

Distance measurements in disodium ATP hydrates by means of ^{31}P double quantum two-dimensional solid-state NMR spectroscopy

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Abstract

POST-C7 measurements provide constraints allowing distinguishing crystal lattice organization and establishing intra and/or intermolecular distances between phosphorus atoms of triphosphate chains for different hydrates of disodium ATP salts. Double-quantum efficiency in function of excitation time obtained from series of two-dimensional spectra for POST-C7 experiments was used to set up of buildup curves and semi-quantitative measure of ^{31}P – ^{31}P length.

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1. Introduction

Adenosine 5'-triphosphate (ATP) plays a very important role in a number of biological processes including signal transduction, energy transfer, phosphorylation–dephosphorylation, and many others [1]. The key information, which allows understanding of the nature of these functions on a molecular level, is knowledge about the geometry and the range of conformational changes. The crystal and molecular structure of disodium ATP salts, as well as other divalent cation hydrates, have been reported [2–4]. Sugawara et al. [5] have published X-ray studies of the reversible structure transition of disodium ATP between dihydrate and trihydrate in a single crystal state. It was concluded that this process is related to the conformational flexibility of ATP and a change of the cell dimensions. Very recently, Saito and co-workers [6] have presented solid-state NMR studies of different hydrates of Na_2ATP , together with a simple pattern, based on ^{31}P CP/MAS spectra analysis, allowing the recognition of the

monohydrate, mixtures of hydrates, and the trihydrate in powdered samples. However, little is known about the structural constraints, cell organisation or intermolecular distances in the hydrated lattice of powders. In this work, we present an approach based on two-dimensional (2D) solid-state NMR spectroscopy which, via measurement of ^{31}P dipolar interactions, allows the answering of questions regarding the constraints of the triphosphate chain.

2. Experimental

2.1. Sample preparation

ATP was purchased from Sigma–Aldrich. Before the measurement samples were recrystallized according to method described in literature [5,6]. However, as we found, when the standard procedure (crystallization from mixture water/*n*-propanol, 1:2.5 v/v) is employed, crystallization of ATP hydrates is a random and complex process which in most cases leads to mixture of dihydrate and trihydrate. Sample obtained in this way is labelled as **1b** and shown in Fig. 1B. Much better and reproducible results in sample preparation we got using modified method. Trihydrate **1a**

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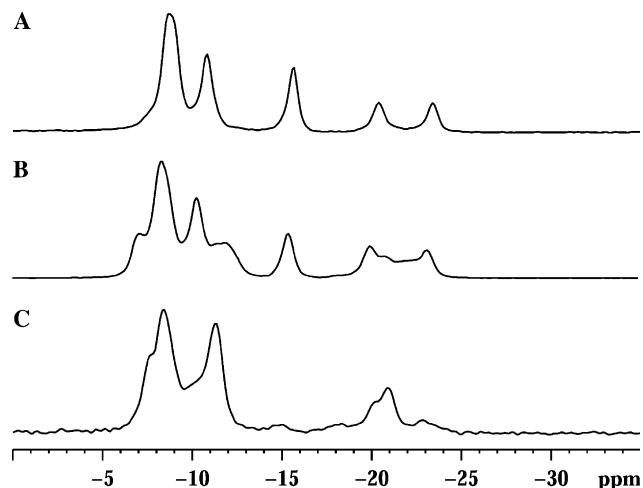


Fig. 1. ^{31}P CP/MAS spectra of ATP hydrates measured at 278 K with spinning speed 8 kHz (A) trihydrate, (B) dihydrate, and (C) monohydrate.

(Fig. 1A) was obtained by means of diffusion process. One hundred milligrams of ATP was dissolved in mixture of water/2-propanol (1:2.5 v/v), and the bulb with solution was closed in vessel filled of 2-propanol. The overnight crystallization in room temperature gives well-defined trihydrate. The monohydrate **1c** (Fig. 1C) was obtained using similar procedure. The only one change is employing of methanol instead of 2-propanol for diffusion.

2.2. Solid-state NMR

The solid-state CP/MAS ^{31}P NMR experiments were performed on Bruker DSX 300 spectrometer operating at 7.35 T. The spectrometer was equipped with MAS probeheads for 4 and 2.5 mm ZrO_2 rotors. POST-C7 experiments were carried out with MAS frequency of 10 kHz. The appropriate RF pulse nutation frequency was 70 kHz. 2D spectra were recorded with t_1 increment synchronised with rotor period. 48 t_1 serial files were collected for each experiment. The 2D spectra were processed with Qsine apodization functions equal 2.5 in both dimensions. Integration of the cross-peak intensities was carried out employing XWIN-NMR and SPARKY programs.

2.3. Simulation of NMR parameters and other calculations

All simulations including analysis of principal elements of NMR chemical shift tensors and POST-C7 build-up curves were done using the SIMPSON program published by Nielsen and co-workers [7]. The δ_{ii} parameters are defined as follows: $|\delta_{11} - \delta_{\text{iso}}| \geq |\delta_{22} - \delta_{\text{iso}}| \geq |\delta_{33} - \delta_{\text{iso}}|$, $\delta_{\text{iso}} = (\delta_{11} + \delta_{22} + \delta_{33})/3$, $\delta_{\text{aniso}} = (\delta_{11} - \delta_{\text{iso}})$, $\eta = (\delta_{22} - \delta_{33})/\delta_{\text{aniso}}$.

