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DISCUSSIONS

Ultrasonic Cleavage of DNA: Quantitative Analysis of Sequence Specificity

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Abstract—Looking for new means of assessing local conformational and dynamic heterogeneities in DNA structure, we have estimated the rates of phosphodiester bond cleavage in DNA fragments of known sequence caused by ultrasonic treatment. Among the 16 dinucleotide steps possible, those with 5'-ward cytosine [5'-d(CpN)-3'] are distinguished by significantly higher cleavage rates: CG > CA = CT > CC. The possible causes of this intriguing phenomenon are considered.

Key words: ultrasound, DNA cleavage, conformational heterogeneity, CpG step

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It is known that ultrasonic exposure of DNA solutions results in cleavage of the double helix [1, 2]. We have studied the cleavage of DNA by ultrasound using gel electrophoresis, and found the cleavage pattern to be sequence-specific [3]. The basic goal of this work was to estimate the relative cleavage rates for all 16 dinucleotide steps.

Briefly, DNA fragments of preset sequence were excised, 3'-endlabeled with ^{33}P and isolated by published techniques [4–6]. Then solutions were sonicated at 22 kHz, resolved in denaturing gradient thickness gels, and the densitometric scans were analyzed with the SAFA software [7]. Fragment sequences and all experimental details are available at http://grok.imb.ac.ru/din.html

For 15 defined DNA fragments of different lengths (150 to 500 bp) the cleavage pattern was analyzed in one of the strands containing the terminal label. Only the central parts of the gels, where the bands were clearly separated, were examined. The total length of considered sequences was ~2500 bp.

The band intensities that correspond to cleavage rates for particular positions in the DNA sequence in a typical experiment are shown in Fig. 1. This pattern demonstrates prevalent cleavage of phosphodiester bonds at the 3' side of cytosines (marked C).

For more detailed analysis, we calculated the relative cleavage rates for all 16 dinucleotide steps. Figure 2 shows that cleavages in three steps 5'-d(CpG)-3', 5'-d(CpA)-3', and 5'-d(CpT)-3' indeed occur much more often than in the rest. The rates within the 5'-C group relate as CG > CA = CT > CC. Importantly, the rates of cleavage in the CA, CT, CC steps are significantly higher than those for the complementary steps (TG, AG, GG). Such "asymmetry" means that the two DNA strands are mostly cleaved not at the opposite bonds: there is either a two-strand break with a shift, or two consecutive single-strand breaks, and in both cases we get sticky ends.

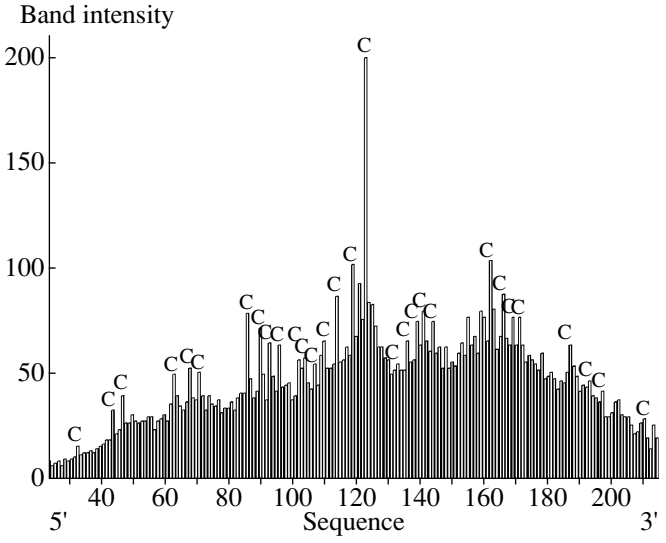

Fig. 1. Typical pattern obtained by computer analysis of gel band densities (proportional to cleavage rate) upon DNA sonication.

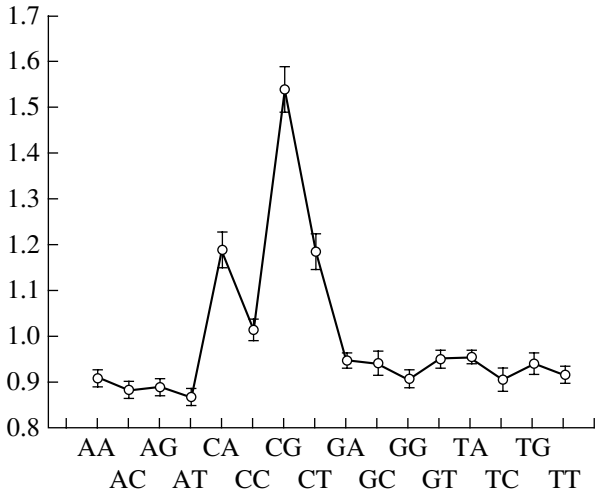

Fig. 2. Mean relative cleavage rates and their 95% confidence intervals for dinucleotide steps.

The CpG step, which experiences the highest cleavage rate, is characterized by the largest values of melting enthalpy and entropy [8]. The local energy properties of the structure might be relevant in the response to deformation. Recently we have examined the propagation of deformation energy through DNA covered with ligands in the framework of a string model, which demonstrated that the deformation energy is increased in the region of string heterogeneity [9].

Another important feature of the CpG step is its maximal flexibility anisotropy (slide-shift), which is estimated by ratio (4.02/12.03 kJ/mol A^2) [10]. In other words, it is much easier to displace the base pairs along the long axis of the dinucleotide than along the short one. Finally, among the (three) variants of heterodimer with 5'-ward cytosine in DNA, CpG is the only step with second-order symmetry. So, peculiarities of this step are characteristic of both DNA strands. This may explain the elevated ultrasonic cleavage rate for this step.

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