Identification and Crystal Structure Determination of New Hydrate Phases of L-Lysine and Rationalization of Hydration/Dehydration Processes

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Abstract

Recently, the crystal structure of L-lysine, the last enantiomerically pure directly-encoded proteinogenic amino acid with no known crystal structure, was determined. It was noted that, under ambient conditions, L-lysine has a strong propensity to incorporate water to form a hydrate phase, necessitating dehydration under rigorously dry conditions to obtain anhydrous L-lysine. Reported here is the existence of two hydrate phases of L-lysine: L-lysine hemihydrate and L-lysine monohydrate. The hydration/dehydration behaviour of L-lysine and both hydrate phases has been investigated by dynamic vapour sorption measurements. Due to the hydration/dehydration processes resulting in microcrystalline powder samples of the two hydrate phases, powder X-ray diffraction methods have been exploited to determine the crystal structures of both L-lysine hemihydrate and L-lysine monohydrate.

Keywords

Powder X-ray diffraction, dynamic vapour sorption, crystal forms, solid-state transformations

Introduction

Recently, the crystal structure of L-lysine[1] (Figure 1) was determined directly from powder X-ray diffraction (powder XRD) data, representing the last of the 20 genetically encoded proteinogenic amino acids to have a crystal structure determined for the enantiomerically pure form. However, it was noted that the commercially purchased sample of L-lysine was in fact a hydrate phase and that prior to data collection (and subsequent structure determination) the sample had to be dehydrated in the presence of P_2O_5.

Recent work[2] on the solid-state phases of amino acids has demonstrated the existence of two hydrate phases of L-phenylalanine (a hemihydrate and a monohydrate) in addition to a new polymorph of L-phenylalanine (polymorph II). The necessity for rigorously anhydrous conditions in the isolation of polymorph II of L-phenylalanine resembles the necessity for anhydrous conditions in the structure determination of L-lysine. Anhydrous conditions were also a necessity in the crystal structure determination of L-arginine.[3]

In total, prior to this work, only seven of the 20 directly encoded proteinogenic amino acids were known to form hydrate phases with known crystal structures: one known only to form a racemic hydrate (DL-glutamic acid[4]), three known only to form enantiomeric hydrates (L-asparagine,[5] L-aspartic acid[6] and L-phenylalanine[2]) and three known to form both enantiomeric and racemic hydrates (arginine,[7,8] proline[9,10] and serine[11,12]).

We report here the discovery of two hydrate phases of L-lysine. The solid phase present under ambient conditions of moderate relative humidity (RH) is, in fact, L-lysine hemihydrate, while anhydrous L-lysine is only present under very dry conditions, as demonstrated by dynamic vapour sorption (DVS) measurements. When exposed to an atmosphere of higher humidity (RH of *ca*. 50% or higher), L-lysine is found to form a monohydrate phase prior to deliquescence. The crystal structures of both L-lysine hemihydrate and L-lysine monohydrate have been determined directly from powder X-ray diffraction (powder XRD) data, due to the difficulty of producing single crystals of sufficient size and quality for crystal structure determination from single-crystal X-ray diffraction data.

Experimental

The sample of L-lysine used in the present work was purchased from Sigma-Aldrich (purity ≥98%). Under ambient conditions, this sample was found to be L-lysine hemihydrate. Samples of L-lysine monohydrate were prepared by exposing L-lysine hemihydrate to an atmosphere of high relative humidity. L-Lysine hemihydrate held within a small glass beaker was placed in a petri dish filled with water. The petri dish and small beaker were covered with a larger beaker to provide a contained environment. The sample was left for several hours, after which time deliquescence was found to have begun. Sample that had not yet deliquesced was removed and found to be L-lysine monohydrate.

Powder X-ray diffraction (powder XRD) data were recorded on a Bruker D8 diffractometer (CuKa_1) operating in transmission mode. The sample of L-lysine hemihydrate was mixed with amorphous starch and packed into glass capillaries, prior to flame sealing, to maintain a constant value of RH and reduce the effect of preferred orientation. The sample of L-lysine monohydrate was contained between two pieces of tape in a foil type sample holder.

Dynamic vapour sorption experiments were carried out at ??? °C on a DVS Advantage 1 instrument (Surface Measurement Systems Ltd., London, U.K.).

Results

Dynamic Vapour Sorption

To study the hydration behaviour of L-lysine, dynamic vapour sorption (DVS) measurements were carried out, starting from a sample of L-lysine dried under vacuum in the presence of P_2O_5. The first measurement (Figure 2) began at 0% RH, increasing to 95% RH, before decreasing to 0% RH. Starting at 0% relative humidity (RH), no uptake of water is found until *ca*. 10% RH, where a mass increase of half an equivalent of water is observed. On continued increase in RH, no further uptake of water is found until *ca*. 55%, where a continuous and large increase in mass occurs with increasing RH. This large increase in mass is presumably due to the deliquescence of the sample and continues until the RH reaches 95% and decreases to 85%, after which point the mass begins to decrease. The decrease in mass continues until the RH reaches 40% after which the mass remains

stable for the remainder of the experiment. The final mass of the experiment was found to be considerably greater than the starting mass indicating that an irreversible hydration event had occurred. The recovered sample appeared to be a transparent glassy substance.

The DVS measurement was repeated with a new sample (again, dried similar to the previous) over a shorter RH range, beginning at 0%, increasing to 25% and decreasing to 0% (Figure 3). An increase in mass was observed between 6% and 13%. The total mass change during these events was found to correspond to a half equivalent of water and so the starting phase was assigned as anhydrous L-lysine and the product phase as L-lysine hemihydrate. No further increase in mass was observed with increasing RH to 25%. On decreasing RH, the mass change event was found to occur in reverse (with hysteresis), indicating that the transformation from anhydrous L-lysine to L-lysine hemihydrate is reversible.

