acted as a substrate for bromoacetaldehyde modification, with the following scheme of sequential reactions used to identify regions which have been modified:
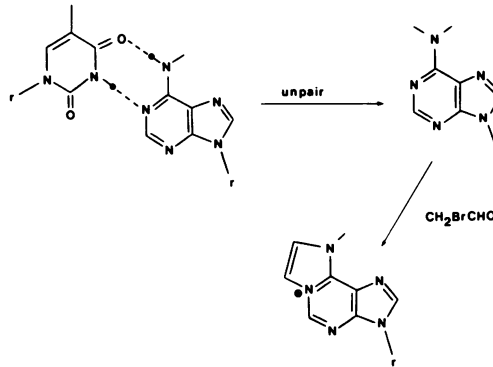
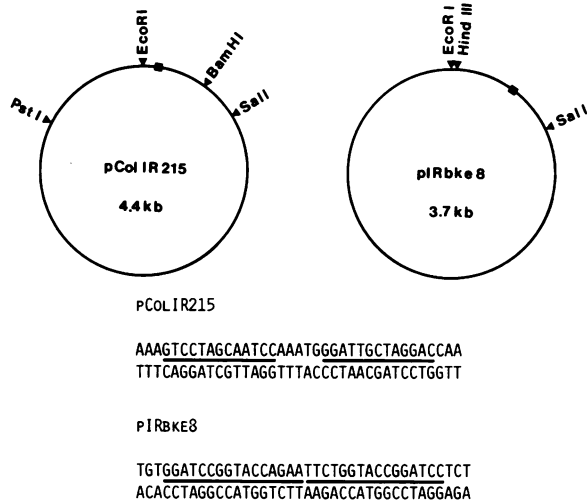Figure 1: Chemistry of bromoacetaldehyde reaction with unpaired adenine to form an etheno derivative.

   (i)   The supercoiled DNA was reacted with bromoacetaldehyde and the DNA purified;

  (ii)   The modified DNA was cleaved by a restriction enzyme to give a linear species;

(iii)   The resulting DNA fragment(s) was cleaved by S1 nuclease.

The rationale is that putative sites of hyper-reactivity towards the single-strand-selective reagent become modified and are therefore unable to re-pair, thus forming a 'bubble' of disrupted base-pairing. Following restriction cleavage which serves two purposes, to remove the torsional constraint and to provide a reference point for the subsequent cleavage, this perturbed region is cleaved by the single-strand-specific nuclease S1. Discrete fragments (i.e. narrow bands on gel electrophoresis) may only arise as a result of sequence-selective modification thereby conferring S1 nuclease hypersensitivity to a specific region of the DNA molecule, which is necessarily in a fixed disposition with regard to the restriction site used.

Hypermodification occurs at inverted repeats

    The plasmid pColIR215 (13), see Figure 2, was used in these studies since the ColE1 inverted repeat that it contains is an extremely strong S1 cleavage site when supercoiled. This sequence is, moreover, quite rich in adenine bases, the target for modification.

    Figure 3 shows a result of pColIR215 reaction as a function of bromoacetaldehyde concentration, monitored by subsequent SalI and then S1 cleavages. Whilst at the lowest bromoacetaldehyde concentration the predominant species observed is the full-length linear plasmid (L1), with higher concentrations of

PCoLIR215

AAAGTCCTAGCAATCCAAATGGGATTGCTAGGACCAA
TTTCAGGATCGTTAGGTTTACCCTAACGATCCTGGTT

PIRBKE8

TGTGGATCCGGTACCAGAATTCTGGTACCGGATCCTCT
ACACCTAGGCCATGGTCTTAAGACCATGGCCTAGGAGA

Figure 2: Maps of plasmids used in these studies, pColIR215 and pIRbke8,
showing relevant restriction sites and inverted repeats (filled boxes). The
sequences of the inverted repeat regions are presented, with symmetric regions
underscored. Sequence data for pColIR215 and the flanking region of pIRbke8
are taken from the data of Oka et al. (42) and Sutcliffe (43) respectively.


