PROBING THE SENSITIVITY OF ^{31}P NMR CHEMICAL SHIFTS TO HYDROGEN BONDING AND TO STEREOCHEMISTRY

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1. Introduction

^{31}P chemical shift measurements have been carried out on a series of 5′-nucleotides and α-hexose-1-phosphates in order to determine the sensitivity of ^{31}P chemical shifts to some environments encountered in biological systems. These sugar-phosphates were selected because of their well-defined conformation. Several chemical shift dependencies on environment are reported. The data is discussed with respect to intramolecular interactions involving the phosphate group.

2. Materials and methods

The samples were commercial preparations, except for 8-Br-GMP [1], 8-amino-AMP, 8-methylamino-AMP, and 8-dimethylamino-AMP [2], which were synthesized according to the referenced procedures. NMR measurements were carried out at 40 MHz on a Joel PS-100 spectrometer in the Fourier transform mode. Chemical shifts were reported in ppm upfield from external phosphoric acid. In addition, measurements were carried out on mixtures of compounds to calibrate the chemical shift differences. The pH was adjusted to 8.5, which eliminated any differences in the phosphate ionization state.

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3. Results

The ^{31}P chemical shifts of a series of 5′-mononucleotides ranged from −3.8 to −3.3 ppm (table 1, fig.1). Interactions between the phosphate group and the base were responsible for some of the chemical shift variations. Experimental and theoretical studies on 8-amino-AMP and 8-methylamino-AMP had shown that an intramolecular hydrogen bond between the ribose-phosphate backbone and the base stabilized the anti conformation about the glycosyl bond and induced an unusual nucleotide conformation [2,9–11]. Hence, these compounds presented an opportunity to measure the effect of a hydrogen bond of the type $^{2-}O_3PO\cdot\cdot HNR$ on the ^{31}P chemical shift. Comparison of these nucleotides with those of the parent compound AMP (table 1) showed that a 0.4 ppm shielding accompanied hydrogen bond formation.

A strong intramolecular interaction also existed in β-NMN, but instead of a hydrogen bond, the attrac-

Table 1
^{31}P chemical shifts in ppm of 5′-nucleotides[a]

Nucleotide	Chemical shift
8-Amino-AMP	−3.30
8-Methylamino-AMP	−3.31
AMP	−3.67
β-Nicotinamide mononucleotide	−3.32
α-Nicotinamide mononucleotide	−3.63

[a] Chemical shifts measured at pH 8.5, 0.01 M

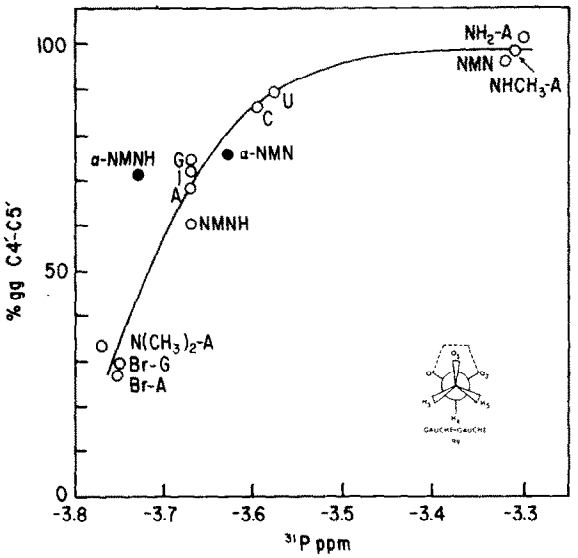

Fig.1. ^{31}P chemical shifts were measured at 40 MHz in aqueous solutions at pH 8.5. The population of the *gauche–gauche* conformation about the C4′–C5′ bond was estimated from the appropriate coupling constants according to standard procedure [3]. The populations differ by ~10% from some of those reported in the literature because computations here were based on recently revised [4] Karplus parameters. The abbreviations and the references for the coupling constant data follow: Br-A, 8-bromo-AMP [3]; Br-G, 8-bromo-GMP (unpublished data); $N(CH_3)_2$-A, 8-dimethylamino-AMP [2]; NMNH, reduced nicotinamide mononucleotide [5,6]; A, AMP [7,8]; I, IMP [7]; G, GMP [7,8]; C, CMP [8]; U, UMP [8]; NMN, nicotinamide mononucleotide [5,6]; 8-$NHCH_3$-A; 8-methylamino-AMP [2]; 8-NH_2-A, 8-amino-AMP [2]. Data for the α-anomers of NMN and NMNH are denoted by a solid point (•).

tion involved the negatively charged phosphate group and the positively charged base [5,12,13]. This interaction was virtually eliminated in the α-anomer of NMN [5] and a 0.3 ppm change (table 1) resulted from the elimination of this interaction. The upfield shift was also observed in 7-methyl guanosine nucleotides, which had a similar attraction between the phosphate group and the base [14].