Structure Determination from Powder XRD Data

The powder XRD patterns of L-lysine hemihydrate and L-lysine monohydrate were successfully indexed using, respectively, the KOHL[13] and ITO[14] algorithms in the CRYSFIRE[15] indexing suite with the following unit cell dimensions and metric symmetries: L-lysine hemihydrate, monoclinic a = 9.54 Å, b = 5.22 Å, c = 17.61 Å, β = 101.1°, (V = 860.6 Å^3); L-lysine monohydrate, orthorhombic a = 5.94 Å, b = 20.61 Å, c = 6.95 Å, (V = 850.3 Å^3). In each case, profile fitting and unit cell refinement were carried out using the Le Bail procedure[16] implemented in the program GSAS.[17] On the basis of systematic absences, the space group of L-lysine hemihydrate was assigned as $C2$, and the space group of L-lysine monohydrate was assigned as $P2_12_12_1$. In each case, good quality fits were achieved [L-lysine hemihydrate, R_{wp} = 2.88%, R_p = 2.17%, Figure 4(a); L-lysine monohydrate, R_{wp} = 2.57%, R_p = 1.94%, Figure 5(a)].

From density considerations, L-lysine hemihydrate was concluded as having one independent molecule of L-lysine (and, therefore, L-lysine hemihydrate also has half an equivalent of water, accomplished in space group $C2$ by placing a single water molecule on the two-fold rotation axis) and L-lysine monohydrate as having one independent molecule of L-lysine (and, therefore, one independent molecule of water). Structure solution was carried out from the powder XRD data using the direct-space genetic algorithm technique[18-22] in the program EAGER.[23-29]

During structure solution, L-lysine molecules were defined as zwitterions with the amine group within the head group (rather than at the end of the side chain) assigned as the protonated base. In the structure solution calculations for L-lysine hemihydrate, the one independent molecule of L-lysine was defined by a total of ten variables: two positional variables (for space group *C*2, the origin can be fixed arbitrarily along the *b*-axis, allowing the positional variable along this axis to be fixed for one molecule), three orientational variables and five torsional variables. A single water molecule (with half occupancy) was included in the structure solution calculation of L-lysine hemihydrate with the oxygen atom fixed in position on the two-fold rotation axis (i.e., with coordinates: 0, *y*, ½), allowing the water molecule to be defined by one positional variable and three orientational variables. In the structure solution calculation of L-lysine monohydrate, the single independent molecule of L-lysine was defined by three positional variables, three orientational variables and five torsional variables for a total of eleven variables, while the single water molecule was defined by three positional variables and three orientational variables, for a total of six variables.

During the structure solution of each of L-lysine hemihydrate and L-lysine monohydrate, 16 independent structure-solution calculations were conducted in parallel. In each case a population of 400 structures was used, with 40 mating operations and 200 mutation operations occurring per generation for a total of 500 generations. The same final, good-quality structure was obtained in all 16 cases for both L-lysine hemihydrate and L-lysine monohydrate.

The structures directly from EAGER were used in each case as the starting point for Rietveld refinement[30] using the program GSAS.[17] Standard restraints were applied to bond lengths and bond angles, and planar restraints were applied to carboxylate groups. Separate isotropic displacement parameters were refined for each molecule with the isotropic displacement parameter of the hydrogen atoms set as 1.2 times the refined isotropic displacement parameter for the non-hydrogen atoms in the same molecule. Intermolecular distance restraints were used to orient hydrogen atoms to produce optimal hydrogen-bonding arrangements.

A disordered model was used in the final Rietveld refinement of L-lysine hemihydrate. The hydrogen atoms within the side-chain amine group of each L-lysine molecule and the hydrogen

atoms of the water molecule were in each case considered to be disordered over six positions. Occupancy values of the disordered hydrogen atoms were fixed at ⅓ in order to give a total of two hydrogen atoms in each disordered group.

A correction for preferred orientation was included in the Rietveld fit of L-lysine monohydrate using the March-Dollase function.[31,32]

The final Rietveld refinement in each case gave a good fit to the experimental powder XRD pattern (L-lysine hemihydrate, R_{wp} = 2.96%, R_p =2.23% ; L-lysine monohydrate, R_{wp} = 2.91%, R_p = 2.15%) with refined parameters: L-lysine hemihydrate, $C2$ a = 9.53103(26) Å, b = 5.21710(17) Å, c = 17.6011(8) Å, β = 101.074(4)°, V = 858.91(6)Å^3 [Figure 4(b), 2θ range, 4–70°; 3866 profile points; 117 refined variables]; L-lysine monohydrate, $P2_12_12_1$ a = 5.93493(10) Å, b = 6.94133(11) Å, c = 20.5755(6) Å, V = 847.64(4) Å^3 [Figure 5(b), 2θ range, 4–70°; 3867 profile points; 102 refined variables].