reagent increasing amounts of a second, smaller, linear species (L2) is seen,
with a relatively low background due to non-selective modification. Thus the
bulk of the reaction is being localised to a specific region of the pColIR215
circle, even after extensive reaction. Careful examination of Figure 3 also
reveals the presence of weakly stained bands corresponding to the shorter
(-600bp) fragment. The sum of these fragments and the L2 fragments is equal
to the length of the full length linear species. It should be emphasised that
discrete fragments smaller than full length linear species can only arise from
the bromoacetaldehyde modification, and not solely from the final S1 nuclease
step. Smaller fragments must come about from a fixed restriction cleavage -
S1 cleavage relationship, and since the order in which these reactions are
performed is fixed this has to result from S1 cleavage of a previously restric-
tion enzyme-cleaved species. In the absence of DNA modification it has been
demonstrated (12) that S1 has no selective sites of cleavage on linear DNA,
and therefore the S1 cleavage seen here must be a result of sequence selective
modification of the supercoiled DNA at the initial stage.
    In order to map the modification site unambiguously it is necessary to
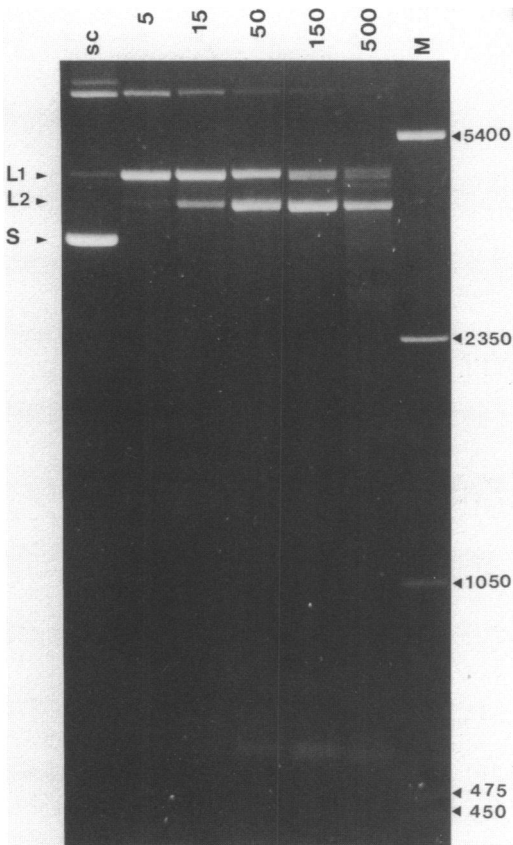repeat the reaction sets using other restriction cleavages, and examples are

Figure 3: Agarose gel electrophoresis of modification of pColIR215 as a function of bromoacetaldehyde concentration. pColIR215 was reacted with the indicated (5 to 500) millimolarity of bromoacetaldehyde, cleaved with SalI followed by S1 nuclease. sc, supercoiled pColIR215; M, marker fragments obtained by HindIII restriction cleavage of phage PM2 DNA - sizes in base pairs indicated on right; S, position of native supercoiled plasmid migration; L1 and L2, migration position of full length and modified cleaved linear species respectively.

presented in Figure 4(a). In this experiment pColIR215 modified by bromo-acetaldehyde has been cleaved with either BamHI, SalI or PstI before S1 cleavage. From the known positions of these targets, see Figure 2, it can be deduced that the modification site lies approximately 100bp from EcoRI on the side distal to the replication origin. Subject to an experimental error of about ±20bp this region coincides with the position of the ColE1 inverted repeat whose sequence is given in Figure 2, and which is responsible for the strong S1 hypersensitivity of this plasmid. To emphasise this point directly analogous bromoacetaldehyde (B) and S1 (S) experiments have been electro-phoresed in consecutive tracks in the gel shown in Figure 4(b). Within the resolution of the gel system, the fragments which arise from bromoacetaldehyde modification and S1 cleavage comigrate, indicating a common target region.

