Structure Solution of ACV-GLU Cocrystal by Combined XRD Refinement, 1D Solid State NMR and DFT Calculations

Ping Li, Tingting Liang*, Huan Liu, Jixin Guo, Jingjing Chu, Lingfang Qiu* and Shuwang Duo*

Jiangxi Key Laboratory of Surface Engineering, Jiangxi Science and Technology Normal University, Nanchang, Jiangxi 330013, China
Email: ltt38516943@126.com; qlf1108@163.com; swduo@imr.ac.cn

Abstract. The powder sample of ACV-GLU crystal complex was prepared by formic acid-assisted grinding. $^{13}$C and $^{15}$N chemical shifts were assigned by referring to the 1D NMR spectra of raw individual components. The crystal structure was first solved by Rietveld refinement. The calculated NMR parameters were compared with the experimental results to validate the proposed structure. ACV, an important antiviral drug, can be cocrystallized with GLU into a triclinic unit cell with a molar ratio of 1:1 where each unit cell consists of two ACV molecules and two GLU molecules. Six intermolecular hydrogen-bonding interactions exist in the cocrystal. An agreement between experimental and calculated NMR parameters would give a final confirmation of the correct model among the preliminary candidates. Such synergistic approach provides efficient and convincing method to obtain the crystal structure of powder cocrystal specimens.

1. Introduction

An API may exist in different forms, such as polymorphs and amorphous, hydrate, solvate, crystal salt, and cocrystal [1]. It is well known that the solid structure of an API can have significant effect on its physicochemical properties, and consequently on its bioavailability, stability, as well as manufacturability. Therefore, it is crucial to screen out and prepare suitable drug form to ensure appropriate properties. Cocrystal, a multiple component crystal that consists of several solid components in a definite stoichiometric ratio held together via noncovalent interactions, has drawn great attention and has been widely explored to improve the properties of the original APIs in recent years [2-5].

Acyclovir (figure 1a), the API involved in this study, is one of the most widely used guanosine analogue antiviral drug with good selectivity and low cytotoxicity to mammalian host cells, which is mainly used in the cure of herpes simplex virus and varicella zoster virus infections [6, 7]. Although ACV is regarded as the start of a new era in antiviral therapy and used as a first line option, its pharmacokinetics is not satisfying enough [7, 8]. ACV has a poor solubility in water. The reported solubility is almost 1.1 mg/mL at room temperature. Such poor solubility is regarded as a crucial factor leading to the low oral bioavailability (ca. 15%-30%) and short elimination half-life (2-3 h) [9-12]. Previous reports show that ACV can exist in two anhydrous crystalline forms at room temperature [13, 14], two hydrated crystalline forms [14, 15], and one acetic acid solvate [14]. While, no data show these forms have enhanced solubility. To date, four ACV cocrystals, selected after screening, have shown better solubility and/or dissolution rate in comparison to anhydrous ACV [7, 9, 16, 17]. ACV-GLU should be the first reported cocrystal form of ACV, which owns enhanced...
solubility and permeability [16].

Studies reveal that some properties of pharmaceutical cocrystals, such as solubility and thermal stability, are closely related to the crystal structure and the corresponding properties of the constituents [2, 4, 18]. Thus, solving the crystal structure is necessary for the in-depth understanding of the structure-property relationships. Also, the information of intermolecular interactions (supramolecular synthon) existing in cocrystal forms can be fully revealed through their crystal structures. Such clues will help us to design novel cocrystals. For example, the crystal structure of ACV-GLU may help to select the coformer of ACV and other nucleotide analogs (often important antiviral drugs). In a word, structure determination of pharmaceutical cocrystals is an indispensable part of the research efforts towards synthesis by design.

Single-crystal XRD measurement on qualified single crystal sample must be the first choice for structure investigation. But such samples are not always available. Moreover, single-crystal and powder XRD measurements are often conducted under different temperature conditions. As a consequence, simulated XRD pattern derived from single crystal structure and powder XRD pattern of bulk sample often show discrepancies on their diffraction angle positions and relative intensities. To add to the confusion, single crystal and bulk sample are often obtained under different preparation conditions. Sometimes, how can one judge whether two samples present different diffraction patterns because of different test temperatures or because of different solid forms.