Discussion

The crystal structure of L-lysine hemihydrate (Figure 6) forms a layered arrangement containing two different regions of hydrogen bonding, similar to that observed in the recently published crystal structure of anhydrous L-lysine.[1] The first region of hydrogen bonding occurs between the ammonium and carboxylate of the head groups, while the second involves both the amine groups at the termini of the side-chains and the molecules of water. While, in anhydrous L-lysine, hydrogen bonding in the second region can occur solely between amine groups to form chains of hydrogen bonds along the *b*-axis, in L-lysine hemihydrate the water molecule is also found to be involved in hydrogen bonding with the side-chain amine group. In L-lysine hemihydrate, each amine group is surrounded by three other amine groups and three water molecules, each capable of acting as a hydrogen-bond donor or acceptor. Each water molecule is surrounded by six amine groups, each capable of acting as a hydrogen-bond donor or acceptor. An extended network of hydrogen bonding is formed parallel to the *ab*-plane (Figure 7), constructed from six-membered rings in chair conformations, with amine groups at the apices, through N···N hydrogen bonds. A second and third network of edge sharing six-membered rings in chair conformations are formed by O···N hydrogen bonds with alternating water and amine groups at the apices. All three networks are

interlinked by sharing of apices. Due to each amine group and water molecule having six possible positions for hydrogen atoms in order to form hydrogen bonds as donors, a disordered model was considered in the final refinement with each hydrogen atom contributing to each possible hydrogen bond in equal proportion.

Also differing between the crystal structure of L-lysine and L-lysine hemihydrate is the number of independent molecules within the unit cell. While the anhydrous crystal structure contains two independent molecules of L-lysine with noticeably different conformations, L-lysine hemihydrate only contains a single independent molecule of L-lysine. The water molecule of L-lysine hemihydrate is situated in the unit cell such that the oxygen atom lies on a two-fold rotation axis. This results in an overall ratio of two L-lysine molecules to one water molecule.

Hydrogen bonding between the head groups in L-lysine hemihydrate (Figure 8) can be described using the classification system proposed by Görbitz *et al*[33] and is, in fact, unique among the hydrates of the amino acids in this regard. L-Lysine hemihydrate is found to contain sheets of type L*x*, in contrast to the L2 sheets found in anhydrous L-lysine.

While the crystal structures of L-lysine and L-lysine hemihydrate share several similarities, the crystal structure of L-lysine monohydrate (Figure 9) appears to differ. L-Lysine monohydrate has only a single independent molecule of L-lysine (and one of water) in the asymmetric unit cell. While in L-lysine and L-lysine hemihydrate there are two different regions of hydrogen bonding, only one exists in L-lysine monohydrate. Hydrogen bonding occurs between the head group carboxylate and ammonium groups, the side-chain amine group and the water molecules (Figure 10). The ammonium group is able to act as a hydrogen-bond donor and form three hydrogen bonds, two with oxygen atoms within two different carboxylate groups and one with the oxygen atom of a water molecule. The amine group is able to act as a hydrogen-bond donor, forming two hydrogen bonds with carboxylate oxygen atoms and as a hydrogen-bond acceptor, forming one hydrogen bond with a water molecule. The water molecule forms two hydrogen bonds acting as a hydrogen-bond donor, one to the aforementioned amine group and one to a carboxylate oxygen. It also acts as a hydrogen-bond acceptor to the previously mentioned ammonium group.

In conclusion, the crystal structures of both L-lysine hemihydrate and L-lysine monohydrate have been determined directly from powder XRD data. Structure determination from powder XRD was a method essential in these cases due to the difficulty in producing a single-crystal of sufficient size and quality for structure determination from single-crystal XRD. The hydration/dehydration behaviour of anhydrous and hydrated L-lysine has been characterized through use of DVS, further emphasizing the highly hygroscopic nature of L-lysine and the difficulties in performing crystal structure determination for not only anhydrous L-lysine, but also L-lysine hemihydrate and L-lysine monohydrate.

Acknowledgements

Supporting Information

Crystal information files (cif) for the two crystal structures determined in this paper. This information is available free of charge via the Internet at http://pubs.acs.org/

Figures

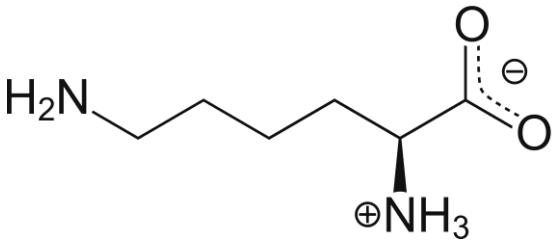

Figure 1: Molecular structure of L-lysine.