Orientation of the ^{31}P principal elements of the chemical shift tensor with respect to molecular frame of triphosphate chain was done employing GAUSSIAN 03 program and GIAO method (b3pw91/6-31G) [8].

3. Results and discussion

3.1. ^{31}P NMR studies

Our study begun with the trihydrate Na_2ATP salt for which the solid-state geometry is well defined [5]. It is apparent from the ^{31}P CP/MAS experiment (Fig. 1A) that the unit cell contains two molecules in the asymmetric part. The distances between phosphorus centres taken from X-ray studies are shown in Fig. 2.

Molecular packing in the unit cell for trihydrate and dihydrate is attached as Supplementary material. 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 ^{31}P nuclei allows spatial proximities to be directly probed within a

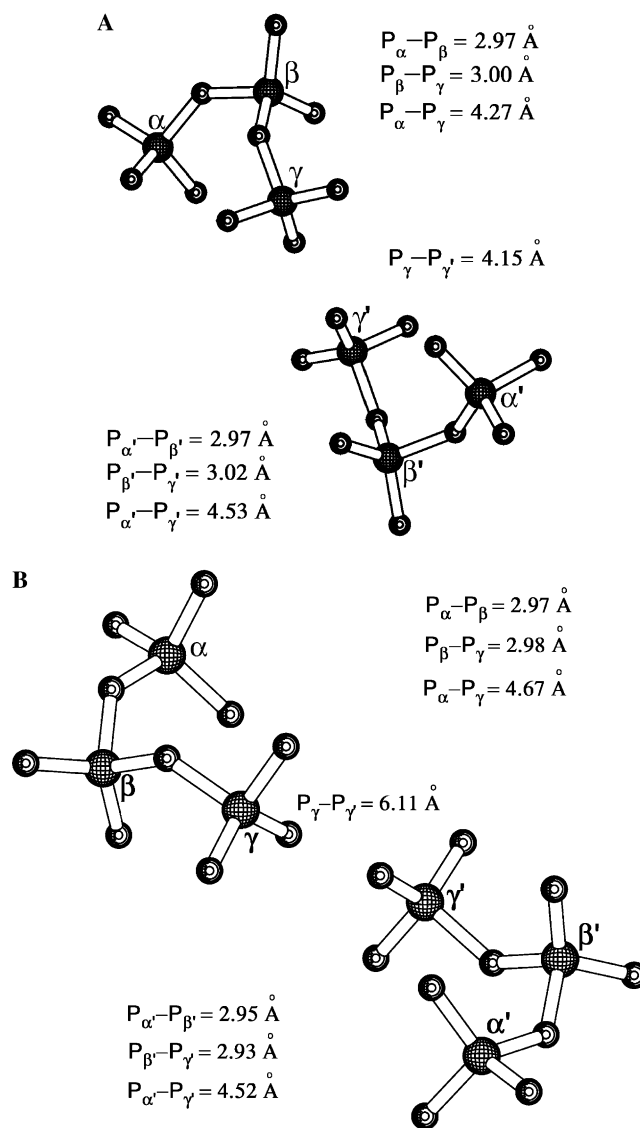


Fig. 2. Distances between phosphorus atoms in triphosphate chains of (A) trihydrate ATP and (B) dihydrate ATP. Data are taken from [5].

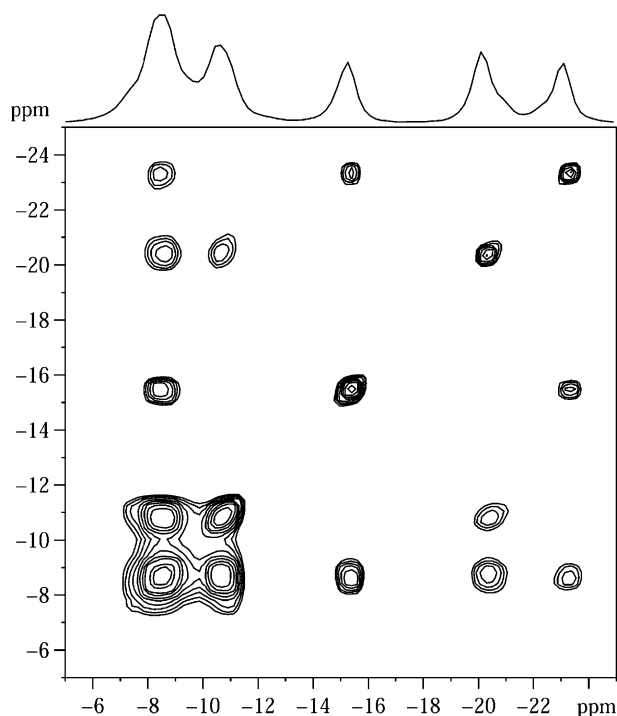
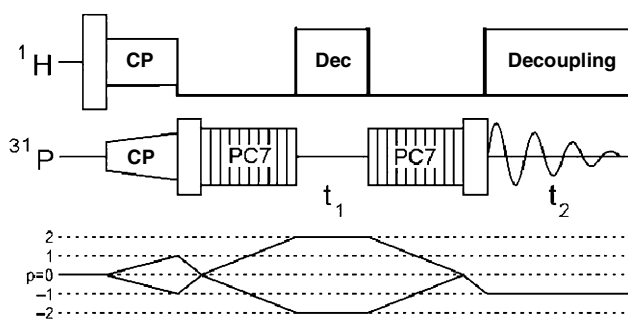


