^{31}P double-quantum solid-state NMR study of phosphoroorganic compounds with (O)P–O–P-(O), (S)P–O–P(S) and (S)P–S–P(O) unit

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Abstract

In this work we have tested applicability of the commonly used double quantum recoupling sequence POST-C7 to study of ^{31}P–^{31}P geometrical constraints for phosphoroorganic model compounds with different chemical shift anisotropy (CSA) and distinct molecular dynamics in the crystal lattice. Our results clearly show that even with large CSA, POST-C7 gives good efficiency of ^{31}P double-quantum excitations. Moreover, large amplitude molecular motion only slightly disturb ^{31}P build-up curve. χ^2 error analysis is used for verification of values and orientations of chemical shift tensors (CST) parameters employed for simulation of POST-C7 buildup curves.
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1. Introduction

Bioorganic phosphates are important components of living organisms involved in a multitude biological processes [1]. There is a number of class of compounds responsible for metabolic pathways with phosphoryl group transfer. Among them phosporylated proteins and nucleoside phosphates play a crucial role [2]. Both biomolecules are involved in phosphorylation and dephosphorylation processes which involve replacement of the hydroxyl group by a phosphate residue and vice versa in numerous biologically active compounds such as carbohydrates, nucleic acids, and proteins [3].

In our recent papers we have presented some structural studies for series of O-phosphorylated amino acids (serine, threonine and tyrosine) [4] and adenosine triphosphate (ATP) hydrates employing mostly solid state NMR spectroscopy [5]. In the latter project we have shown that ^{31}P double quantum two-dimensional solid-state NMR spectroscopy can be used to distance measurements in disodium ATP hydrates. Continuing our interests in

application of recoupling strategy to analysis of ^{31}P structural constraints in di- and triphosphates of nucleosides and their structural analogues recently we focused our attention on thiophosphate derivatives of nucleotides. A variety of thiophosphate analogues of ATP and ADP such as ADPαS, ADPβS, ATPαS, ATPβS and ATPγS were discussed by Eckstein [6]. Nucleoside thiophosphates have found wide application and have helped answered questions in various areas of biochemistry. For instance, Cullis et al. employed nucleotide thiophosphates to probe mechanistic aspects of *Eschericha coli* DNA gyrase [7]. Rayment and coworkers employing MgATPγS have refined X-ray structure of the *Dictyostelium discoideum* myosin domain. Myosin is a molecular motor that converts chemical energy into directed movement [8]. Number of other examples can be found in literature.

In current work we wish to test applicability of ^{31}P POST-C7 sequence [9] to study of model samples with different arrangement of oxygen, sulfur, phosphorus atoms as shown in Scheme 1.

Due to 100% natural abundance and a relatively high magnetogyric ratio, ^{31}P is an attractive nucleus for NMR studies, in particular for dipolar ^{31}P–^{31}P correlation experiments. The strong dipole–dipole couplings between

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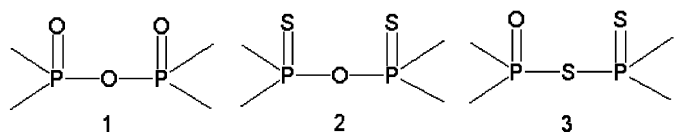
Scheme 1.

^{31}P nuclei allows spatial proximities to be directly probed within a distance range of 2–5 Å. The homonuclear dipole–dipole coupling conveys information about the distance of a pair of nuclei and is expressed by the equation $R_{dip} = -(\mu_0/8\pi^2)\gamma 2h/d^3$, where d is the internuclear distance and γ is the magnetogyric ratio. On the other hand, it is known that large chemical shift anisotropy (CSA) and dynamic processes have great influence on quality of spectra, recoupling strategy and efficiency of double quantum (DQ) excitation. Models chose in our project cover problems discussed *supra*.