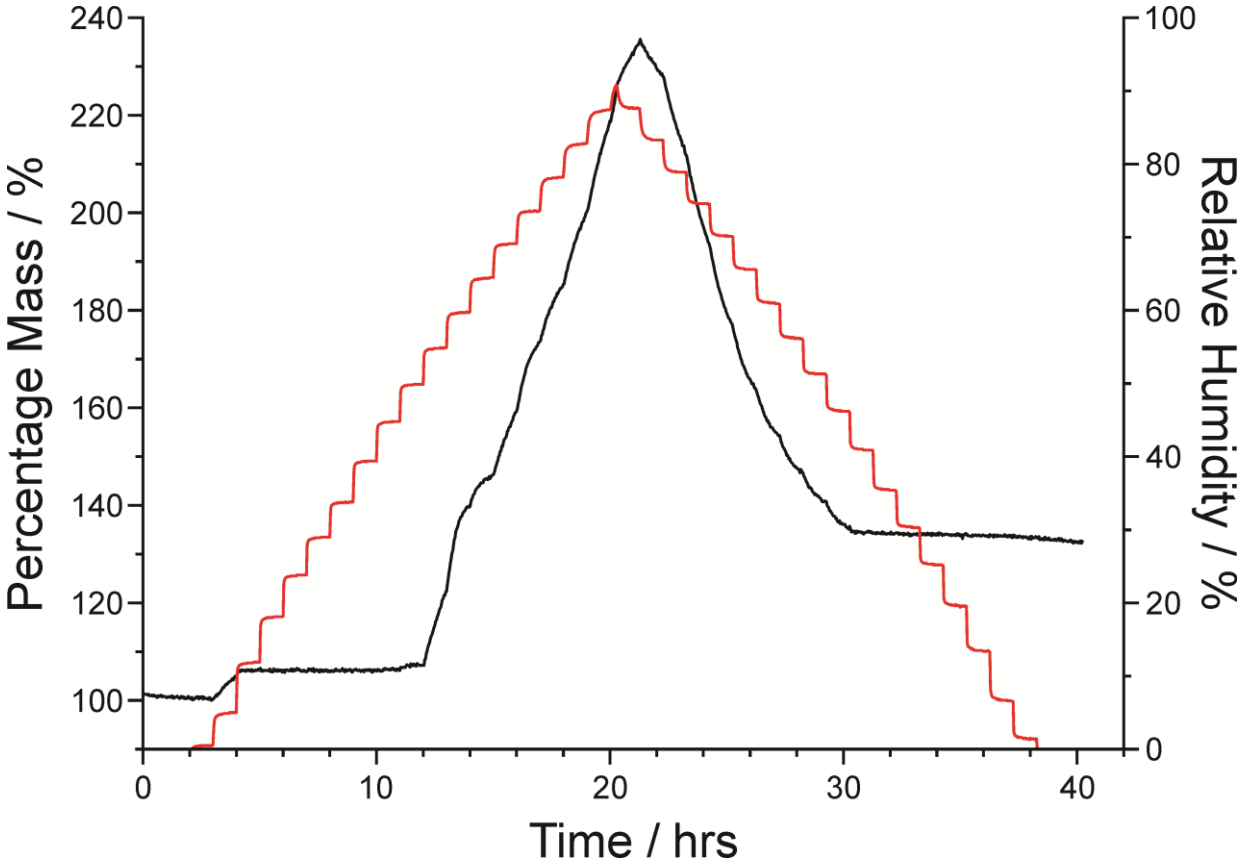

Figure 2: DVS data recorded on varying RH between 0% and 90% starting from anhydrous L-lysine. Relative humidity (red line) increased/decreased in stepped manner over time with corresponding change in sample mass (as a percentage of the lowest mass at 0% RH) shown as a black line.

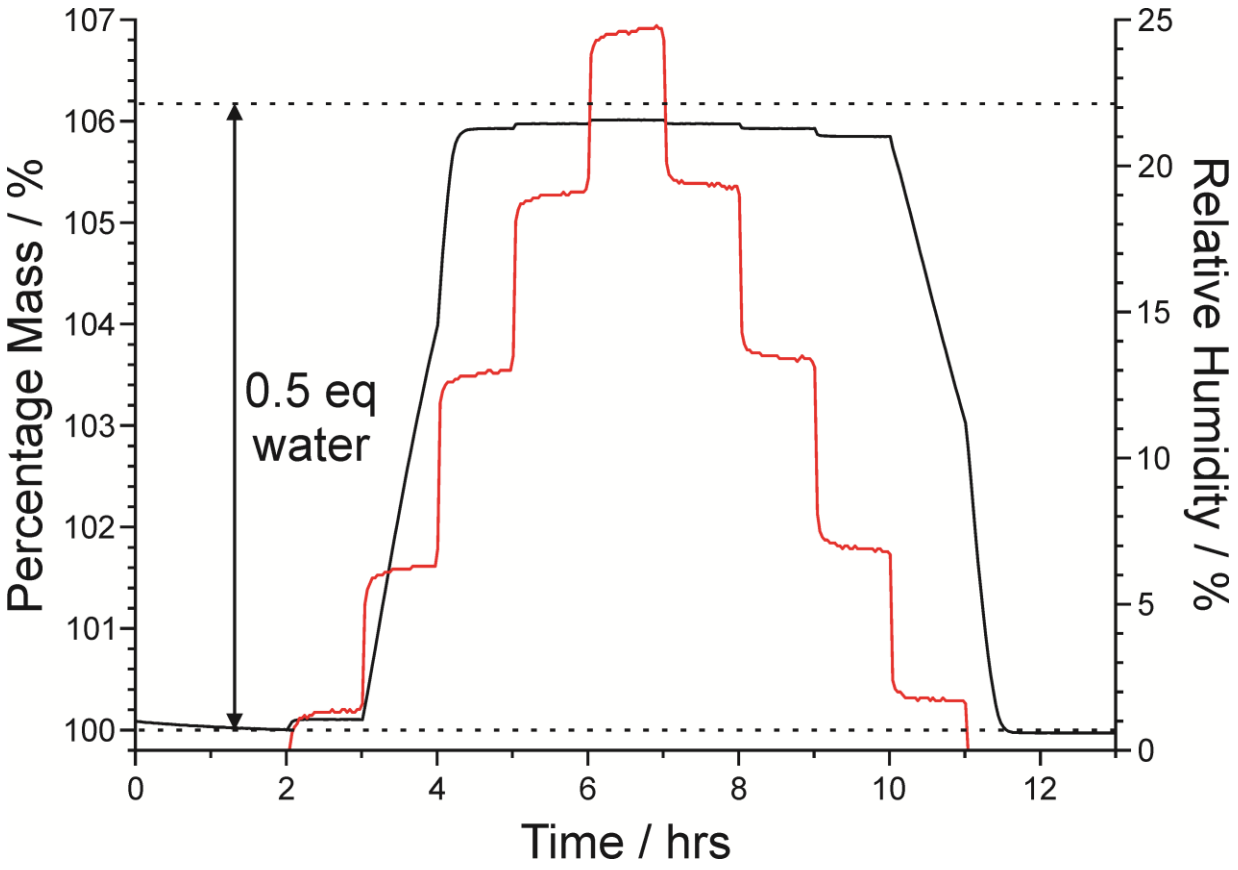

Figure 3: DVS data recorded on varying RH between 0% to 25% (red line) starting from anhydrous L-lysine. Relative humidity (red line) increased/decreased in stepped manner over time with corresponding change in sample mass (as a percentage of the lowest mass at 0% RH) shown as a black line.