Fig. 3. ^{31}P – ^{31}P proton driven spin diffusion correlation for trihydrate ATP. The phase sensitive spectrum was recorded with spinning speed 10 kHz and mixing time 200 ms.



Scheme 1.

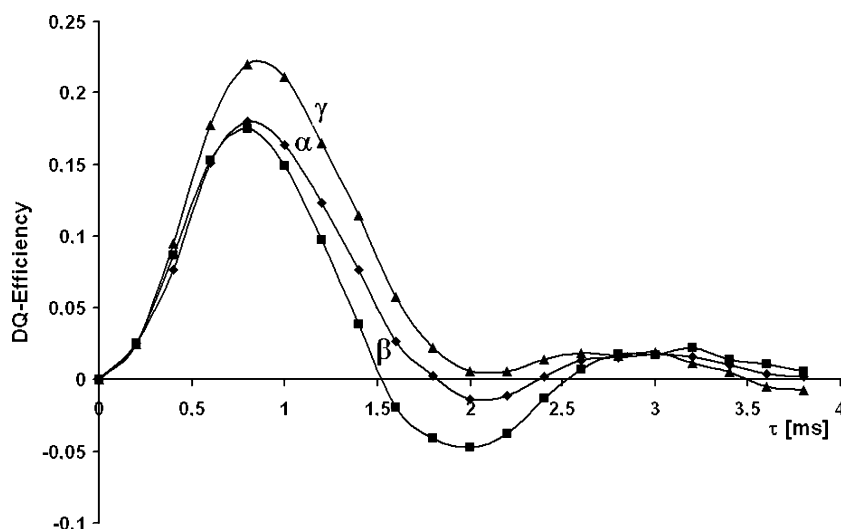


Fig. 4. ^{31}P POST-C7 buildup curve for monohydrate disodium ATP taken from series of 1D spectra recorded at 278 K with spinning speed 10 kHz and RF 70 kHz.

distance range of 4–5 Å. The homonuclear dipole–dipole coupling conveys information about the distance of a pair of nuclei and is expressed by the equation $R_{\text{dip}} = -(\mu_0/8\pi^2)\gamma^2\hbar/d^3$, where d is the internuclear distance and γ is the magnetogyric ratio. Employing the 2D proton driven spin diffusion (PDS) experiment [9] via single quantum (SQ–SQ) coherences we established the basic connectivities between phosphorus sites in the unit cell (Fig. 3).

However, as we concluded, in the SQ approach, some important structural constraints regarding intermolecular contacts are missed because of strong overlapping of the axial peaks. Hence, in the next step we decided to use an experiment which employs a double quantum (DQ) strategy [10]. Under MAS condition, dipolar recoupling sequences are applied to excite DQ coherences and, subsequently, to reconvert them back into observable magnetization. The intensity of a DQ signal depends on the dipolar coupling between the respective pair of nuclei, i and j . Therefore, DQ signals can be used as a quantitative or semi-quantitative measure of internuclear distances and molecular motion. There are a number of recoupling sequences that allow the measurement of internuclear distances. Schmedt auf der Gönne [11] recently exhaustively discussed some of them with respect to ^{31}P inorganic samples. In our work, we have applied the POST-C7 sequence which is characterised by large DQ efficiency and is easy to implement [12]. The employed pulse sequence is shown in Scheme 1.

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, in the case of ^{31}P DQ NMR build-up curves, several problems can occur. 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 is observed due to relaxation and the influence of

further spins. From inspection of Fig. 3 it is apparent that the phosphorus nuclei of ATP cannot be considered in terms of “isolated spin pairs” but rather as “spins in a cluster” hence the problem discussed supra is expected. Surprisingly, the POST-C7 build-up curve at a rotor spinning frequency of 10 kHz is very similar in a τ_{ex} range of 0–1.6 ms. Above this value, the $^{31}\text{P}_\beta$ signal has negative phase but with increasing τ_{ex} , the oscillation becomes smaller, reaching zero at 4 ms (Fig. 4). This promising result prompted us to measure 2D POST-C7 spectra for the hydrates under discussion.

