Conformations of A, T-rich DNAs

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ABSTRACT

DNAs from the genomes of *Clostridium perfringens* and *Cytophaga johnsonii* display orthodox A-DNA and B-DNA structures despite their high (A+T) nucleotide content. Unique structures, such as those found for synthetic DNAs having specific special sequences, do therefore not necessarily occur for DNAs having more random base sequence even if these have unusual base compositions. *Clostridium perfringens* DNA exhibits unusual structural properties only prior to purification by gel filtration.

INTRODUCTION

Early X-ray fiber diffraction studies showed that DNA duplexes obtained from a wide variety of sources, using a number of different isolation procedures and having greatly varying base compositions, all exhibited structures essentially identical to calf thymus A-DNA and B-DNA.² Recent X-ray studies on synthetic polynucleotides indicate, however, that this structural conservatism of DNA is not without exception. Studies of poly(dA)·poly(dT),³ poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC)⁴ and poly(dA-dT-dT)·poly(dA-dA-dT)⁵ show that these duplexes can adopt helical forms different from either A- or B-DNA. Infra-red dichroism studies with hydrated poly(dA)·poly(dT) films reinforce the X-ray results.⁶

Considering the results from synthetic DNAs, certain biological DNAs exhibit base sequences and compositions which might be expected to affect their structural properties. Some, like the genomic DNA of *Clostridium perfringens*, contain a high percentage of A:T base-pairs, while others, like the satellite DNAs isolated from crabs, exhibit highly-repeated short oligonucleotide sequences and resemble the synthetic DNA duplexes

described above. We detail in this report, therefore, X-ray investigations of A,T-rich DNAs isolated from *Cl. perfringens* and *Cytophaga johnsonii* and, in the following paper, describe similar investigations of several satellite DNAs.

MATERIALS AND METHODS

Cl. perfringens DNA was purchased from P-L Biochemicals, Inc. Some DNA samples were dissolved in 0.01 M NaCl, 0.001 M EDTA (pH 7.0) to a concentration of about one mg DNA/ml, precipitated by gentle addition of two volumes of ethanol and washed serially with four volumes 66% ethanol-distilled water, two volumes ethanol and two volumes acetone. DNA samples prepared for gel filtration were dissolved in 0.01 M NaCl, 0.001 M EDTA (pH 7.0) to a concentration of 0.5 mg DNA/ml. 20 ml of this DNA solution was applied in 2 ml aliquots to a 2x20 cm column of Sepharose 4B (Pharmacia), eluted with 0.01 M Tris-HCl (pH 7.0) at a flow rate of 20 ml/hr and collected in 2 ml fractions. The eluted fractions containing DNA (as determined by measuring absorbance at $\lambda=260$ nm) were pooled and concentrated to 5 ml of solution in a rotary evaporator. The DNA was precipitated and washed as described above. DNA precipitates were dried overnight alongside a petri dish containing silica gel in a vacuum desiccator and stored at -20°C .

5 mg of *C. johnsonii* DNA was kindly provided by Dr. Wilhelm Guschlbauer. The DNA was dissolved in 5 ml of 0.01 M NaCl, 0.001 M EDTA (pH 7.0), precipitated, washed and dried as described above.

Base composition analysis of *Cl. perfringens* DNA showed that no inadvertent fractionation of the DNA sample had occurred during gel filtration. Approximately 0.25 mg of DNA was dissolved in 0.2 ml distilled water and enzymatically degraded to mononucleotides by addition of 20.0 μl of 1.0 M Tris-HCl (pH 8.0), 5.0 μl of 1.0 M MgCl_2 , 20.0 μl of snake venom phosphodiesterase (E.C. 3.1.4.1) (purchased from Worthington Biochemical Corporation and treated to inactivate any 5'-nucleotidase contaminant⁷) and 5.0 μl of DNase I (E.C. 3.1.4.5) (purchased from Worthington and dissolved in distilled water to a concentration of 2.5 mg/ml), followed by incubation of the mixture at 37°C for two hours. The reaction was stopped

by heating at 100°C for five minutes and then centrifuged at 2000 rpm to remove any precipitate. Nucleotides in the supernatant were separated on an AG 1-X4 (400 mesh) ion-exchange column as previously described⁸ with the modification that 10% ethanol-distilled water replaced 20% ethanol in the entire procedure. Absorbances of the fractions containing nucleotides were measured to determine nucleotide ratios.

Preparation of DNA fibers and fiber X-ray diffraction techniques have been described previously.⁹

Fibers having a range of retained salt concentration were prepared by repeating the washing phase of the DNA preparation. Diffraction diagrams were obtained with the fibers maintained at relative humidities between 31% and 92%.

RESULTS

Samples of *Cl. perfringens* DNA which have passed through 4% agarose columns (see experimental details above) yield fibers giving diffraction patterns such as are shown in Figure 1. No other types of diffraction pattern are observed for

Figure 1: Composite of X-ray diffraction patterns from purified *Clostridium perfringens* DNA. Both the A-DNA pattern (left) and the B-DNA pattern (right) were obtained at 66% relative humidity with the axes of the fiber specimens at 76° to the X-ray beam. The diffraction diagrams were obtained using nickel-filtered copper K α radiation generated from a Picker multifocus X-ray generator and collimated using pinhole optics (Ref. 11). The pattern on the left shows that a minor fraction of the specimen is still in the B conformation.

these DNA samples. The patterns obtained are essentially identical to those seen for calf thymus A-DNA¹⁰ and B-DNA¹¹ and show that the conformations adopted by this DNA are not significantly affected by its high A:T base-pair composition.

