Structural investigation of Lisinopril by powder X-ray diffraction and solid-state NMR

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Abstract. Structural studies on polycrystalline Lisinopril (N-N-[(S)-1-carboxy-3-phenylpropyl]-L-lysil-L-proline) are performed by combined powder X-Ray diffraction and ¹³C solid-state nuclear magnetic resonance (NMR). The crystal structure of this drug, used primarily for the treatment of hypertension, has not yet been determined due to the impossibility of synthesizing single crystals of sufficient quality. It is shown here that valuable insights into the crystal and molecular structure of Lisinopril can be obtained on polycrystalline powder based on the complementary character of the information provided by the two techniques.

1. Introduction

Lisinopril is a drug of the angiotensin converting enzyme (ACE) inhibitor class that is primarily used in treatment of hypertension, congestive heart failure, heart attacks and also in preventing renal and retinal complication of diabetes [1]. Lisinopril has a number of properties that distinguishes it from other ACE inhibitors: it is hydrophilic, has long half-life and tissue penetration and is not metabolized by the liver.

The preferred conformation of Lisinopril in solution was investigated by liquid-state NMR [2], and the result was correlated with its pharmacological activity. However, crystal structure of Lisinopril is not yet reported in the Cambridge Structural Database (CSD) most probably because it was not possible to obtain single crystals of sufficiently good quality. Therefore, useful structural features of Lisinopril in the solid form are determined here based on powder X-Ray diffraction and ¹³C solid-state NMR. These methods are often used as complementary techniques for structural investigation in such cases when single crystal X-Ray diffraction cannot be applied.

2. Experimental

Experimental powder diffraction pattern was obtained with Panalytical X-ray diffractometer using Cu Kα₁ radiation. X-ray powder diffraction pattern (figure 1) was indexed using Dicvol 91 and Ito programs, whereas the lattice parameters were refined using the Checkcell program. The solid-state ¹³C NMR spectrum of Lisinopril (figure 2) was recorded at room temperature on a Bruker AVANCE-400 spectrometer (at 100 MHz ¹³C Larmor frequency) by using the Cross-Polarization under Magic
Angle spinning (CP/MAS) technique [3]. Quantum mechanical computation of $^{13}$C chemical shifts of Lisinopril in solid was performed by using the GIPAW module within the QUANTUM ESPRESSO program [4] based on the structural model provided from the powder X-Ray data. Gauge Including Projector Augmented-Wave method (GIPAW) was introduced as an extension to the Projector Augmented-Wave method, being valid for systems in non-zero uniform magnetic fields [5]. For chemical shift calculations we use norm-conserving GIPAW-pseudopotentials, doing first a geometry optimization for the H atoms positions. The proposed spectral assignment in figure 3 is based on comparing the measured $^{13}$C chemical shifts with those obtained in solution and computed by the GIPAW method.

![Figure 1. Experimental (top) and simulated (bottom) powder X-Ray diffraction pattern of Lisinopril.](image)

![Figure 2. The $^{13}$C solid-state NMR spectrum of Lisinopril and the proposed spectral assignment.](image)
3. Results and discussion

From the indexing procedure we obtained that Lisinopril crystallizes in the monoclinic system having the following lattice parameters: \( a = 14.6632\,\text{Å}, \ b = 5.9097\,\text{Å}, \ c = 14.3140\,\text{Å}, \ \beta = 113.140^\circ \) and the volume of the unit cell, \( V = 1137.7 \,\text{Å}^3 \). Taking into account the molecular weight of Lisinopril, \( M = 405.488 \), and the forbidden reflexions, we conclude that the most probable space group is \( \text{P}2_1 \), which has two molecules per unit cell. The calculated density of 1.18 g/cm\(^3\) is consistent with the typical values for organic molecules containing only carbon, oxygen, nitrogen and hydrogen atoms.