Fig. 5A presents a DQ–SQ spectrum of the trihydrate **1a** recorded at 10 kHz MAS with $\tau_{\text{ex}} = 1.4$ ms. As expected, the cross-peaks between α and β as well between β and γ phosphorus centres are well resolved. The cross-peak pattern confirms the presence of two molecules in the asymmetric part of the unit cell and shows the connectivity between them. The further interactions between the α and γ sites are clear. It is interesting to note different intensities of the cross-peaks between α – γ and α_1 – γ_1 spin pairs. Unexpectedly, a relatively strong cross-peak between the γ – γ_1 phosphorus centres is seen, corresponding to inter-molecular contact in the lattice. These observations are consistent with X-ray data (Fig. 2), which shows that for the trihydrate α – γ lengths are 4.27 and 4.53 Å while the γ – γ_1 distance is 4.15 Å.

X-ray studies of dihydrate ATP show that intermolecular distance between the γ phosphorus atoms is larger (6.15 Å) than in the trihydrate, even though the unit cell is slightly smaller [13]. Consequently in the dihydrate (or mixtures of hydrates with significant amounts of dihydrate), we should not observe γ – γ cross-peaks. Fig. 5B provides straightforward evidence for this and clearly shows that the 2D POST-C7 approach can be a source of reliable information about the crystal lattice arrangement. To date, the X-ray structure of monohydrate ATP is not established, so solid-state NMR spectroscopy is a most useful analytical technique, which allows the elucidation of subtle structural effects [6].

Fig. 5C presents the 2D POST-C7 spectrum of the monohydrate, with strong intermolecular γ – γ interactions. The intensity of this cross-peak suggests that the intermolecular distance between γ sites is only slightly longer compared to the intramolecular α – γ contact and both independent molecules in the unit cell are involved in such interactions. To verify this hypothesis, we have carried out a series of 2D experiments with different excitation times τ_{ex} , employing both symmetric and constant-time procedures. In the symmetric procedure, excitation, and reconversion times are incremented such that $\tau_{\text{DQ1}} = \tau_{\text{DQ2}}$ [11]. In the constant-time procedure, the times are varied such that $\tau_{\text{DQ2}} = \tau_{\text{total}} - \tau_{\text{DQ1}}$, where τ_{DQ1} is incremented from zero to τ_{total} . The total conversion time needs to be long enough to induce a zero crossing in the build-up curve which is necessary for reliable data analysis in terms of the dipolar coupling constant. As shown in Fig. 6, the build-up curve for α – γ and γ – γ pairs have comparable pro-

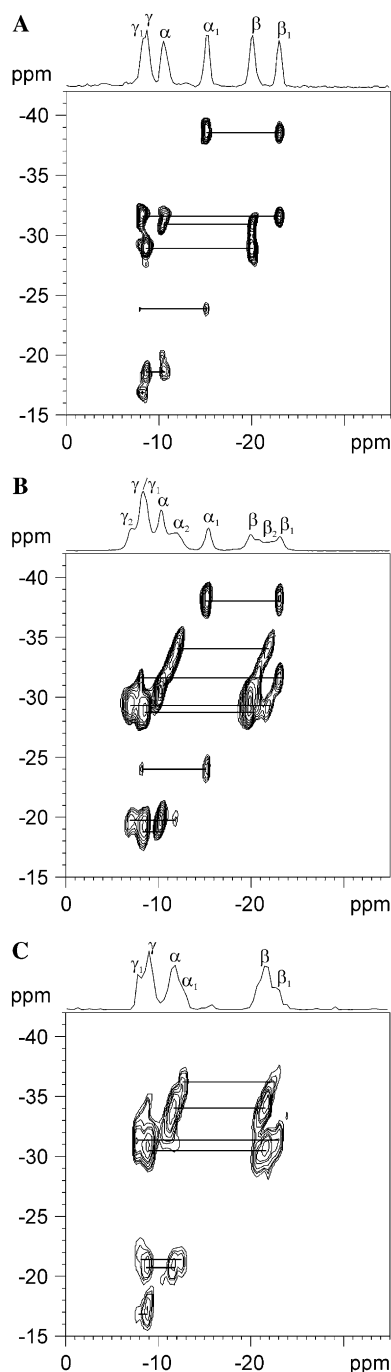


Fig. 5. ^{31}P – ^{31}P POST-C7 correlation spectra for disodium ATP hydrates recorded at 287 K, spinning rate 10 kHz, RF 70 kHz and excitation time (τ_{ex}) 1.4 ms, (A) trihydrate, (B) dihydrate, and (C) monohydrate.

files but the intensity of latter curve is slightly smaller. This result provide further evidence confirming a minute distinction between these two distances.