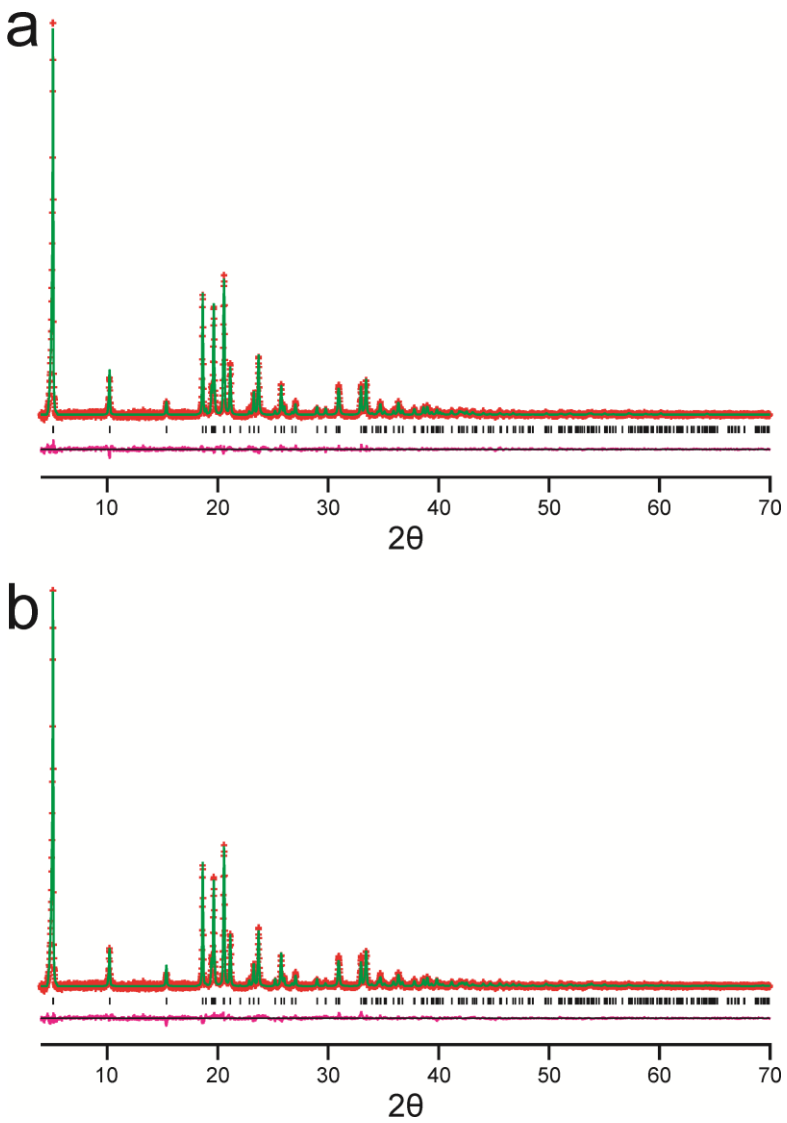

Figure 4: (a) Le Bail and (b) Rietveld fits for the powder pattern of L-lysine hemihydrate, showing the experimental data (red + marks), calculated data (green line), difference plot (magenta line), and predicted peak positions (black tick marks).

