Chemical Physics Letters 660 (2016) 214–220

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Research paper

6-Aminopenicillanic acid revisited: A combined solid state NMR and *in silico* refinement

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ARTICLE INFO

Article history:
Received 21 July 2016
Revised 9 August 2016
In final form 12 August 2016
Available online 12 August 2016

Keywords:
GIPAW
NMR crystallography
6-APA
CPMAS NMR

ABSTRACT

$^{13}C/^{15}N$ (experimental and *ab initio*) solid-state NMR was used to achieve an affordable way to improve hydrogen refinement of 6-aminopenicillanic acid (6-APA) structure. The lattice effect on the isotropic chemical shifts was probed by using two different magnetic shielding calculations: isolated molecules and periodic crystal structure. The electron density difference maps of optimized and non-optimized structures were calculated in order to investigate the interactions inside the 6-APA unit cell. The ^{13}C and ^{15}N chemical shifts assignments were unambiguously stablished. In addition, some of the literature ^{13}C resonances ambiguities could be properly solved.

1. Introduction

The inherent inability of X-ray diffraction (long range order technique) to provide accurate atomic positions for hydrogens leads to structural inaccuracies in molecular crystals [1]. Specially for solids in which hydrogen bonding is the most important intermolecular interaction, hydrogen refinement is crucial for a successful theoretical investigation of the structure. Taking this into account, hydrogen bond based crystals (*e.g.* pharmaceutical hydrates) cannot be fully described only with X-ray studies and need some additional crystallographic techniques, such as neutron diffraction. Particularly in those cases, computational chemistry is a powerful and affordable tool to achieve/predict the hydrogen positions [2]. Accordingly, a number of theoretical techniques can be used to calculate intermolecular potentials in crystals [3–5]. However, sometimes quantum chemical calculations are so expensive that make some investigations prohibitive. To circumvent this problem the AA-CPL (atom-atom Coulomb-Pauli-London) force field model was developed and has been used in hydrogen refinement [6].

While X-ray diffraction gives a long range order analysis of crystals, solid state NMR spectroscopy provides information about local disorders. Thus, solid state NMR can be combined with first principles (also known as *ab initio*) calculations and diffraction experiments in order to achieve a comprehensive understanding of solid systems [7]. Since *ab initio* NMR calculations are very sensitive to structural features, it can be used to probe the structural details and even to improve the hydrogen refinement [8]. In contrast with solution state NMR, the intense dipolar interaction leads to a very broad ^{1}H MAS NMR spectra. Even though ^{1}H solid state NMR is becoming commonplace, the process of proton acquisition in molecular crystals still represents an experimental challenge [9].

Although *ab initio* NMR calculations yields isotropic chemical shifts (δ_{iso}) and/or isotropic magnetic shielding (σ_{iso}) the shielding is a tensor quantity, i.e. it depends on the relative orientation of the atom in relation to external magnetic field (B_0). In fact, this can be easily observed in static solid state NMR spectra (NMR powder patterns), where spectral broadening arises from chemical shift anisotropy [10]. There are several ways to calculate the magnetic response in solids by cluster approximation [11,12]. Nevertheless the best description of a crystalline solid state system should account for its natural translational invariance [13]. Gauge-Included Projector Augmented Waves (GIPAW) calculations are a successful method of *ab initio* NMR calculation that respects the periodicity of the crystal structure by using planewave basis set and periodic boundary conditions [14]. GIPAW calculations have

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http://dx.doi.org/10.1016/j.cplett.2016.08.030
0009-2614/

been widely used for organic [15,16] and inorganic [17] solids, for chemical shifts assignments [18] and in crystal structure investigations [19,20]. GIPAW-computed chemical shifts can also be used to study the effect of the intermolecular interactions by comparing magnetic response of the periodic crystal structure and isolated molecule [21,22].

An interesting challenge for both solid state NMR and *ab initio* calculations rely on the study of the structural and crystallographic properties of the 6-aminopenicillanic acid (6-APA, Fig. 1). 6-APA is an important synthetic key to prepare new penicillins, although itself presents antibacterial properties [23]. The most important application is the industrial synthesis of β-lactams, where 6-APA is used as a versatile intermediate [24,25].