Finally, we wish to comment on the problem of reversible hydration of disodium ATP hydrates. Under mild thermal condition (heating up to 373 K), $\text{Na}_2\text{ATP}\cdot 3\text{H}_2\text{O}$ gradually undergoes a rearrangement, losing water molecules. The final product is the monohydrate. It is interesting to note that during phase transition the cross-peaks

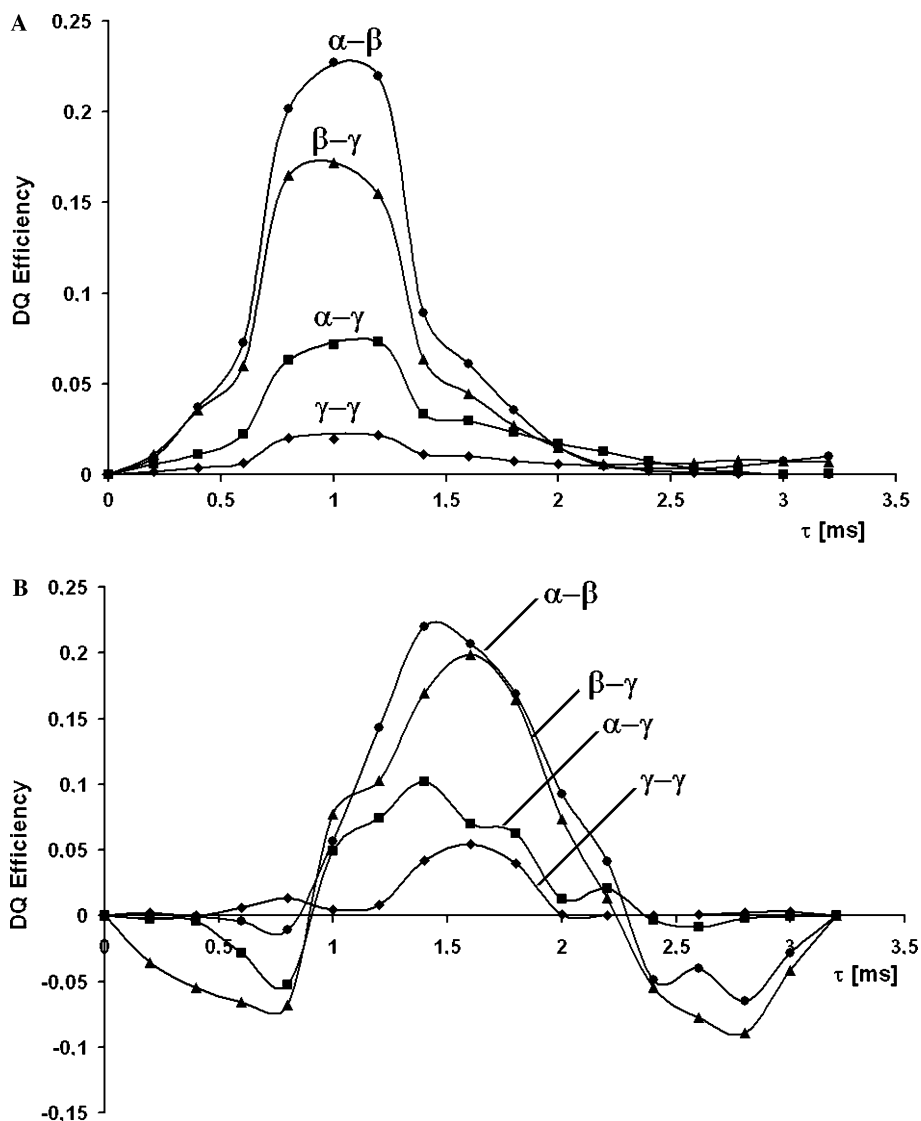


Fig. 6. ^{31}P POST-C7 buildup curve for monohydrate disodium ATP taken from series of 2D spectra recorded at 278 K with spinning speed 10 kHz and RF 70 kHz employing (A) symmetric procedure and (B) constant time procedure.

corresponding to $\gamma-\gamma$ interactions may be monitored on each stage [13].

3.2. NMR simulations

Simulation of POST-C7 build-up curve requires the knowledge of several NMR parameters, which are necessary as an input file to carry out reliable calculations. In case of the phosphorus atoms the crucial information regards chemical shift anisotropy (CSA), which has great influence on the efficiency of DQ coherences. Another important set of parameters is orientation of the principal elements of ^{31}P nucleus with respect to molecular frame of phosphate group. The values of α_{P} , β_{P} must be converted into the structurally significant angles α_{M} , β_{M} where P is ^{31}P CSA principal axis system and M is local molecular frame. The relative orientation of this axis systems is conveniently specified by set of Euler angles.

The values of ^{31}P δ_{ii} parameters for sodium ATP trihydrate were reported by Un and Klein [14]. Unfortunately in cited paper not all subtle structural information were shown, very likely because of not sufficient resolution. Fig. 7A presents ^{31}P CP/MAS spectrum of sample **1a** with spinning rate 3 kHz. Calculated spectrum is shown in Fig. 7B. Similar approach was employed for sample **1c** (Figs. 7C and D, respectively). The values of ^{31}P δ_{ii} elements and other shielding parameters are collected in Table 1. From obtained data it is apparent that for β phosphorus site $|\delta_{\text{anizo}}|$ parameters are significantly larger compared to other phosphorus atoms. This difference is mostly caused by distinction of values of ^{31}P δ_{33} elements. Moreover ^{31}P δ_{33} is also sensitive to number of water molecules present in the crystal lattice. For sample **1a** $|\delta_{\text{anizo}}|$ of β phosphorus is larger ca 10–15 ppm compared to **1c**.