One approach to determine the crystal structure of powder samples is the structure refinement from powder XRD data [19]. Such practice has been widely used in pharmaceutical research filed and is regarded as a routine work [20, 21]. Meanwhile, solid state NMR could be adopted as a role to validate the refined result for it can provide information on the chemical environment of organic nuclei, as well as qualitative or quantitative information about atomic spatial proximities. The synergistic approach has been adopted by researchers due to its credibility, and the published reports mainly focus on pure APIs [22-25]. To date, such synergistic approach has also been introduced into the complex research field [26-28]. In this contribution, the crystal structure of ACV-GLU cocrystal was confirmed by powder XRD, combining with 1D ssNMR and DFT calculations. Additionally, when comparing with the previously refined cocrystals (indomethacin-nicotinamide and theophylline-nicotinamide) [26, 27], molecules contained in ACV-GLU are more flexible and the potential intermolecular interaction sites are more diversified. Thus, the current case study can also be used to test the practicality of the synergistic approach for complicated cocrystal systems.

2. Materials and Methods

2.1. Materials and Sample Preparation

The two-thirds hydrated form of ACV (≥ 98%), GLU (≥99%) and formic acid (≥ 98%) were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received. Form I [29] (or named Form 2 [9]) of anhydrous ACV was obtained by heating the hydrate at a rate of 5 ℃/min up to 180 ℃ in a tube furnace and keeping the temperature for 1 h, then allowing the sample to cool. In order to prepare the
ACV-GLU cocrystal, 112.6 mg of ACV (Form I) was ground with 66.1 mg of GLU for 1 h, following by addition of one drop of formic acid [17].

2.2. Powder X-ray Diffraction
Powder XRD pattern of ACV-GLU under ambient conditions was collected on a PANalytical X' Pert Pro X-ray powder diffractometer equipped with X' Celerator Real Time Mutil-Strip detector, the Cu Kα radiation was used at 45 kV and 40 mA. Sample was scanned in the transmission mode and the scan range (2θ), step size as well as time per step were 4.0 to 70.0°, 0.01313°, and 48 s, respectively.

2.3. Solid State NMR Spectroscopy
13C and 15N CP/MAS NMR spectra of starting materials and ACV-GLU were acquired with a 4 mm double-resonance MAS probe on a Bruker AVANCE III spectrometer operating at a magnetic field strength of 11.7 T. The Hartmann-Hahn conditions of the CP experiment for acquiring 13C and 15N spectra. 13C spectra were acquired at 8 kHz MAS spinning speed with a 2 ms contact time. 15N spectra were acquired at 5 kHz MAS spinning speed. Recycle delays of ACV, GLU, and ACV-GLU were 120, 240, and 45 s, respectively. 13C and 15N chemical shifts were referenced to adamantane and L-glycine, respectively.

2.4. Computation Methods
The crystal structure refinement was performed by using Materials studio (MS) Reflex plus software package. Firstly, the XRD pattern was indexed using TREOR90 [30], as a consequence, all kinds of likely cell parameters was obtained, arranging according to the size of quality factors. Next, an automatic space group determination was carried out based on cell parameters, followed by the Pawley refinement [31], until a good quality of fit the figure of merit was achieved (Rwp = 3.24%, Rp = 2.31%). To obtain the crystal structure, a direct-space method according to the simulated annealing algorithm was performed with a couple of optimized ACV and GLU molecular structures. The optimized fit between the simulated and experimented powder patterns was acquired with (Rwp = 6.16%, Rp = 5.26%). Finally, this structure was used as the input model for the Rietveld refinement [32], in the process, besides the Global isotropic is reflexed at last, and the occupancy is fixed at 1.00, the other way was consistent with Ref. [27]. The refined crystal structure was carried out geometry optimization using MS CASTEP program, which implements DFT within a generalized gradient approximation and the planewave pseudopotential approach [33], utilizing Perdew-Burke-Ernzerhof revised by dispersion, medium planewave cutoff energy and K-point setting in MS package. During the geometry optimization, the unit cell parameters were fixed, but the position of every atom was allowed to be optimized. Subsequently, NMR shielding and electric field gradient calculations were carried out with MS CASTEP-NMR code, using periodic gauge including projector augmented waves way, on-the-fly pseudopotential, fine K-point and cut-off energy of 550 eV.