One difference between the two reactions, however, lies in their respective
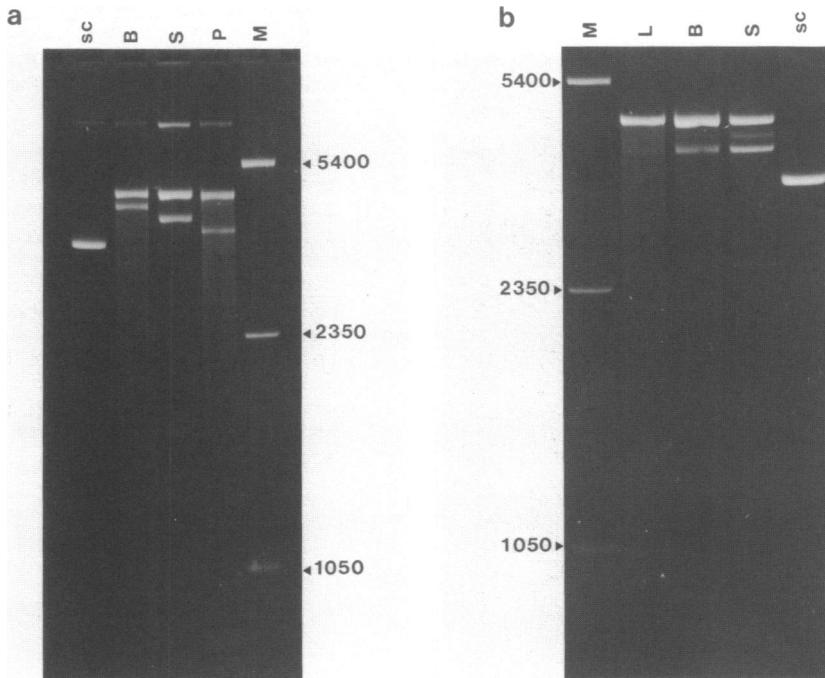
Figure 4: Bromoacetaldehyde modification of pColIR215.
(a) Restriction mapping of modification position of pColIR215 using restric-
    tion cleavage by BamHI (B), SalI (S) and PstI (P).  sc, supercoiled
    pColIR215; M, phage PM2 digested with HindIII.
(b) Importance of supercoiling, and comparison with S1 cleavage.  L, SalI
    linearised DNA reacted with bromoacetaldehyde followed by S1; B, super-
    coiled plasmid reacted with bromoacetaldehyde, then cleaved by SalI
    followed by S1; S, supercoiled plasmid cleaved by S1 followed by Sa.I;
    sc, supercoiled pColIR215; M, phage PM2 digested with HindIII.

temperature dependences.  Whilst the inverted repeat of ColEl and its deriv-

atives may be S1 cleaved even at low temperatures (4°C), the bromoacetaldehyde

reaction is negligible below 30°C.