In most papers, dealing with orientation of ^{31}P δ_{ii} elements for phosphate groups the values obtained by

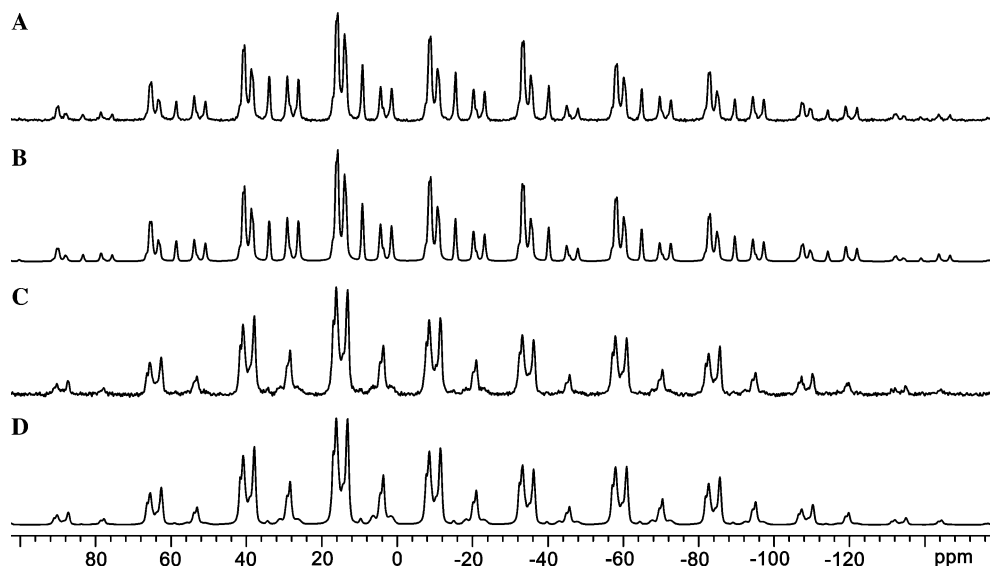


Fig. 7. (A) Experimental ^{31}P CP/MAS NMR spectrum of **1a** recorded at 273 K with spinning rate 3 kHz. (C) experimental ^{31}P CP/MAS NMR spectrum of **1c** recorded at 273 K with spinning rate 3 kHz. (B and D) Appropriate theoretical spectra obtained employing SIMPSON program.

Table 1a

Values of the experimental ^{31}P chemical shift parameters δ_{ii} and corresponding δ_{aniso} and asymmetry parameter η or sodium ATP (a) trihydrate and (c) monohydrate

	^{31}P	δ_{iso} (ppm)	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	δ_{aniso} (ppm) ($\delta_{33} - \delta_{\text{iso}}$)	η ($(\delta_{22} - \delta_{11})/\delta_{\text{aniso}}$)
a	P_γ	-8.4	88.3	-3.9	-109.7	-101.3	0.9
		-9.0	84.3	1.7	-113.0	-104.0	0.8
	P_α	-10.7	73.9	7.4	-113.4	-102.7	0.6
		-15.5	77.8	-1.2	-122.9	-107.5	0.7
	P_β	-20.3	80.6	14.8	-156.2	-135.9	0.4
		-23.2	73.7	12.9	-156.2	-133.0	0.5
c	P_γ	-7.7	81.8	6.6	-111.5	-103.8	0.7
		-8.6	80.5	7.0	-113.1	-104.6	0.7
	P_α	-10.3	79.3	7.3	-117.5	-107.2	0.7
		-11.5	81.5	6.4	-122.4	-110.9	0.7
	P_β	-20.1	73.3	9.7	-143.4	-123.3	0.5
		-21.0	69.4	7.9	-140.3	-119.3	0.5

The δ_{ii} parameters are defined as follows: $|\delta_{11} - \delta_{11}| \geq |\delta_{22} - \delta_{\text{iso}}| \geq |\delta_{33} - \delta_{\text{iso}}|$, $\delta_{\text{iso}} = (\delta_{11} + \delta_{22} + \delta_{33})/3$, $\delta_{\text{aniso}} = (\delta_{11} - \delta_{\text{iso}})$, $\eta = (\delta_{22} - \delta_{33})/\delta_{\text{aniso}}$.

Table 1b

Calculated angles between principal axes i and P–O bonds for the sodium ATP trihydrate

