Short communication

Phosphorus NMR of Plasmid DNA

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Due to the key position of phosphorus-containing compounds in biology, favourable NMR properties of ^{31}P nucleus and its 100 % natural abundance, ^{31}P NMR has become a valuable biochemical method. It gives, for example, information about DNA conformation since ^{31}P NMR chemical shift is sensitive to the phosphodiester linkage geometry (Gorenstein 1984). ^{31}P NMR reflects base sequence — dependent structural variations in the classical B-DNA double helix (Patel et al. 1982) and it also distinguishes between various types of DNA double helix (Patel et al. 1979; Shindo et al. 1979; Kypr et al. 1981; Vorlíčková et al. 1983). Studies carried out so far have almost been restricted to short DNA fragments of nucleosomal length since resonances of longer fragments were too broad to be measured with a sufficient signal-to-noise ratio (Hanlon et al. 1976; Kypr, Sklenář and Vorlíčková, unpublished results). An extrapolation of results obtained with linear DNA fragments of various lengths has almost excluded obtaining ^{31}P NMR of 5—10 kilobase phage or plasmid DNAs. Surprisingly, a ^{31}P NMR spectrum of 7 kilobase pIns 36 plasmid DNA has recently been reported (Bendel et al. 1982). However, its signal-to-noise ratio was very low so that the resonance amplitudes could only be determined. No other ^{31}P NMR spectrum of plasmid DNA has since been published. We show here that a signal-to-noise ratio comparable to that obtained with short linear DNA fragments can also be obtained in the ^{31}P NMR spectrum of plasmid DNA.

Col El plasmid DNA was prepared as described previously (Vojtíšková et al. 1980). It was contaminated with less than 1 % chromosomal DNA, 1 % proteins and 0.5 % RNA. For the ^{31}P NMR measurements, 1 mg of the plasmid DNA was precipitated with ethanol and dissolved in 0.12 ml of SSC, pH 7.0, at room temperature. Fifteen microlitres of D_2O and a trace of trimethylphosphate were added for locking the spectra and internal referencing, respectively. Electrophoresis in 1 % agarose gels indicated the presence of 5 % nicked plasmid molecules in the sample. Their number increased to 40 % during a week standing in the NMR probe at 30 °C. ^{31}P NMR measurements were performed in Wilmad

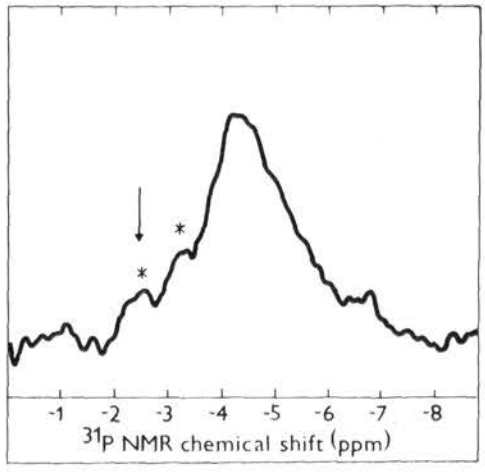

Fig. 1. ^{31}P NMR spectrum of Col El plasmid DNA dissolved in SSC, pH 7.0, at a concentration of 7.5 mg/ml. Asterisks indicate two satellite peaks (see text). The position of the resonance diagnostic of the left-handed Z-DNA conformation is indicated by arrow. The spectrum was accumulated 108 346 times (33 hours of accumulation), with 8 K data points, a sweep width of 3750 Hz, a delay time of 1.1 s, and a flip angle of 70° (14 μs). The temperature was 30 °C.

508 cp microcells at 80 MHz on an adapted Bruker WP 80 spectrometer with an Oxford cryomagnet. The spectra were recorded under proton noise decoupling conditions.

Col El plasmid DNA contains more than 6 kilobase and hence is not expected to give a ^{31}P NMR resonance with a sufficient signal-to-noise ratio in a reasonable time. Plasmid DNA molecules appear, however, not to behave according to the extrapolation following from measurements with linear DNA. Their convenience to be characterized by ^{31}P NMR is likely to be attributed to the increased segmental bending and torsion motions in the supercoiled state (Bendel et al. 1982). We were able to obtain a ^{31}P NMR resonance of Col El plasmid DNA almost at the first attempt with 1 mg of the material. The signal averaging period did not much exceed the acceptable one day duration and the measurements were performed at an ambient temperature. The resonance virtually consists of a single, broad and asymmetrical resonance centered at — 4.3 ppm (Fig. 1). Such a resonance position is typical of the classical B-DNA conformation (Simpson and Shindo 1980). Both the width and asymmetry suggest that the NMR line only represents an envelope of many unresolved signals of DNA phosphates with chemical shift dispersion. The dispersion has also been observed with synthetic oligonucleotides (Patel et al. 1982) and natural DNA fragments (Simpson and Shindo 1980), and it has been interpreted in accordance with the crystal structure of the dodecamer d(CGCGAATTCGCG) (Dickerson and Drew 1981) to reflect base sequence

-dependent variations in the B-DNA phosphodiester linkage geometry. Note also the satellite peaks superimposed on the main resonance. Control experiments will be necessary to disclose their origin. Nonetheless, it is interesting that the two small peaks marked by asterisks (Fig. 1) are located exactly in positions where left-handed Z-DNA (Simpson and Shindo 1980) and right-handed A-DNA (Vorlíčková et al. 1982) conformations exhibit characteristic resonances.

Conformational properties of phage and plasmid DNA molecules have so far been studied by few methods which have provided limited information only. In fact the attention has mainly been paid to the detection of local left-handed regions (Singleton et al. 1982) or cruciforms (Lilley 1983). Information about the conformation of the whole molecule has been provided by circular dichroism (Maestre and Wang 1971) but, unfortunately, in an integral form. By contrast, ^{31}P NMR gives separate resonances for various structural forms of DNA and can thus provide data about their populations in the molecule. This possibility may be of use to study, for example, environmental perturbations of the B-Z equilibria in various phage and plasmid DNA molecules.

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