3. Results and Discussion
3.1. 1D Solid State NMR Experiments: Cocrystal Formation
As mentioned in the experimental section, the ACV and ACV-GLU samples acquired in this work were first characterized by XRPD, TGA and DSC. The obtained XRPD pattern and melting point are completely consistent with the reference (figure 2) [19], indicating successful preparation of the cocrystal sample.
Figure 2. (A) XRPD patterns of (a) ACV/H$_2$O and (b) ACV I; (B) TGA traces of (a) ACV/H$_2$O and (b) ACV I; (C) XRPD patterns of (a) ACV I, (b) GLU and (c) ACV-GLU cocrystal; (D) (a) DSC curves of ACV I, (b) GLU and (c) ACV-GLU cocrystal.

The $^{13}$C CP/MAS NMR spectra of ACV, GLU, and ACV-GLU are exhibited in figure 3. The sharp resonance peaks of ACV-GLU (figure 3c) indicate the product exists in well crystallized state. Comparing with the spectra of input materials (figures 3a and 3b), ACV-GLU sample exhibits slight changes of $^{13}$C chemical shifts, which can be ascribe to the change of carbon chemical environments associated with the formation of cocrystal. One obvious change takes place at the carboxyl groups of GLU (C9&C13), which varied form similarity (one peak) to difference (two peaks). In addition to the formation of ACV/GLU crystal complex (cocrystal or salt), the simultaneous solid form changes of ACV and GLU can also result to such chemical shift changes of all resonance peaks. An ssNMR parameter, proton spin-lattice relaxation time ($T_1$), is used to dismiss this doubt. Due to the efficient $^1$H spin diffusion in solid sample, a single phase often yields one, uniform $^1$H $T_1$ value at lower spinning speed [34]. ACV-GLU has an averaged $^1$H $T_1$ of 15.56 s with a standard deviation of 0.77 s, indicating the grinded sample should be in one solid phase. Also, the information about number of molecules per asymmetric unit ($Z'$) can be obtained from the $^{13}$C spectrum. Due to every chemical distinct carbon in ACV-GLU is only represented by single resonance, it is easy to know that $Z'$ should be equal to 1.

Figure 3. $^{13}$C CP/MAS NMR spectra of (a) ACV, (b) GLU and (c) ACV-GLU. Chemical shifts of (a) are assigned according to Ref. [29].
ACV is an ampholyte with $pK_a = 2.27$ & 9.25 [35], and GLU is weak acid with $pK_a$ values of 4.29 & 5.94 [36]. According to the $\Delta pK_a$ rule, both cocrystal and salt have the possibility to be formed [37]. The main difference between salt and cocrystal is whether complete proton transfer appeared between API and the guest. As nitrogen atom is often existed in a hydrogen bond or is protonated/deprotonated in a salt [34], $^{15}$N CP/MAS NMR spectra are adopted to clarify the ionization state of ACV-GLU (figure 4). The $^{15}$N chemical shifts of ACV can be readily assigned via theoretical calculation. After ACV-GLU formation, the most obvious chemical shift change takes place on the N4 atom (+ 12.5 ppm). Generally, if a complete proton transfer occurred, a much larger $^{15}$N chemical shift varying should be observed. Thus, ACV and GLU should be in neutral state in ACV-GLU, and ACV-GLU should be a cocrystal. Also, a + 12.5 ppm change indicates there must be significant difference of intermolecular interactions between the ACV and cocrystal specimen on N4 site.

