DETECTING CONFORMATIONAL HETEROGENEITY IN THE DNA BACKBONE BY ^{31}P NUCLEAR MAGNETIC RESONANCE

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1. Introduction

Recent ^{31}P NMR studies of tRNA have disclosed that ^{31}P chemical shift sensitively reflects the higher order structure of tRNA, e.g., double-stranded or single-stranded [1–4]. The origin of such differential chemical shift was discussed extensively in [5] where empirical correlation between ^{31}P chemical shift and phosphodiester conformation was presented, in particular the P–O torsional angles (ω,ω'), by using calculated electronic density on the phosphorus atom. Evidence is accumulating to show that such relation indeeds holds at least qualitatively in some model systems, including polynucleotides [6] and short oligonucleotide duplex [7]. It is generally found that ^{31}P resonance is shifted downfield when the polynucleotides transform from helix (with the P–O configuration locked to (*gaushe, gaushe*) position) to coil (where the P–O configuration can also take non-*gaushe* positions).

In the present paper, we first show that such downfield shift upon helix–coil transition is also observable with an actual DNA sample. Our major purpose of the present paper, however, is to give evidence that there is substantial heterogeneity in the DNA backbone structure in the single-stranded form and that this heterogeneity depends on the base-sequence, based on the ^{31}P NMR spectra of DNA and apurinic acid.

2. Experimental

Salmon testes DNA was purchased from Sigma Chemical Company. Apurinic acid was prepared from salmon testes DNA as in [8]. DNA and apurinic acid were dialyzed before NMR measurements. DNA and apurinic acid was 15 mM and 0.1 M, respectively, and final NaCl was 10^{-5} M and 0.1 M for DNA and apurinic acid solution, respectively. All the NMR measurements described below was carried out in D_2O solution. The pH of the solution was adjusted by adding NaOH to the solution.

^{31}P NMR spectra of DNA were measured at 145.7 MHz with a Bruker HX-360 in a Fourier-transform mode. ^{31}P NMR spectra of apurinic acid were recorded on a JEOL PS-100 Fourier transform unit. All the spectra of apurinic acid were obtained under proton noise decoupling. Chemical shifts were referenced to an external standard of H_3PO_4 (85%) in a cylindrical capillary.

3. Results and discussion

Figure 1a shows ^{31}P NMR spectrum of salmon testes DNA measured at pH 3.4. The resonance lineshape is not symmetrical and apparently consists of several resonance lines. Since double-stranded DNA is not stable at low ionic strength [9] a large part of the molecule must be converted to single stranded form coexisting with the rest in double-stranded form under our experimental condition (ionic strength $< 10^{-4}$ M).

When the solution is increased to pH 11.2, the largest peak in the higher field (1.15 ppm) decreases and the resonance intensity in the lower field (less than 1.0 ppm) increases (fig.1b). The change in the spectrum is more clearly seen in the difference spectrum shown in fig.1c. Since at pH 11.2, guanine and thymidine bases are deprotonated and all hydrogen

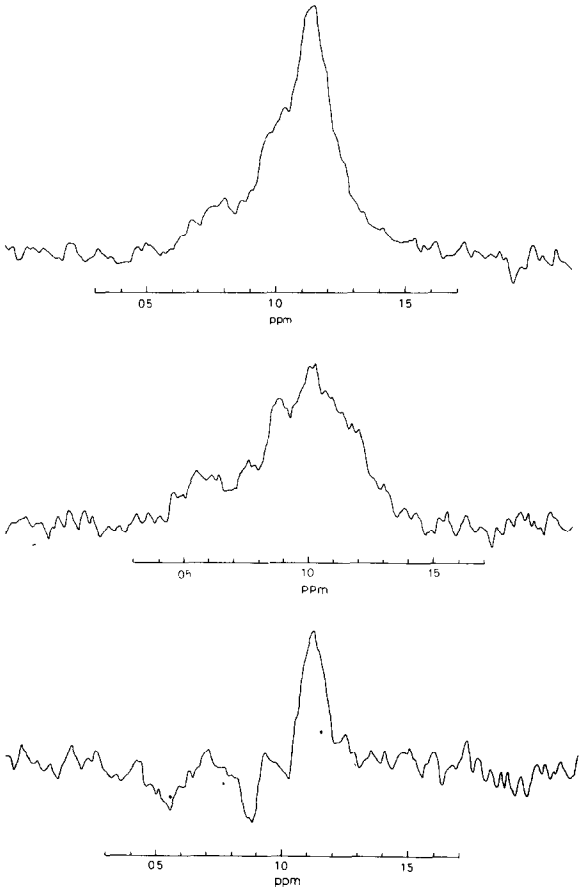

Fig.1. ^{31}P NMR spectrum of salmon testes DNA (15 mM in monomer unit) measured at 145.7 MHz at 27°C. The solution contains 0.01 mM NaCl and 3 mM EDTA. Chemical shift (in ppm) was referenced to external H_3PO_4 (85%). (a) pH 3.4; (b) pH 11.2; (c) (a)–(b).

bonds between the complementary base-pairs are broken [10], the DNA is considered to be almost completely in single-stranded form. The result leads us to suggest that the peaks in the higher field (1.15 ppm) arise from the double-stranded portion of DNA whereas those in the lower field (less than 1.0 ppm) arise from the single-stranded portion of DNA. This suggestion is in accordance with the similar finding in tRNA [3,4] and in d-CpCpGpG [7].