    A second point arises from Figure 4(b).  Track L is the result of bromo-

acetaldehyde modification of linear pColIR215 DNA, as opposed to the previous

studies on supercoiled DNA.  This is achieved by reversing the sequence in

which the bromoacetaldehyde and restriction endonucleolysis reactions are

performed.  No discrete bands are seen in this track, in contrast to those

arising from S1 cleavage or bromoacetaldehyde modification of supercoiled

plasmid.  Clearly the localised modification at or around the inverted repeat

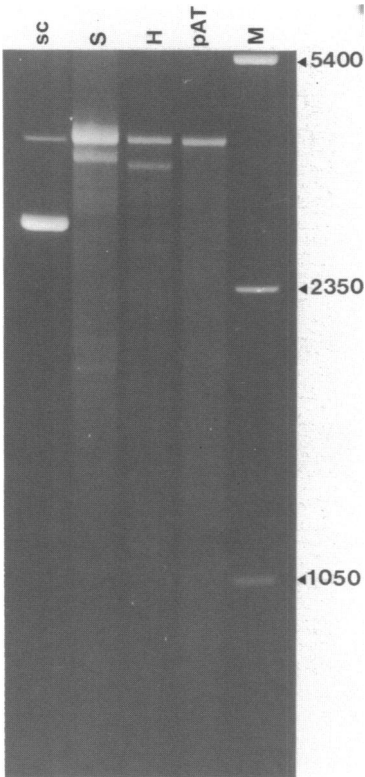sequence requires that the molecule be torsionally stressed, just as does S1

Figure 5: Bromoacetaldehyde modification of pIRbke8 and its parent plasmid pAT153. pIRbke8 was reacted with bromoacetaldehyde followed by restriction cleavage with SalI (S) or HindIII (H). pAT153 (pAT) was analogously treated as in (H). sc, supercoiled pIRbke8; M, phage PM2 digested by HindIII.

cleavage at the same sequence (12,14).

Analogous experiments have been performed on a second plasmid, pIRbke8, see Figure 2. This plasmid contains a synthetic inverted repeat which acts as a strong S1 site (30), although not as strong as that of pColIR215. Like that of pColIR215 it is rich in adenine residues and might therefore be susceptible to bromoacetaldehyde modification. The results of these experiments are presented in Figure 5 and it is clear that just as with pColIR215 discrete bands are produced after modification, restriction cleavage and S1 nucleolysis reactions. Measurement of fragment lengths localises the position of hyper-modification to the region of the inverted repeat of this molecule. This site is, however, modified less readily than that of pColIR215, requiring greater bromoacetaldehyde concentration or longer reaction times, and in general producing weaker bands superimposed on a greater smear arising from non-selective reaction. In general terms, however, these two inverted repeats are evidently exhibiting analogous hypersensitivity towards the single-strand-
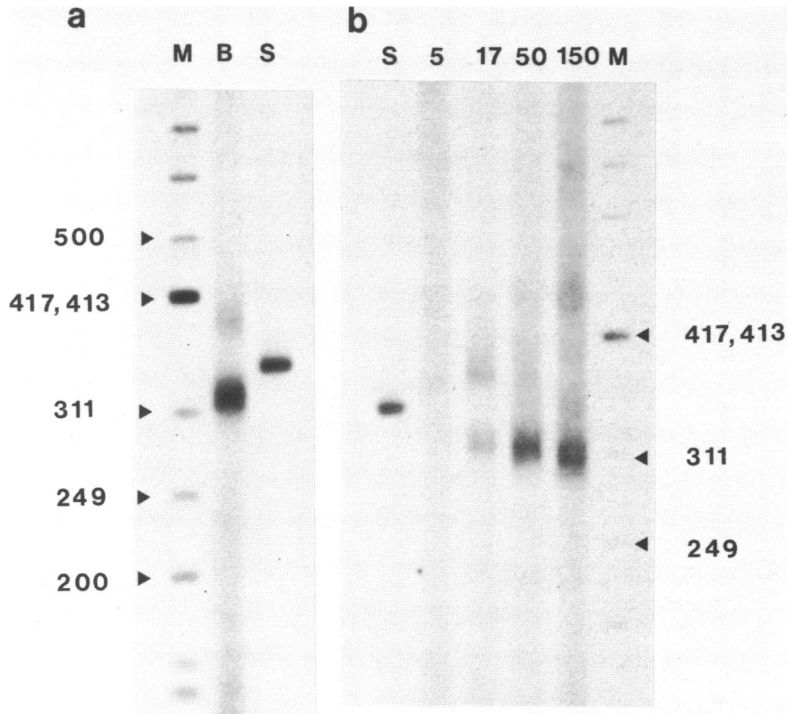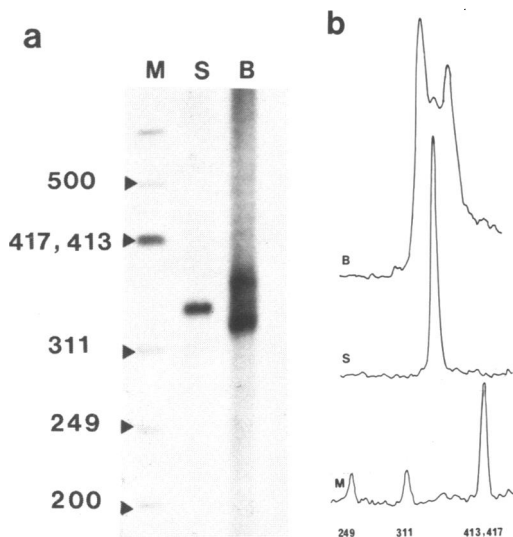
Figure 6:   Finer mapping of modification - cleavage pattern in pColIR215.
(a) Comparison of S1 and bromoacetaldehyde targets.   Autoradiograph of 5%
    polyacrylamide gel electrophoresis of [5'-$^{32}$P] labelled -340bp fragments
    arising from S1, BamHI cleavage (S) or bromoacetaldehyde (25mM), BamHI,
    S1 cleavage (B).   M, phage $\phi$X174RF DNA digested by HinfI and exchange
    labelled with [$^{32}$P] as marker fragments.
(b) Modification pattern as a function of bromoacetaldehyde concentration,
    M and S as in (a); other tracks, modification by indicated millimolarity
    of bromoacetaldehyde.


