# pH DEPENDENCE OF $^{31}$P MAGNETIC RESONANCE SPECTRA OF HOMOPOLYRIBONUCLEOTIDES

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## 1. Introduction

Although proton magnetic resonance has been applied extensively to studies of nucleic acids in solution, little attempt has so far been made to utilize the phosphorus-31 resonance spectra in these studies. It appears to be a common practice to encounter a broad resonance line with occasional structures in a $^{31}$P spectrum of tRNA or DNA [1,2]. This presumably reflects chemical shift differences of $^{31}$P resonance in these macromolecules, but the factors governing these chemical shifts have been little understood. It appears important, therefore, to establish the main factors that determine $^{31}$P chemical shift of nucleic acids, based on the results of simpler systems, such as homopolynucleotides.

In this communication, $^{31}$P magnetic resonance spectra of polyriboadenylic acid (poly A), polyribocytidylic acid (poly C), polyriboguanylic acid (poly G), and polyribouridylic acid (poly U) have been measured as functions of pH. The results have clearly indicated that the chemical shift of $^{31}$P nuclei of synthetic homopolynucleotides depends critically on the charged state of the bases. In addition, some interesting informations have been obtained as to the rate of conversion between different conformational states of polynucleotides corresponding to differently charged states of bases.

## 2. Results and discussion

Figure 1 shows results of pH titration carried out at 0.1 M NaCl concentration at 30°C. Since each phosphate except those at the terminal group in the polynucleotides bears a single charge for the wide pH range studied (pH 2–12), the uptake or the release of a proton will only occur at the base moieties. The results of fig.1 indicate two distinctive properties of $^{31}$P chemi-

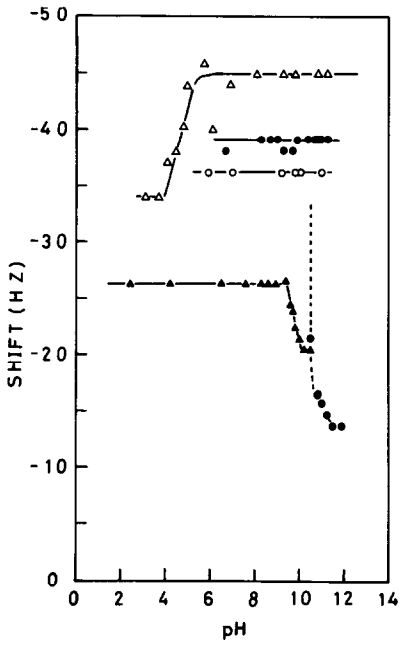


Fig.1. pH dependence of $^{31}$P chemical shift of four homopolyribonucleotides: poly U (0.092 M) (▲–▲–▲), poly C (0.060 M) (△–△–△), poly A (0.054 M) (○–○–○), and poly G (0.060 M), (●–●–●) the broad component; (●--●--●) the sharp component. Concentrations are given in monomer unit. The $^{31}$P spectra were taken at 40.48 MHz with the Fourier-transform mode on a JEOL PFT-100 system under proton noise-decoupling. pH titration was performed at 30°C in the presence of 0.1 M NaCl by the successive addition of NaOH. Chemical shifts are given in Hz from $H_3PO_4$ (5 M) as an external reference (negative values corresponding to high field shifts).

cal shift of homopolyribonucleotides. First of all, it is critically dependent on the base species. Even in the neutral pH range (pH 6–9), where all the four bases exist as electrically neutral entities, their chemical shifts are mutually different, extending over the range of ~0.5 ppm. The other important property of the $^{31}$P chemical shift of homopolyribonucleotides as readily seen from fig.1 is that it changes with pH nearly in accordance with the known p$K_a$ values of the polynucleotide bases. From fig.1, we obtain pH for the mid-point of transition to be 4.6 for poly C, 9.7 for poly U, and ~ 11 for poly G, whereas the reported p$K_a$ for the corresponding mononucleotides (5′-phosphate, measured at zero ionic strength at 20°C) are 4.5, 10.1, and 10.0, respectively [3]. For poly A, the resonance signal disappears below pH 5.5, corresponding to the protonation of the bases (p$K_a$ ≈ 3.9 for 5′-AMP) [3].

The mechanisms by which the chemical shift of the phosphorus resonance of polynucleotides is affected by the base may be devided into two kinds. (1) Effect of the base moiety on the electronic structure of the phosphate group, exerted through the sigma-bonding framework of the ribose moiety and the phosphodiester bonding, (2) direct electrostatic or hydrogen-bonding interaction between the phosphate group and the base. The electrostatic interaction would be particularly large when the base is positively charged by protonation at acidic pH, as is well known for poly A [4]. The effect of the ring current of the base might also contribute to a certain extent to the $^{31}$P chemical shift in the case of purine nucleotides.

Since in the neutral pH range, no strong electrostatic interaction would be expected, the big difference in chemical shift observed between poly U and poly C in the pH range 6–9 could not be ascribed to the mechanism (2). It appears more reasonable to ascribe the difference to the mechanism (1), the differential effect of bases, U and C, upon the electronic structure of the phosphate group through the intervening sigma bonds. The dependence of chemical shift on the charged state of the bases would arise either from mechanism (1) or (2) or both. Similar dependence of $^{31}$P chemical shift on the charged state of attached aromatic heterocyclics have been reported in some dinucleotide systems [5].