A correlation existed between interactions of the phosphate group with the base, the ^{31}P chemical shift, and the conformation about the C4′–C5′ bond in 5′-β-nucleotides (fig.1). The nucleotides which experienced a strong attraction between the base and the phosphate group vide supra exhibited the most

Table 2
^{31}P chemical shifts in ppm of uridine diphosphohexoses[a]

Compound	Hexose-P	Uridine-P
UDP-Glucose	12.78	11.18
UDP-Glucuronic Acid	12.70	11.03
UDP-Galactose	12.60	11.11
UDP-Mannose	13.76	11.49

[a] Chemical shifts measured at pD 7, 0.01 M

shielding (fig.1). In contrast, there was a repulsion between the base and the phosphate in the *syn* nucleotides 8-Br-AMP [3], 8-$N(CH_3)_2$-AMP [2,11], and 8-Br-GMP [15], and these nucleotides exhibited the least shielding (fig.1). In addition, there was no base–phosphate interaction in α-NMNH [5] and it exhibited a ^{31}P chemical shift comparable to the *syn* nucleotides. The magnetic anisotropy of the base [16,17] did not appear to be the main shielding mechanism, since the correlation was not linear (fig.1). It may be noted that the phosphate group is nearest to the base in the *gauche–gauche* conformation.

Another dependence of the ^{31}P chemical shifts on stereochemistry was observed for the α-hexose-1-P ring of UDP-hexoses. The chemical shift of the phosphate group which was directly attached to the C1 position of the α-hexose ring was 1 ppm more shielded in the UDP-mannose than in the other hexose derivatives (table 2). The configuration of C2 was the only structural difference between UDP-mannose and UDP-glucose. This indicated that the C2 hydroxyl may be responsible for this result. These compounds

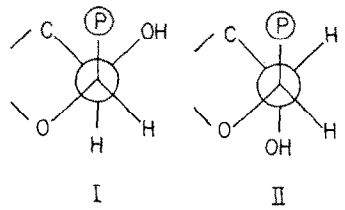

Fig.2. Newman projections of the conformation about the C1–C2 bond of the α-hexose-1-P ring for the two different configurations of C2. Projection I shows the configuration in both UDP-glucose and UDP-galactose. The configuration in II is that of UDP-mannose.

are known to exist in a chair conformation with the phosphate group axial [18]. Newman projections of the C1–C2 bond showed a consequence of the different configurations at C2 on conformation (fig.2). The C2 hydroxyl was *trans* to the phosphate group (fig.2,II) in UDP-mannose, while it was *gauche* (fig.2,I) in the remaining compounds (table 2). We had reported that the ^{31}P chemical shifts of hexose-1-phosphates were shielded due to attachment of the phosphate group at the anomeric carbon [19]. The data here showed that the ^{31}P chemical shift also depended on conformation and on the configuration of the adjacent carbon C2.

4. Discussion

The results were in general agreement with the conclusion that phosphate ionization caused significantly more perturbation of the chemical shift than do some other changes in environment [20]. Our data indicated that hydrogen bond formation, or an attraction with a positively charged base, produced ~10% as much shielding as would protonation of a dianionic phosphate group.

It has been proposed that the O–P–O bond angle was the major factor affecting ^{31}P chemical shifts [21,22]. This explained the 20 ppm deshielding of 2′,3′-cyclic nucleotides compared to other nucleotides, and it also was useful for rationalizing the upfield shift which follows protonation of a dianionic phosphate group [21,22]. On the other hand, quantum-mechanical calculations predict that P–X electronegativity strongly effects ^{31}P chemical shifts [23]. A hydrogen bonding interaction could change the O–P–O bond angle or the P–X electronegativity. Thus, a large chemical shift change might be expected. The small change observed here may be rationalized with respect to both theoretical predictions if the chemical shift changes associated with electronegativity effects were in an opposite direction to those associated with O–P–O bond angle differences. Hence, the relatively low sensitivity of the ^{31}P chemical shift to hydrogen bonding and to the other strong coulombic attractions could be rationalized.

Proximity between a *gauche* hydroxyl group and the phosphate group in the α-hexose-1-P compounds caused deshielding of 1 ppm (table 2). This was opposite in direction to that brought about by the coulombic attractions in nucleotides. Therefore, it appears that some other mechanism was responsible for the chemical shift variations in the α-hexose-1-P ring.

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