selective reagent.

     The unique importance of the inverted repeat itself is demonstrable by the

direct comparison of results of bromoacetaldehyde modification experiments on

pIRbke8 and its parent plasmid pAT153 (33).   Figure 5 shows that the specific

fragments observed with pIRbke8 have no counterpart in the case of pAT153.

Since the only difference between the two plasmids is the introduction of the

inverted repeat it is necessarily concluded that this feature, and this alone,

confers the hypersensitivity to bromoacetaldehyde (and also to S1 nuclease).

Distribution of modification

     In order to examine the distribution of bromoacetaldehyde modification in

Figure 7: Finer mapping of modification - cleavage pattern in pIRbke8.
(a) Autoradiograph of 5% polyacrylamide gel electrophoresis of [5'-$^{32}$P]
    labelled -360bp fragments arising from S1, HindIII cleavage (S) or bromo-
    acetaldehyde, HindIII, S1 cleavage (B).  M, phage φX174RF digested by
    HinfI.
(b) Densitometer scan of tracks from (a).

greater detail, experiments were performed which were equivalent to those
above, but shorter DNA fragments were examined by kinase labelling 5'-termini
with [$^{32}$P] and autoradiography of polyacrylamide gels.  This increases the
resolving power of the technique to 5-10bp.

   Figure 6 shows the results of fine mapping the modification sites of
pColIR215, and a comparison with the analogous S1 cleavage experiment.  One
point is striking - the two procedures do not result in identical fragments
being produced; the principal band seen after bromoacetaldehyde modification
is about 25bp shorter than the corresponding S1 fragment.  With milder bromo-
acetaldehyde reaction radioactivity can be detected over 50bp, with peaks at
20-25bp on either side of the central S1 hypersensitive site.  With greater
bromoacetaldehyde concentrations only the shorter fragments are detected, the
lengths of which become progressively smaller.  Clearly a defect is present in
this approach in that the modified site is recognised by S1 cleavage; thus the
shorter fragments will be over-emphasised.  This is seen directly by using an
alternative restriction site, such as the EcoRI target, which allows the obser-
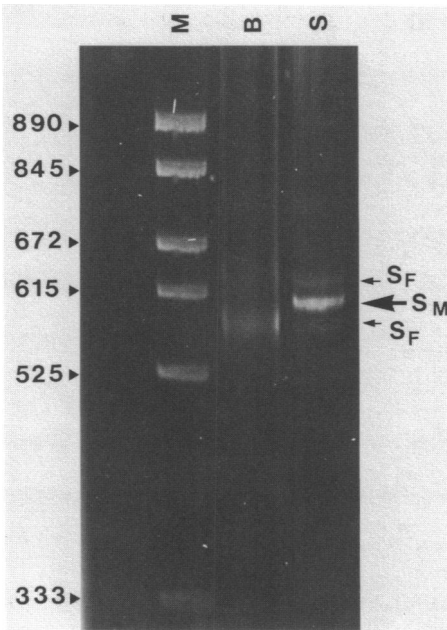vation of fragments arising from the other side of the inverted repeat.

Figure 8: Major and flanking S1 cleavages of pColIR215 at 37°C, and comparison with bromoacetaldehyde modification positions. 5% polyacrylamide gel electrophoresis of ~650bp fragments arising from bromoacetaldehyde, SalI, S1 cleavages (B) or S1, SalI cleavages (S). M, phage PM2 DNA digested by HaeIII as marker fragments, sizes in bp indicated on left.

   The overall distribution of modification can be demonstrated more clearly
in the case of pIRbke8, where levels of modification are generally lower.
Figure 7 shows a polyacrylamide gel analysis of the S1 and bromoacetaldehyde
fragments arising from HindIII cleavage.  It is seen very clearly that there