The first 6-APA solid state NMR investigation was carried out by Clayden et al. [26] in a comprehensive paper about the use of ^{13}C CPMAS (cross polarization with magic angle spinning) for conformational studies of some penicillins. In this work, the authors have used the published crystal structures [27,28] to rationalize the ^{13}C solid state NMR data. Due to the very low solubility and rapid decomposition of 6-APA in the majority of solvents, the ^{13}C NMR assignment was performed by comparison with solution ^{13}C NMR data of some penicillins. Aguiar et al. [29] published the assignments for 6-APA resonances fully based on cross-polarization solid state NMR dynamics, by using the variation of experimental NMR parameters (contact and delay times). In those works [26,29] the ^{13}C CPMAS spectra show the same chemical shift sequence and almost the same intensities. However, the assignments of the resonances at 28/37 ppm (C9 and C10) and 73/66 ppm (C3 and C5) were ambiguous. In another work, Mwangi and Garside [30] used the crystal structures available in the literature [27,28] to explain the 6-APA crystal growth morphology. However, they found that molecular mechanics calculations could not be carried out due to the misplaced hydrogen atoms in the structure published by Diamand [27] as well as due to the absence of atomic coordinates found in the structure determined by Galdecki and Werfel [28]. Stroganov and co-workers [31] compared the equilibrium geometry properties obtained by quantum/molecular mechanics with the Galdecki and Werfel structure. Their results were in good agreement with the experimental data, even though the authors neglected the zwitterionic nature of the molecule to perform the calculations. Swaminathan et al. [32] compared the experimental and theoretical FTIR spectra of 6-APA. These calculations were performed with an isolated molecule at HF/6-311G(d, p) and B3LYP/6-311G(d, p) level of theory. In this paper, the experimental FTIR spectrum strongly suggests the presence of a zwitterion structure for 6-APA, through the observation of a ~2600 cm^{-1} absorption due to the NH_3^+ stretching mode (ν_{NH3^+}). Surprisingly, the authors ignored the amine protonated and carboxylate anion forms in their calculations, i.e. the results yields no ~2600 cm^{-1} absorption.

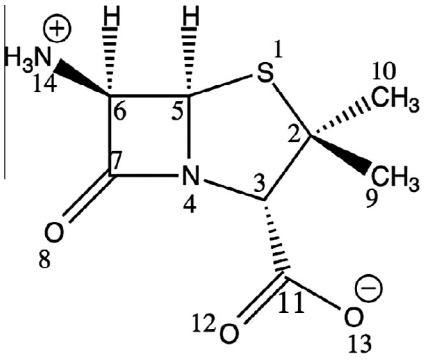

Fig. 1. Chemical structure of 6-APA.

Recently the question regarding the lack of fully defined structural data was also addressed by Saouane et al. [33], that employed synchrotron radiation to perform a single crystal diffraction experiment. However, in this work the hydrogen atoms were refined by using the atom-atom Coulomb-London-Pauli method (AA-CPL) [6], which employs a semi-empirical method to create an optimal hydrogen refinement for C, N, O and Cl (in all chemical connectivities). Additionally, the authors calculated the intramolecular interaction energies by using the PIXEL method [34], confirming the predominance of a Coulombic term (−379,1 kJ) over other contributions (polarization, repulsion and dispersion).

Taking into account that ^{13}C and ^{15}N isotropic chemical shifts are directly related to the proton positions and that the experimental ^{13}C and ^{15}N CPMAS is much more affordable than ^{1}H MAS, in this communication the ^{13}C and ^{15}N NMR crystallography was explored to achieve a high-resolution crystal structure of 6-aminopenicillanic acid (6-APA) with the best theoretical/experimental chemical shift fit. Additionally GIPAW calculations were used to solve the 6-APA ^{13}C resonances ambiguities and to confirm the ^{15}N assignments. To the best of our knowledge experimental^{15}N CPMAS as well as ^{13}C and ^{15}N GIPAW calculations were not previously reported so far for the 6-APA crystal structure.

2. Experimental

2.1. Sample

A sample of 6-aminopenicillanic acid was purchased from Sigma Aldrich (99% purity). It was maintained in a refrigerator to prevent decomposition and used without any further purification.

2.2. Experimental ^{13}C and ^{15}N solid state NMR

^{13}C and ^{15}N CPMAS NMR measurements were recorded on a Bruker Avance III 400 spectrometer (9.4 T), operating at Larmor frequencies of 100.3 MHz (^{13}C) and 40.6 MHz (^{15}N) respectively. The experiments were carried out in a 4 mm CPMAS probehead and ZrO_2 rotors stopped with Kel-F caps. The sample was spun at 11 kHz (^{13}C) and 5 kHz (^{15}N), by using a ramped ^{1}H pulse shaped from 50 up 100% during contact time $^{1}H \rightarrow S$ (S = ^{13}C or ^{15}N) cross polarization pulse sequence (CP) in order to circumvent spin modulation of Hartmann-Hahn conditions. The ^{13}C acquisition parameters were recorded following the previous literature [26,29]. In order to excite the ^{15}N, a pulse width of 5 μs was used, and the contact times were varied in the range of 100–2000 μs, with recycle delay of 10 s. Fourier transformation was performed after 256 and 8580 accumulations for ^{13}C and ^{15}N resp., with an exponential multiplication function of 50 Hz. The α-glycine carboxyl ($\delta_{C=O}$ = 176.02 ppm) and amine (δ_{+NH3} = −347.54 ppm) resonances were used as external secondary signal references for chemical shift calibration.

2.3. Theoretical approach

The electronic structure calculations were performed using the Quantum-Espresso suite of programs [35], which implements the DFT [36] framework with periodic boundary conditions (PBC) from previously published crystal structure [33] and is also available in Cambridge Structural Database (database reference code: AMPENA01). The one-electron Kohn and Sham [37] wavefunctions were expanded by using a planewave basis set up to 60 Ry kinetic energy cutoff and 240 Ry for the charge density cutoff. The Exchange and correlation (XC) contribution was calculated using the Perdew–Burke–Ernzerhof (PBE) approach [38] for the generalized gradient approximation (GGA). The projector augmented