<	Molecule A			<	Molecule B		
	σ_{11}	σ_{22}	σ_{33}		σ_{11}	σ_{22}	σ_{33}
i- P_α - O_{10}	48.5	138.4	92.0	i- P_α - O_{29}	69.0	149.4	111.2
i- P_α - O_1	86.0	61.5	28.7	i- P_α - O_{20}	93.6	92.3	4.3
i- P_α - O_2	80.4	53.6	141.9	i- P_α - O_{21}	149.4	73.7	115.2
i- P_α - O_3	148.5	121.0	95.3	i- P_α - O_{22}	47.9	45.6	104.4
i- P_β - O_3	37.3	52.9	93.6	i- P_β - O_{22}	84.4	165.5	103.3
i- P_β - O_4	90.1	120.2	149.8	i- P_β - O_{23}	85.2	89.3	4.9
i- P_β - O_5	90.1	120.0	30.0	i- P_β - O_{24}	41.1	62.8	118.2
i- P_β - O_6	137.8	47.9	92.3	i- P_β - O_{25}	156.7	76.6	108.7
i- P_γ - O_6	130.7	139.2	92.4	i- P_γ - O_{25}	37.2	126.8	94.5
i- P_γ - O_7	29.6	119.3	93.5	i- P_γ - O_{26}	138.5	131.4	91.9
i- P_γ - O_8	95.3	58.3	147.8	i- P_γ - O_{27}	87.4	62.8	27.4
i- P_γ - O_9	99.0	61.8	29.8	i- P_γ - O_{28}	89.1	51.1	141.1

Herzfeld et al. [15] from single crystal measurement of barium diethyl phosphate are taken as a reference data. The alternative method, which provide the same information

is approach based on theoretical calculations. The advantage of the latter approach is due to the fact that obtained values represent real sample not model. In our work, the

orientations of ^{31}P δ_{ii} components with respect to molecular frame of triphosphate chains for ATP sample were calculated employing GIAO program and are presented in Fig. 8. The appropriate value of angles are given in Table 1. The procedure of calculation of Euler angles is attached as Supplementary materials. [13]

Fig. 9 shows the theoretical POST-C7 buildup curves calculated with anisotropic ^{31}P values taken from Table 1 and established Euler angles. In our calculations we have employed procedure with two-spin and three-spin approximation. Fig. 9a presents buildup curves for symmetric procedure with P–P distances in range from 2.97 to 4.53 Å. The buildup profiles for two-spin approach shows that for the former distance the DQ efficiency is ca. 26% and maximum is reached at 1.2 ms. For the latter length the DQ efficiency is 7% and maximum is found at 1.8 ms. It is interesting to note that for three-spin calculations (Fig. 9B) we obtained much higher DQ efficiency (ca. 88%). It is worthy to stress that this approach predicts negative phase for build-up profile what is consistent with experimental data.

Simulated build-up curves of constant time procedure for two-spin and three-spin systems are shown in Figs. 9C and D, respectively. From analysis of data it is apparent that calculations with two-spin approach correspond roughly to experimental results. Three-spin approximation presents very dip negative phase, much larger compared to that seen in Fig. 5. It is apparent from presented simulations that none of the used methods exactly fit the experimental data, however trend in theoretical build-up curves is consistent with measured values.

4. Conclusions

Water molecules are common components of biomolecular crystals and influence both molecular structure and macroscopic properties of crystals. Not always the obtained material is suitable to mono-crystal studies (vide monohydrate 1c). In this work, we demonstrated that the DQ NMR approach could be used to semi-quantitative describe crystal lattice organisation and is a source of important structural information. The power of methodology based