Samples of *Cl. perfringens* DNA which have not passed through 4% agarose, however, yield diffraction diagrams such as is shown in Figure 2. This pattern, while similar to

Figure 2: X-ray diffraction pattern from *Clostridium perfringens* DNA before complete purification. Conditions were the same as for Figure 1 except that the diffraction was obtained at 92% relative humidity.

patterns from oriented but not crystalline B-DNA fibers, indicates a helical rise-per-residue (h) of 0.329 nm, somewhat smaller than in B-DNA ($h=0.338$ nm). No fibers from these samples yield A-DNA diffraction patterns. Since the base composition of the single peak of *Cl. perfringens* DNA eluted from 4% agarose ($((A+T)/(G+C)=2.22)$ is essentially identical to that reported for the bacterial genome ($((A+T)/(G+C)=2.24)$)¹² the orthodox A-DNA and B-DNA geometries of the purified DNA demonstrate that mere A,T-richness does not alter DNA structure.

The unusual diffraction characteristics observed with *Cl. perfringens* DNA samples prior to gel filtration indicate the presence of a contaminant which is affecting the DNA conformation. The contaminant is not detectable in eluted column

fractions by measurement of absorbance at $\lambda=260$ nm. However, in an analogous X-ray diffraction experiment with *Bacillus subtilis* DNA, teichoic acid was found to have a similar effect on the structural behavior of DNA (Szulmajster, Marchetti and Arnott, unpublished results). Since both *Cl. perfringens* and *B. subtilis* are gram-positive bacteria containing cell wall teichoic acid (not specifically eliminated in conventional DNA isolation procedures) it is possible that teichoic acid contamination of *Cl. perfringens* DNA samples results in the anomalous DNA diffraction observed. DNA and teichoic acid can be readily separated by gel filtration through 4% agarose,¹³ reinforcing this conclusion. The particular nature of the contaminant is unimportant to study of base composition effects on DNA structure, nonetheless, since purified *Cl. perfringens* DNA, despite its A,T-richness, exhibits only orthodox molecular geometries.

C. johnsonii DNA samples yield fibers giving diffraction patterns as shown in Figure 3. These two patterns demonstrate

Figure 3: Composite of X-ray diffraction patterns from *Cytophaga johnsonii* DNA. The A-DNA pattern (left) was obtained at 66% relative humidity while the B-DNA pattern (right) was obtained at 92% relative humidity. Both diffraction diagrams were obtained using nickel-filtered copper K_{α} radiation generated from a Phillips XRG 2500 X-ray generator and focussed using toroidal optics (Ref. 21) with the axes of the fiber specimens at 76° to the X-ray beam. The meridional reflexion indicated by an arrow is discussed in the text.

that, like *Cl. perfringens* DNA, this A,T-rich DNA ((A+T)/(G+C)=1.89)¹⁴ exhibits orthodox A-DNA and B-DNA forms. The weak meridional reflections (indicated by an arrow in Figure 3) at $\zeta=2.1 \text{ nm}^{-1}$, corresponding to 0.47 nm in real space, are the only significant deviations of these patterns from calf thymus A- and B-DNA diffraction. A distance of 0.47 nm corresponds to the spacing between polypeptide β -pleated sheets¹⁵ and the weak meridional reflexions in *C. johnsonii* DNA diffraction patterns may indicate a residual protein contaminant in the DNA sample. It is doubtful that the meridional reflexion corresponds to any feature of the DNA structure since it is present in both A- and B-DNA diffraction patterns and the patterns are orthodox in all other respects.

DISCUSSION

Despite claims that *Cl. perfringens* and *C. johnsonii* DNAs have distinctive structures,¹⁶ we have observed only the classical A and B geometries for these DNAs except when samples are not purified sufficiently. Both DNAs, which have about 66% (A+T), exhibit stable A-DNA forms, thereby disproving the hypothesis that A,T-rich DNAs cannot adopt the A form.^{17,18} Even crab satellite I DNA, which approximates poly(dA-dT)•poly(dA-dT) and has a 95% (A+T) composition, exhibits a stable A-DNA form under the appropriate conditions (see following paper). Thus far, only DNAs having few guanine-guanine intra-strand base stacks and highly-repeating sequences (poly(dA)•poly(dT), poly(dA-dT)•poly(dA-dT), poly(dG-dC)•poly(dG-dC) and poly(dA-dT-dT)•poly(dA-dA-dT)) have been found not to adopt stable A forms.^{3,4,5}

Studies of these polynucleotides and additional work on synthetic poly(trinucleotide) DNAs¹⁸ have shown that the DNAs which can adopt the D conformation do so under the same conditions that would normally give the A form. This suggests that the A and D structures are mutually exclusive alternatives as conformations stable at low salt concentration. This does not mean that the A form is never accessible to these unorthodox polynucleotides: Davies and Baldwin¹⁹ have observed fibers of poly(dA-dT)•poly(dA-dT) in the A conformation, but these were metastable and transitioned to the D form within a few days.

It should be noted, however, that the results of this work do not rule out composition effects on the *ease* with which the *A*→*B* structural transition occurs in a DNA;¹⁷ conditions (such as salt concentration) governing this transition are not easily controlled in DNA fibers.

It is not surprising that DNAs containing randomly distributed base sequences should adopt only the usual DNA structural geometries, even if these DNAs have unusual base compositions. This is in keeping with the results of previous X-ray studies.² If nucleotide sequence effects on DNA structure indeed occur *in vivo*, it is more likely that these effects would be localized to particular sequences presumably similar to those in synthetic DNA polymers which do provide non-classical forms. On the other hand, the base composition effects that have been found to mediate the structural transition between orthodox *A*- and *B*-DNA forms both in extreme^{3,4,5,20} and intermediate¹⁷ examples of DNAs having varying nucleotide composition may be more generally important; for instance, in regulation of RNA transcription and DNA duplication where transient RNA-DNA hybrids require adoption of the *A* form.

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