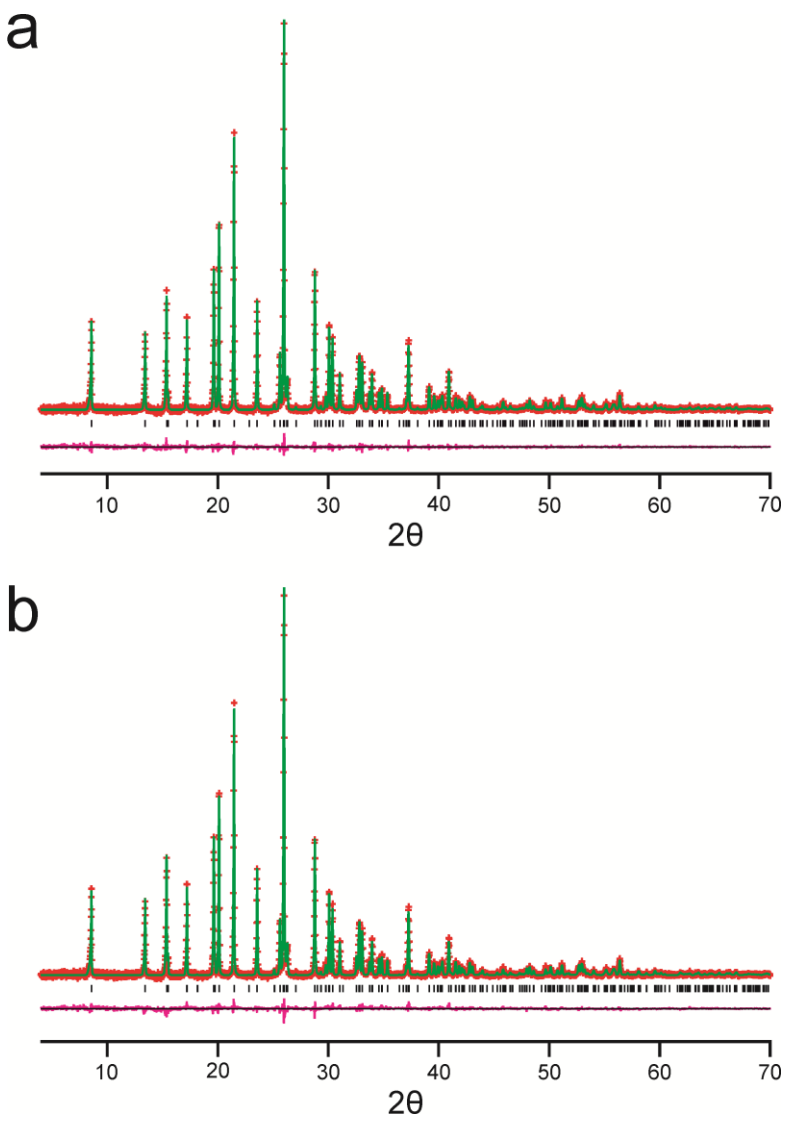

Figure 5: (a) Le Bail and (b) Rietveld fits for the powder pattern of L-lysine monohydrate, showing the experimental data (red + marks), calculated data (green line), difference plot (magenta line), and predicted peak positions (black tick marks).

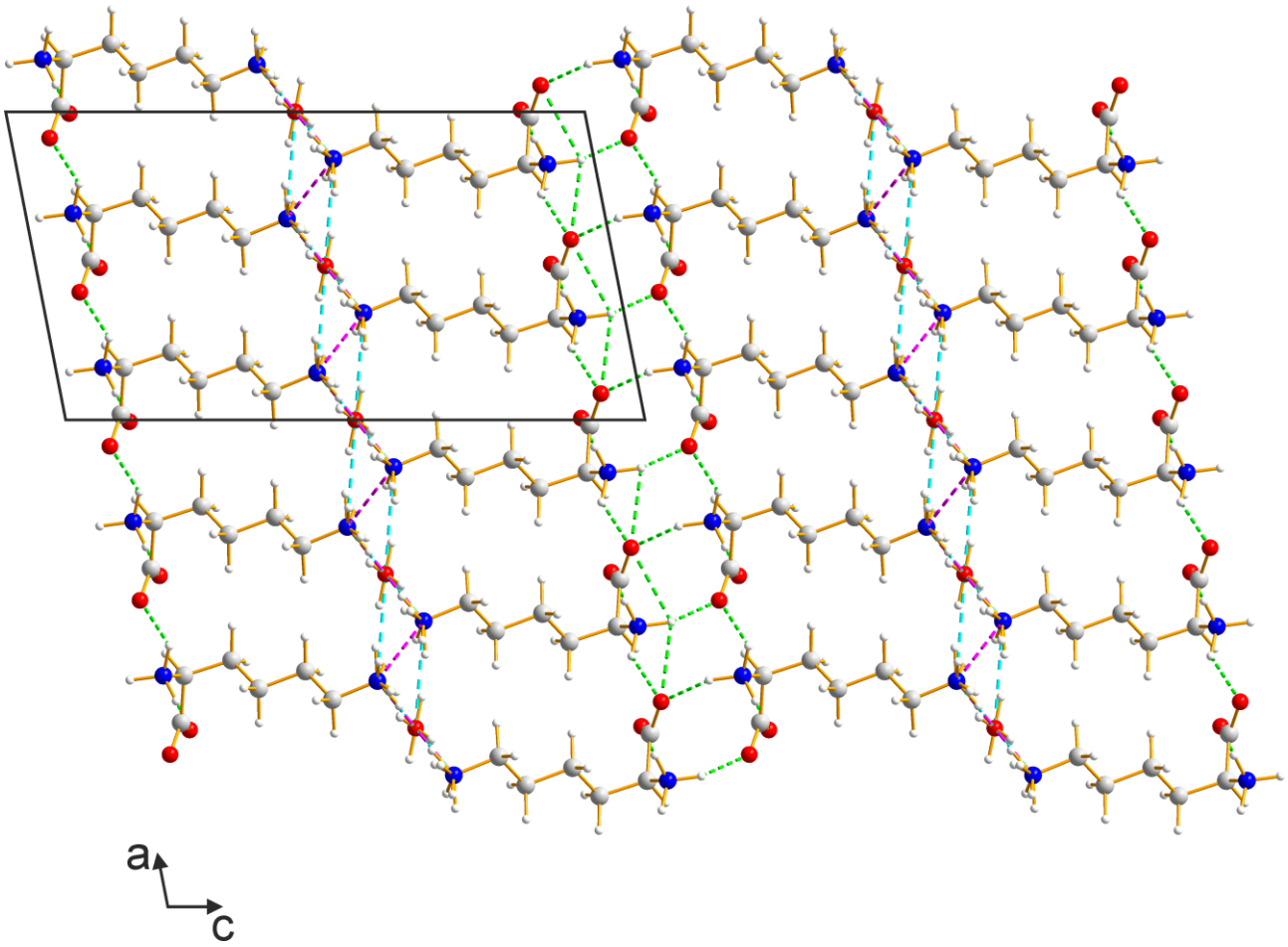

Figure 6: Crystal structure of L-lysine hemihydrate viewed along the *b*-axis. Hydrogen bonds between head groups are shown as green dashed lines, hydrogen bonds between side-chain amine groups are shown as cyan dashed lines and hydrogen bonds between side-chain amine groups and water molecules are shown as magenta dashed lines. In the latter two cases, the hydrogen bonds are drawn between heteroatoms independent of the hydrogen atoms.