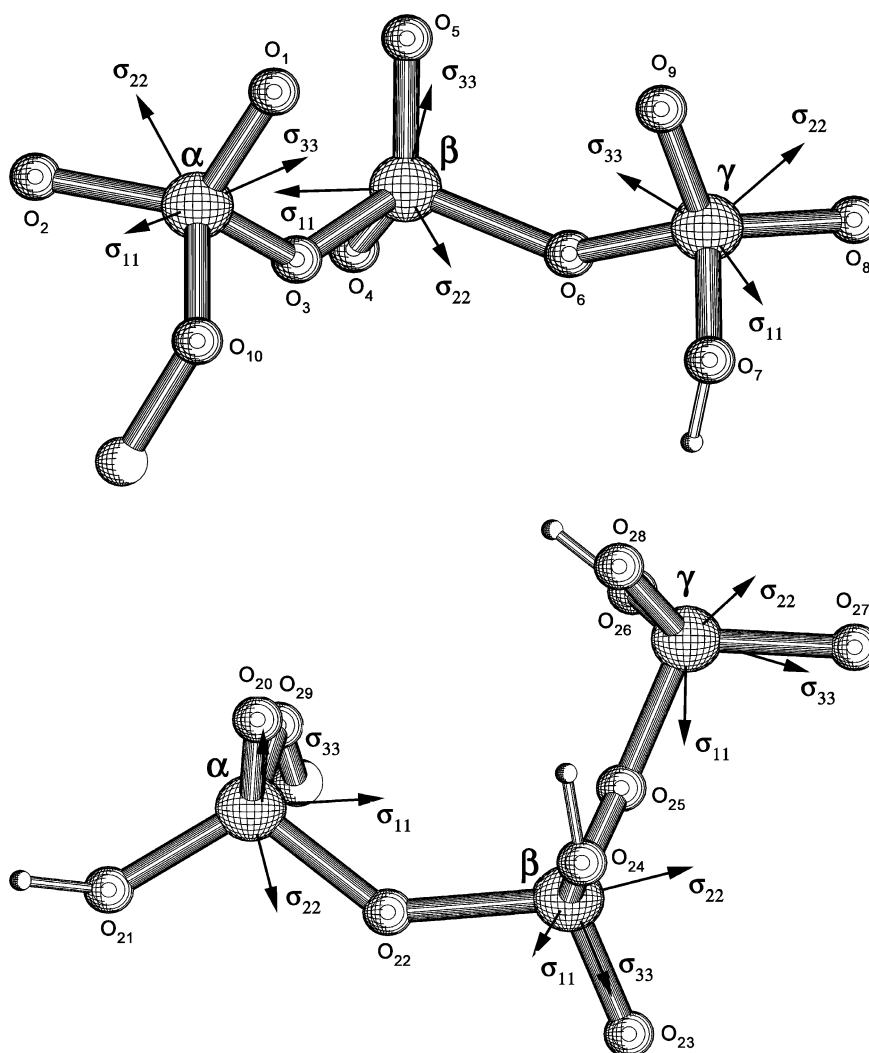


Fig. 8. Calculated orientations of the ^{31}P principal elements of chemical shielding tensors with respect to molecular frame of triphosphate chain in trihydrate 1c. The values of the appropriate angles are collected in Table 1b.

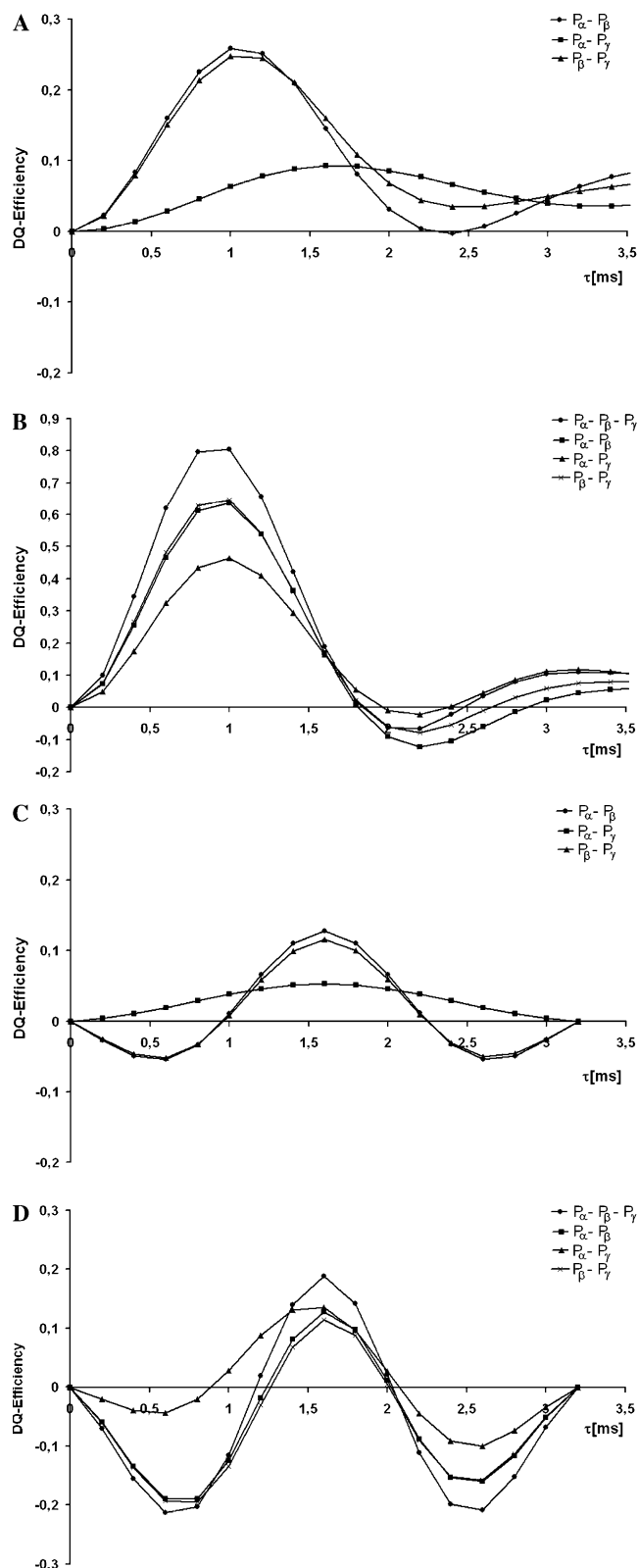


Fig. 9. Theoretical POST-C7 build-up curves for different P-P distances (A) symmetric procedure with two-spin approximation (B) symmetric procedure with three-spin approximation, (C) constant time procedure with two-spin approximation, and (D) constant time procedure with three-spin approximation. Build-up profiles were obtained employing SIMPSON program.

on NMR recoupling sequences and X-ray diffraction in structural studies of powdered samples was recently discussed by Levitt and co-workers [16]. Approach presented in cited work is extremely useful when the “rare” spins (e.g., ^{29}Si), which can be considered as isolated spin pairs are used as a structural probes. The challenge for solid-state NMR is introducing the methodology, which allow to measure single contact for “abundant” spins being in cluster.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jmr.2005.11.016](https://doi.org/10.1016/j.jmr.2005.11.016).

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