is a broad distribution of radioactivity flanking the S1 position, with peaks
at approximately 20bp on either side.  Densitometry of this distribution shows
that the modification extends throughout the region, peaking at either extreme
and the centre.
   Taken together these results show that the entire local region of inverted
repeats may become hypersensitive to bromoacetaldehyde modification when
supercoiled, and that the hypersensitivity is actually greatest in the flanking
region.  And yet from the pIRbke8 - pAT153 comparison it is clear that the
inverted repeat is solely responsible for conferring the hypersensitivity,
despite the fact that pAT153 sequences themselves become extensively modified
in pIRbke8, i.e. the flanking sequences.  There is some evidence that under
some circumstances S1 nuclease may itself recognise structural perturbation in
the flanking sequences.  Figure 8 shows a comparison of bromoacetaldehyde
modification and S1 cleavage of pColIR215, both at 37°C, with restriction
cleavage by SalI.  Weakly staining fragments (S$_F$) may be seen flanking the
main S1 cleavage (S$_M$), which comigrate with the broader fragments arising from

chemical modification.  This effect is not readily reproducible and is only
seen when higher temperatures are used for the S1 reaction.


DISCUSSION

   Inverted repeat sequences confer localised hypersensitivity to modification
by the single-strand-selective reagent bromoacetaldehyde when the DNA is
negatively supercoiled.  This has been demonstrated for two unrelated inverted
repeats, and the crucial role of the inverted repeat itself has been
established.

   Local structural perturbation at torsionally stressed inverted repeats has
previously been revealed by selective cleavage by single-strand-specific
nucleases; principally S1 nuclease (12-14) but also T7 nuclease (14) and
micrococcal nuclease (34,35).  Some major structural perturbation which is
supercoil-dependent is therefore indicated, and cruciform (hairpin loop) form-
ation has been suggested to be responsible for the observed nuclease sensi-
tivity.  This perturbation has now been demonstrated to be recognised by a
non-enzymic probe, bromoacetaldehyde, thereby removing a possible objection to
conclusions based on enzymatic probes alone.  Single-strand-specific nucleases
mechanistically must proceed _via_ an intermediate in which the enzyme is bound
to single-stranded DNA, and may therefore stabilise this form.  Thus it is
conceivable that the Km of the enzyme may itself induce a structural trans-
ition in the DNA.  Whilst this point is rather weaker for micrococcal nuclease,
primarily a double-strand endonuclease, it is clearly untenable for a small
molecule such as bromoacetaldehyde which must, therefore, be recognising some
pre-existing structural feature.  In view of the inverted symmetry which is of
fundamental importance for the observation of these effects, a strong candi-
date for this feature must be cruciform structure.