2. Experimental

2.1. NMR measurements

The solid-state CP MAS experiments were performed on a BRUKER Avance DSX 300 spectrometer at 75.47 MHz frequency for ^{13}C and 121.49 MHz for ^{31}P, equipped with a MAS probehead using 4 mm ZrO$_2$ rotors. The sample of glycine was used for setting the Hartmann–Hahn condition and adamantane as a secondary chemical shift reference $\delta = 38.48$ and 29.46 ppm from external TMS [10]. For ^{31}P NMR 85% phosphoric acid was employed as primary standard. The conventional spectra were recorded with a proton 90° pulse length of 3.5 μs and a contact time of 1 ms. The repetition delay was 10 s and the spectral width was 25 kHz. The FID's were accumulated with a time domain size of 2 K data points. The RAMP shape pulse [11] was used during the cross-polarization and TPPM decoupling [12] with τ_p 6.8 μs and a phase angle of 20° during the acquisition. The cross-polarization efficiency was measured with contact times between 10 μs and 12 ms. The spectra data were processed using the XWIN-NMR program and XWIN-PLOT [13]. ^{13}C T_1 and ^{31}P T_1 parameters were measured by means of Torchia sequence [14]. The ^{13}C $T_{1\rho}$ relaxation times were measured employing standard BRUKER software. POST-C7 experiments were carried out with MAS frequency of 10 kHz The appropriate r.f. pulse nutation frequency was 70 kHz. The efficency of DQ was measured by comparison of POST-C7 and CP/MAS spectra recorded under exactly the same conditions. All simulations including analysis of principal elements of NMR chemical shift tensors and POST-C7 build up curves were done using the SIMPSON program under LINUX environment [15]. The δ_{ii} parameters are defined as follows $|\delta_{11} - \delta_{iso}| \geqslant |\delta_{22} - \delta_{iso}| \geqslant |\delta_{33} - \delta_{iso}|$, $\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$, $\delta_{aniso} = (\delta_{11} - \delta_{iso})$, $\eta = (\delta_{22} - \delta_{33})/\delta_{anizo}$. The liquid NMR experiments were performed on a BRUKER

DRX 500 spectrometer at 125.76 MHz frequency for ^{13}C and 202.46 MHz for ^{31}P.

2.2. Theoretical calculation

DFT GIAO calculations were carried out on a PC using the GAUSSIAN 03 program under LINUX environment [16]. The GIAO method with the B3PW91 hybrid method with 6-311++G** and 6-311G** basis set was used to calculate NMR parameters and orientation of the ^{31}P principal elements of the chemical shift tensor with respect to molecular frame.

3. Results

3.1. Study of Adenosine 5′-diphosphate (ADP) potassium salt 1

Our study begun with ADP sample for which the X-ray structure is known [17]. This sample represents model **1**. The ^{31}P principal elements of chemical shift tensor δ_{ii} and CSA parameters are collected in Table 1. The orientation of the ^{31}P δ_{ii} is presented in Fig. 1. The employed pulse sequence is shown in Scheme 2. In our work, we have applied the POST-C7 sequence which is characterized by large DQ efficiency and is easy to implement [9].

A common way to determine distance information using spin-1/2 DQ NMR is based on the acquisition of DQ filtered spectra as a function of the DQ excitation time. However, as discussed in our previous paper in the case of ^{31}P DQ NMR build-up curves, several problems can occur [5]. First, a negative DQ intensity is frequently observed at short excitation times, caused by slight misadjustment in the RF amplitude, large chemical shift anisotropy, etc. Second, severe damping of the build-up curves can be observed due to relaxation and the influence of further spins. Fig. 2a shows the build-up curve for ADP sample obtained by means of POST-C7 sequence with spinning speed of sample 10 kHz and RF used for recoupling equal 70 kHz employing symmetric procedure. As seen the maximum is reached at 1.2 ms. In range of 2.1–3 ms the small negative dip is seen. The established value of DQ efficiency is 18.5%.

We have also carried out a series of 1D experiments with different excitation times τ_{ex}, employing asymmetric and

Table 1
Values of the experimental ^{31}P chemical shift parameters δ_{ii} and corresponding anisotropic parameters

Sample	Nr ^{31}P	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	Ω
1	P1	−10.7	69	6	−107	176
	P2	−11.8	85	17	−137	222
2	P1	51.8	165	108	−119	284
	P2	40.9	149	110	−138	287
3	P1	66.0	176	123	−101	277
	P2	4.9	82	81	−148	230