More interesting is the finding that ^{31}P NMR spectrum of single-stranded DNA (fig.1b) consists of at least several peaks of different chemical shifts. This observation makes sharp contrast to that in single-stranded homopolynucleotides in which only single homogeneous line is observed [11].

What would be the origin of such a heterogeneous resonance in single-stranded DNA? As in the case of helix–coil transition in polypeptides [12], a possibility might first be sought in the polydispersity of the DNA sample. However, the DNA sample had been well dialyzed to remove the low molecular weight species, and under the same experimental condition, no peak distribution could be observed for homopolynucleotides. Furthermore, the reduction of molecular weight of DNA causes little change in the ^{31}P chemical shift [13]. These observations exclude the possibility that the observed chemical shift distribution in single-stranded DNA arises from polydispersity of the DNA sample.

The origin of the chemical shift distribution must, therefore, be sought in some sort of heterogeneity in the internal structure of the DNA molecule that could bring about heterogeneous distribution of ^{31}P chemical shift. In view of the expected dependence of ^{31}P chemical shift on the diester torsional angles (ω,ω') and/or O–P–O bond angle [5], any heterogeneity in the phosphodiester linkage in the backbone structure of DNA would bring about heterogeneous distribution of chemical shift in the ^{31}P NMR spectrum.

Two kinds of heterogeneity in the DNA backbone structure may be considered.

1. The static heterogeneity that arises from the static distribution of torsional angles in the DNA structure. This kind of heterogeneity may be encountered when the polynucleotide structure is rigid and the local motion of phosphate moiety is slow so that the life time of the different configuration may be long enough to permit the static distribution of chemical shift in the NMR time scale. This was considered to be the case with poly(G) in which a rigid multi-stranded structure prevents rapid conversion among different configurations in the phosphodiester bond [14]. Although such a possibility is presumably small in more flexible single-stranded DNA, apriori exclusion of this possibility may not be justified in view of the extremely high molecular weight of the DNA (one or two orders of magnitude larger than homopolynucleotides).
2. The heterogeneity which would be time-averaged heterogeneity which would remain after time averaging different torsional conformations of the diester bonds because of the different preference

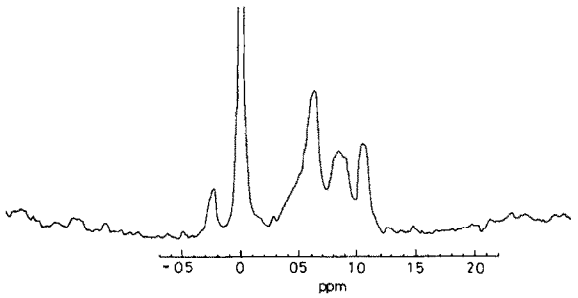

Fig.2. ^{31}P NMR spectrum of apurinic acid (0.1 M in monomer unit) measured at 40.5 MHz at 29°C, pH 5.8. The solution contains 0.1 M NaCl and 3 mM EDTA. Chemical shift (in ppm) was referenced to external H_3PO_4 (85%). The large peak at 0 ppm is that of external H_3PO_4 and small peak at −0.25 ppm could be assigned to be that of terminal phosphate from the pH dependence (in preparation).

of torsional angles in different portion of DNA. This kind of heterogeneity inevitably requires that the configurational preference depends on the base sequence.

To distinguish between the two possibilities, we have prepared apurinic acid from DNA by removing nearly all the purine bases. This molecule is, in its nature, unable to form double strand, and shows high flexibility since 42% of the residues have no bases attached to them. The local motion of the phosphate moiety was found to be as fast as that of poly(U) [15] (10^{-10} s) (in preparation). Nonetheless, as shown in fig.2, ^{31}P NMR spectrum of apurinic acid consists of several peaks, the range of their distribution roughly coinciding with that of single stranded DNA (0.5–1.5 ppm). The result suggests strongly that the distribution in chemical shift in DNA, and therefore in the prefered phosphodiester torsional angle, is of the time-averaged nature and consequently that this heterogeneity must arise from the heterogeneity of the base composition, i.e., the base-sequence. The dependence of ^{31}P chemical shift in DNA on the base sequence is in accordance with our earlier finding that ^{31}P chemical shifts of homopolynucleotides in single-stranded form (poly(A), poly(C), poly(U) and poly(G)) are different mutually under the same experimental conditions [11] and also with the recent findings that ^{31}P chemical shifts of several dinucleotides are different from each other [6,16].

These facts suggest that this conformation is determined by the local based sequence, i.e., dominantly by the two bases in the immediate neighbor of the phosphate group in question. It is conceivable that the sequence-dependent heterogeneity in the DNA backbone structure disclosed in the present paper may have certain signification in the enzymatic recognition of a DNA sequence.

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