We may also derive some information about the rate of interconversion between differently charged

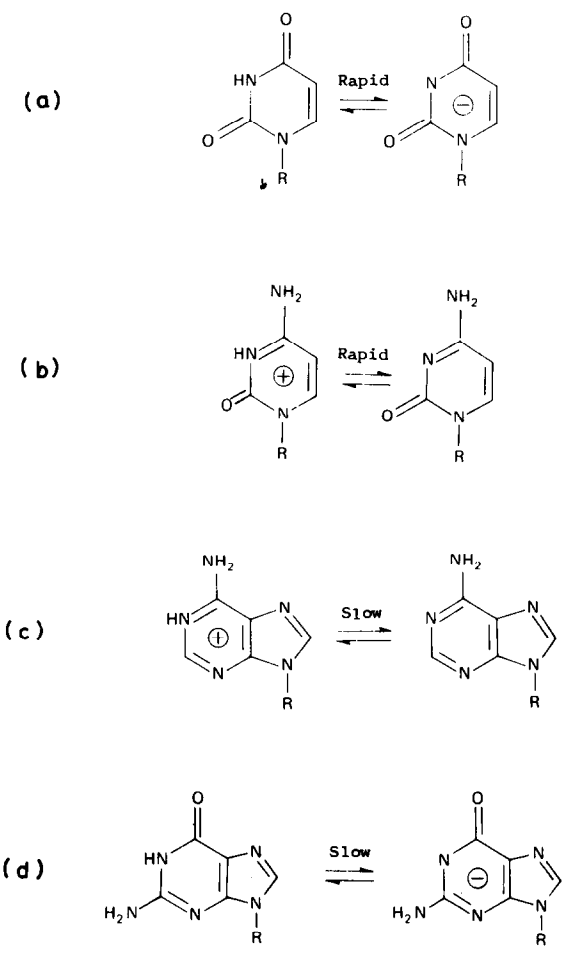


Fig.2. Equilibria between different charged states of bases in polyribonucleotides: (a) poly U, (b) poly C, (c) poly A, and (d) poly G.

states of a base from the observed spectra. Poly U shows a single narrow resonance (width at half intensity, $\Delta\nu_{1/2}$ ~ 3 Hz) for all the pH range studied, without appreciable change in its intensity. This indicates that the rate of conversion between the protonated and the unprotonated forms of poly U (fig.2a) is rapid (>20 sec$^{-1}$) and no rigid internal structure may be formed at any pH under the present experimental condition. Poly C also shows a narrow single resonance for the whole pH range studied, indicating that the rate of protonation and deprotonation is rapid (fig.2b). It was also noted, however, that the intensity of this signal decreases in accordance with the chemi-

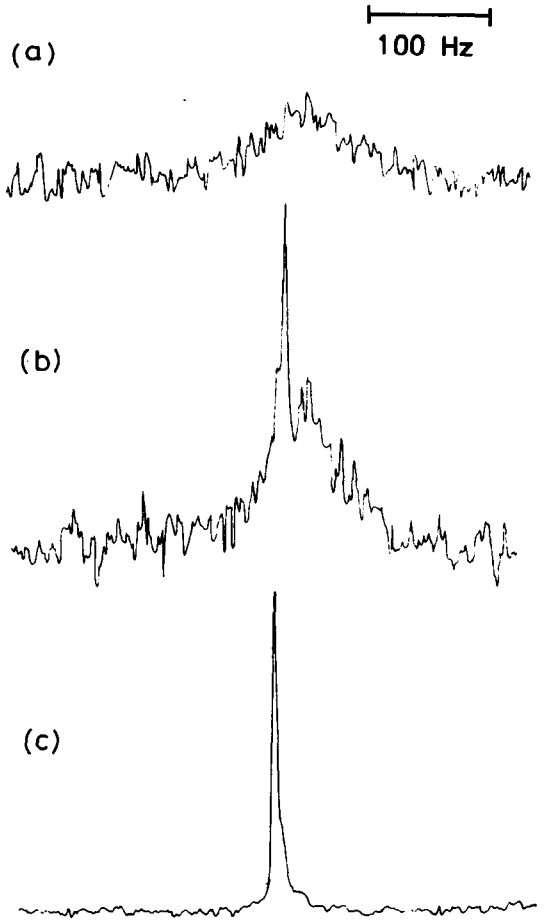


Fig.3. $^{31}$P magnetic resonance spectra of poly G at three typical pH: (a) 5.7, (b) 11.4, and (c) 11.9.

cal shift due to protonation, the mid-point of pH change coinciding with that of the chemical shift. This is indicative of the presence of another protonated form of poly C which gives a broader resonance, slowly exchanging with the sharp one. Poly A shows a sharp single resonance above neutral pH, but this sharp signal disappears below pH 5.5, indicating that the rate of conversion between the protonated, highly structured form [4] and the unprotonated, flexible form [6] is slow ($<10$ sec$^{-1}$) (fig.2c). Poly G in the pH range 6–10 has a neutral guanine base but its line width of $^{31}$P resonance is broad ($\Delta\nu_{1/2} \sim 100$ Hz). This observation strongly suggests that some ordered structure of poly G is formed even with the electrically neutral bases. The broad signal in the neutral pH range is gradually replaced by a sharp signal at higher pH. In the intermediate pH range (pH 10.5–11.5), the two signals coexist (fig.3), indicating that the rate of conversion between the neutral and the negatively charged base states of poly G is slow ($<10$ sec$^{-1}$).

More extensive studies of the dependence of the $^{31}$P magnetic resonance spectrum on the primary structure and the conformation of polynucleotide systems are in progress in our laboratory.

## References

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