The problems associated with obtaining single crystals are being increasingly overcome by full structural determination from powder diffraction patterns [6]. However, the success rate in such applications essentially depends on the ability to reach a reliable initial structural model by the existing search algorithms. The difficulty in determining such models largely increases with the number of degrees of freedom (DOF) to be explored for the investigated molecule. From this point of view Lisinopril, which has 19 flexible torsion angles that can be modified during the search procedure is approaching the upper limit of structural complexity that can be reliably solved from X-Ray powder diffraction data [6]. The crystal structure analysis was performed based on the direct space search method implemented in the Fox program [7]. The initial configuration of Lisinopril was given using the \( \tau \) matrix with 29 non-hydrogen atoms. For obtaining a structural model both, simulated annealing and parallel tempering algorithms were employed. Multiples runs were performed in order to find the best solution: the structural model selected in the end corresponds to the lowest value of the \( R_{pw} \) parameter [7].

![Molecular structure of Lisinopril](image)

**Figure 3.** The molecular structure of Lisinopril.

In the molecular conformation obtained from analyzing the X-Ray data (see figure 3) the three branches of the molecule make approximately the same angles around the central C14 atom, namely \( 115.5^\circ \) (N13, C14, C20), \( 110.2^\circ \) (N13, C14, C15), and \( 112.3^\circ \) (C15, C14, C20). Another distinctive structural feature is that the C14 - C19 carbons, which form a straight chain in the starting configuration of the isolated molecule, are found to be bent to a ring-like conformation due to the crystal packing. The arrangement of the two Lisinopril molecules in the unit cell is shown in figure 4: according to this structural model, crystal packing appears to be favored by intermolecular hydrogen bonding between N13-H...O11, and O21-H...O28, respectively.

The analysis of the chemical shifts obtained from MAS NMR, together with their values computed by explicitly considering the crystal lattice, often provides valuable local structural details that can be used for increasing the reliability of the structural model derived from the powder X-Ray data. For instance, large differences between the chemical shift values extracted from the \( ^{13}\text{C} \) CP/MAS spectrum shown in figure 2 and those reported for Lisinopril in solution [2], are obtained for the C24 (~9 ppm), C9 (~8 ppm) sites, the C14-C19 carbon chain (~4-5 ppm), the C20 carbonyl (~5 ppm), and the C27...
carboxyl site (~ 4 ppm), respectively. They are indicative for the conformational changes in solid, and generally consistent with the structural model described above. At the other extreme, there are little changes associated with the phenyl ring, the carbon sites connecting it with the rest of the molecule (C7, C8), and the central C14 carbon, for which chemical shift variations were found only between 0.5 and ~ 2 ppm: these small variations are most likely determined by crystal packing effects, and not by significant changes of the molecular conformation. Also, within the accepted error limits of the GIPAW method [5], the computed $^{13}$C chemical shifts are generally in good agreement with the measured values. Relatively large deviations of up to 10 ppm were found though in the case of the C9, C24, carboxyl and carbonyl sites; however, this result is not completely surprising since the structural model used in calculations is not yet refined, and these particular carbons are known to be very sensitive even to small deviations from the real local geometry.

Figure 4. The unit cell of Lisinopril.

4. Conclusions
In summary, structural investigations on polycrystalline Lisinopril (N-N-[(s)-1-carboxy-3-phenylpropyl]-L-lysil-L-proline), a drug of the angiotensin converting enzyme (ACE) inhibitor class that cannot be synthesized as single crystal, have been carried out by powder X-Ray diffraction and $^{13}$C solid-state NMR. The lattice parameters were extracted with high accuracy, and a structural model has been obtained through direct space search by employing the simulated annealing and parallel tempering algorithms. This model shows a series of important conformational changes compared to the preferred molecular conformation of Lisinopril in solution. Most of these structural features are consistent with the experimentally measured $^{13}$C chemical shifts in solid, and also with the values computed through the GIPAW method. In conclusion, the derived model constitutes a reliable starting structure for the next stage of crystal structure refinement.

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