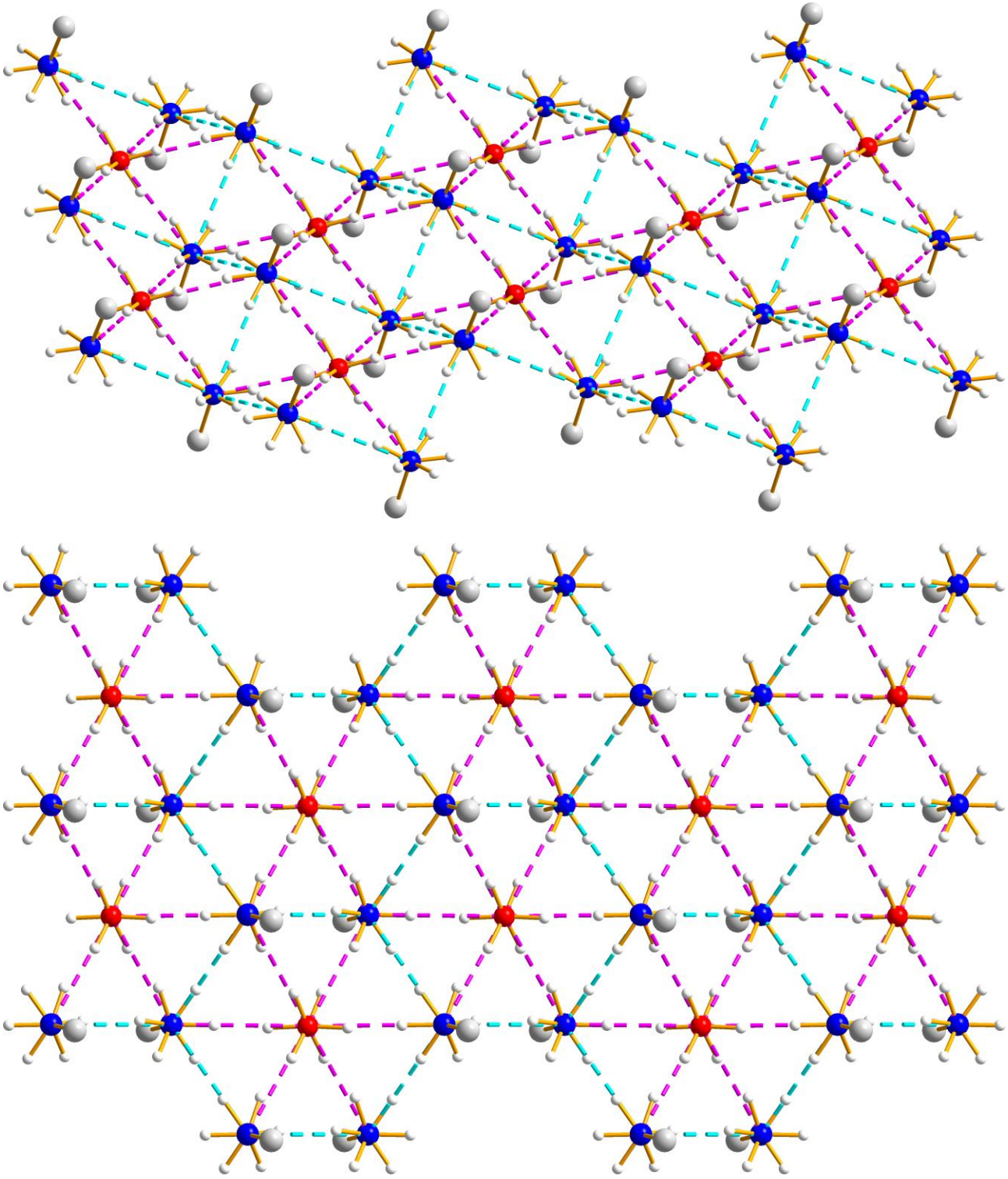

Figure 7: Hydrogen-bonded region involving side-chain amine groups of L-lysine molecules and water molecules in the crystal structure of L-lysine hemihydrate. N···N hydrogen bonds are shown in cyan while N···O hydrogen bonds are shown in magenta. <<<Which figure is better?>>>