![Figure 4. $^{15}$N CP/MAS NMR spectra of (a) ACV and (b) ACV-GLU.](image)

3.2. Powder XRD Refinement: Structure Determination

The crystal structure candidates of ACV-GLU can be refined from the powder XRD data. Full details of the refinement methodology have been given in the Experimental Section. The information about amount of molecules per asymmetric unit ($Z' = 1$) was used during the process. One cocrystal structure with favorable residual variances (figure 5a, $Rwp = 3.69\%$, $Rp = 2.64\%$) was determined from the XRPD using Rietveld refinement. The packing diagram of obtained cocrystal was shown in figure 5b. Moreover, the crystallographic details of the crystal structure are presented in table 1.

![Figure 5. (a) Restrained Rietveld fitting of ACV-GLU XRPD patterns, the measured data is represented with the red line (top), the simulated data with the black line (middle), and the difference pattern with the blue line (bottom); (b) Packing diagram of obtained ACV-GLU cocrystal structure.](image)
Table 1. Crystallographic details of the obtained ACV-GLU cocrystal structure.

| Formula/Molecular weight | Crystal system | a (Å) | b(Å) | c(Å) | α(º) | β(º) | γ(º) | Volume (Å³) | Z/Z’ |
|--------------------------|----------------|-------|------|------|-------|------|------|-------------|------|
| C_10>H_19>N_5>O_7       | triclinic/P-1 | 8.2673 | 8.8520 | 11.6991 | 71.0875 | 87.5737 | 80.1642 | 797.962 | 2/1  |

3.3. DFT Calculations: Final Confirmation of the Refined Structure

NMR parameters, especially those significantly changed ones, are generally regarded as reliable criteria to assess the rationality of the refined result. Comparing with the spectra of starting materials, 13C signal splitting of C9 & C13, significant 15N chemical shift change of N4, 15N signal position exchange of N3 and N5, as well as 13C signal position exchange of C6 and C7 had been observed for ACV-GLU. Taking these spectral features into consideration, 13C and 15N chemical shifts are used as evaluation tools. As shown in table 2, the calculated 13C and 15N chemical shifts of ACV-GLU are consistent with the experimental data, suggesting that the obtained structure is the representation of actual structure. The correctness of the refined result, including crystallographic details and intermolecular interactions, is also confirmed by the single-crystal structure test [21], indicating the synergetic approach is an effective method to determine the structure of powder cocrystals.

Table 2. Experimental and calculated 13C/ 15N chemical shifts of cocrystal*.

| Atom | 15N(δexp) | 15N(δcal) | 13C(δexp) | 13C(δcal) |
|------|-----------|-----------|------------|------------|
| 1    | -235.8    | -238.5    | 158.8      | 159.2      |
| 2    | -300.1    | -296.9    | 155.4      | 154.1      |
| 3    | -215.7    | -213.7    | 152.8      | 153.3      |
| 4    | -151.7    | -153.4    | 115.6      | 115.9      |
| 5    | -211.3    | -207.5    | 138.6      | 138.6      |
| 6    | 74.0      | 73.6      |            |            |
| 7    | 69.7      | 67.3      |            |            |
| 8    | 60.6      | 60.0      |            |            |
| 9    | 179.1     | 179.8     |            |            |
| 10   | 34.7      | 34.3      |            |            |
| 11   | 17.7      | 17.0      |            |            |
| 12   | 34.7      | 32.9      |            |            |
| 13   | 176.7     | 178.3     |            |            |

Note: * the calculated chemical shift δcal = – (σcal – σref), where the σcal is the calculated shielding value of cocrystal, σref = σcal’ + δexp’, where the σcal’ and δexp’ are the calculated shielding value and experimental chemical shift of starting materials, respectively.

4. Conclusions

In this study, ssNMR as well as DFT calculations assisted powder XRD refinement is employed to determine the crystal structure of powder ACV-GLU cocrystal. Here, the 1D solid state NMR experiments provided valuable information about cocrystal formation and the final structure confirmation. Such clues make the structure determination process more fluent. Thus, such synergetic approach is very necessary to ensure a fluent and credible workflow in the structure solution of the powder cocrystal samples. This study further confirms that the simple and credible method has a promising application in solving the crystal structure of powder cocrystal samples.

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