   Cruciform formation can account for the nuclease hypersensitivity and its
topological dependence in a simple way, and it is difficult to conceive struc-
tures which can provide an alternative.

   What, then, of the differences between the effects of S1 nuclease and bromo-
acetaldehyde?  These are likely to be partly a result of the different molec-
ular size and mechanism of these probes, and also partly as a consequence of
the different experimental approaches necessitated by each probe.  Inter-
actions between an enzyme and another macromolecule such as DNA are likely to
be severely restricted by steric constraints.  It is therefore rather easy to
envision a cruciform - enzyme interaction being limited principally to the
extreme ends of the structure, at the unpaired loop.  Conversely, a small

molecule like bromoacetaldehyde is subject to much less constraint and could diffuse to many parts of the structure. A broader distribution of modification might thus be expected together with the observed higher backgrounds of non-selective attack. There is one very important experimental distinction which must be made between S1 and bromoacetaldehyde however. The former must obey essentially single hit statistics, since once a cleavage has been introduced the molecule is no longer supercoiled. No such restriction applies to bromoacetaldehyde modification; indeed the reverse situation may well apply in that once a region has become partially modified then this perturbation may itself facilitate greater local modification. This is borne out to some extent by the progressive increase in extent of modification seen with pColIR215 as the reagent concentration is raised. It may be observed in a different manner by studying topoisomer migration on chloroquine gels as a function of extent of modification. An expected decrease in mobility is seen, but no clear limit reached (Lilley, D.M.J., unpublished data), consistent with the production of a 'bubble' whose extent increases with the degree of modification. A difficulty with the 'expanding bubble' explanation is the observation of both hypersensitive ends of the pColIR215 inverted repeat when observed from one side. To explain this result it becomes necessary to postulate that the modified DNA is itself relatively resistant to S1 nuclease, but that it may nevertheless hypersensitise adjacent nucleotides. This is difficult to reconcile, however, with the central hypersensitivity observed for pIRbke8, and the cleavage of flanking DNA by S1 nuclease at 37°C. I tentatively suggest, therefore, that these regions have an altered DNA structure which is somehow propagated from a central cruciform over 10-15bp on each side.

Such effects are not without precedent. The junction between B- and Z-conformations has been shown to have an unusual structure in that it is sensitive to S1-nuclease (4) and recently a crystallographic study of an RNA-DNA junction demonstrated that the A-type conformation may be propagated into a proximal DNA section (36). Work is currently in progress in this laboratory to find other single-strand-selective reagents in order to extend these studies. Bromoacetaldehyde is chiefly limited to adduct formation with adenine residues, although cytosines may react at lower pH (29). Clearly it is very desirable to broaden the extent of this probing with reagents which will modify other bases.

A further advantage of small molecule probes lies in the possibility that they may be sterically capable of reacting with transient but constrained species. Cruciform structures are likely to be dynamic short-lived structures

(30,35), and the intermediates which lie along the reaction surface may present new targets for bromoacetaldehyde reaction. This may help to explain the overall distribution of modification produced by the compound.

These studies provide new evidence for supercoil-driven perturbation of DNA structure which is sequence-dependent. This is likely to have functional consequences, for example in recombination (37), and a rôle has recently been suggested in the structure of the upstream domain of eukaryotic genes (38). Alternatively, these properties may confer undesirable consequences upon functional DNA molecules (39-41), and may therefore be features to be avoided. Whatever the actual operational significance, however, it is important to gain a better understanding of the structure and dynamics of these elements.

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