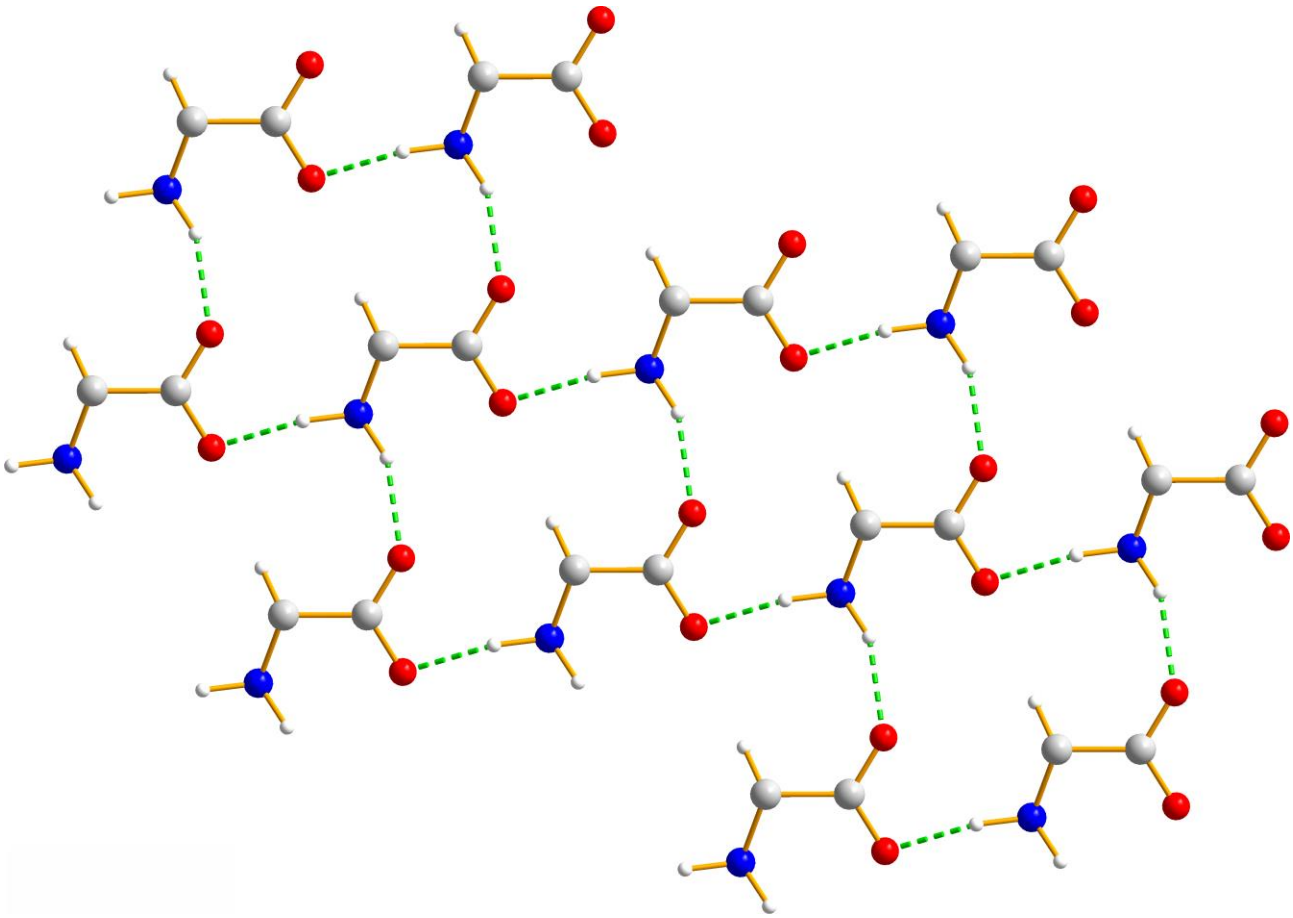

Figure 8: Single layer of hydrogen bonding between head groups in the crystal structure of L-lysine hemihydrate showing the L*x* classification as described by Görbitz *et al*.

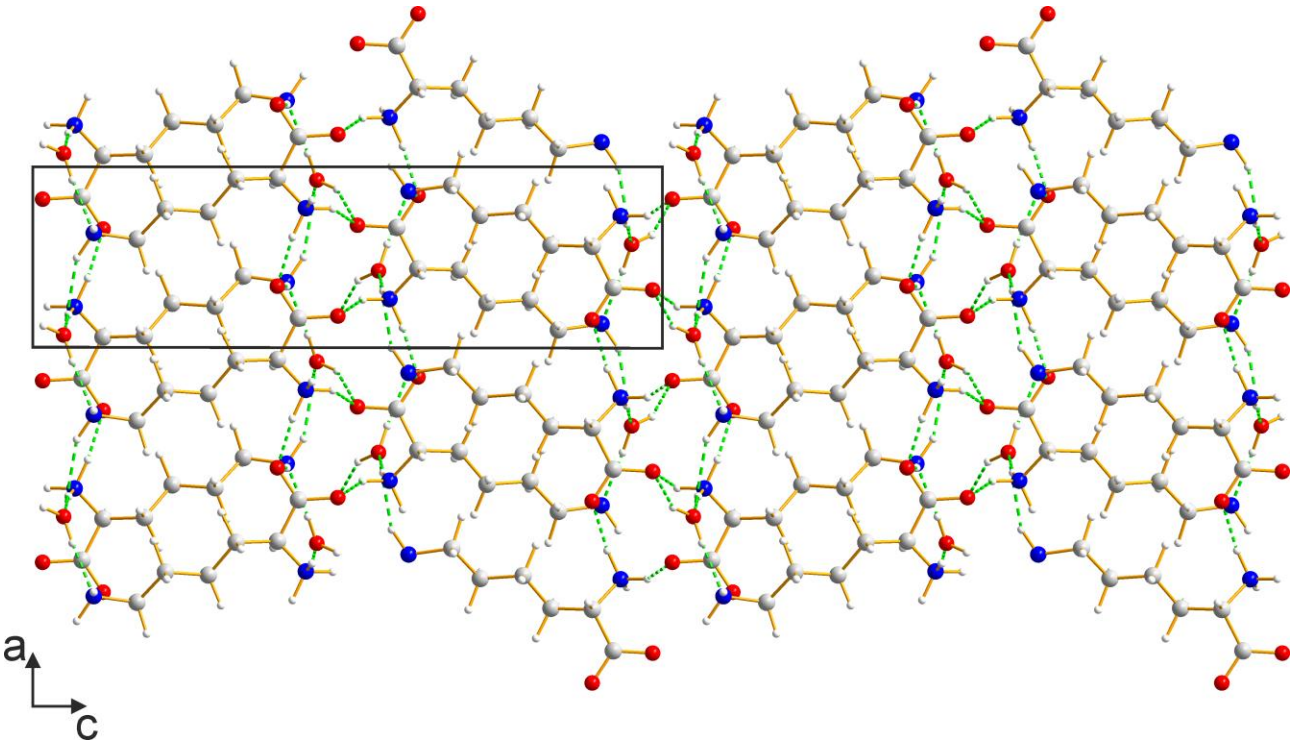

Figure 9: Crystal structure of L-lysine monohydrate viewed along the *b*-axis.

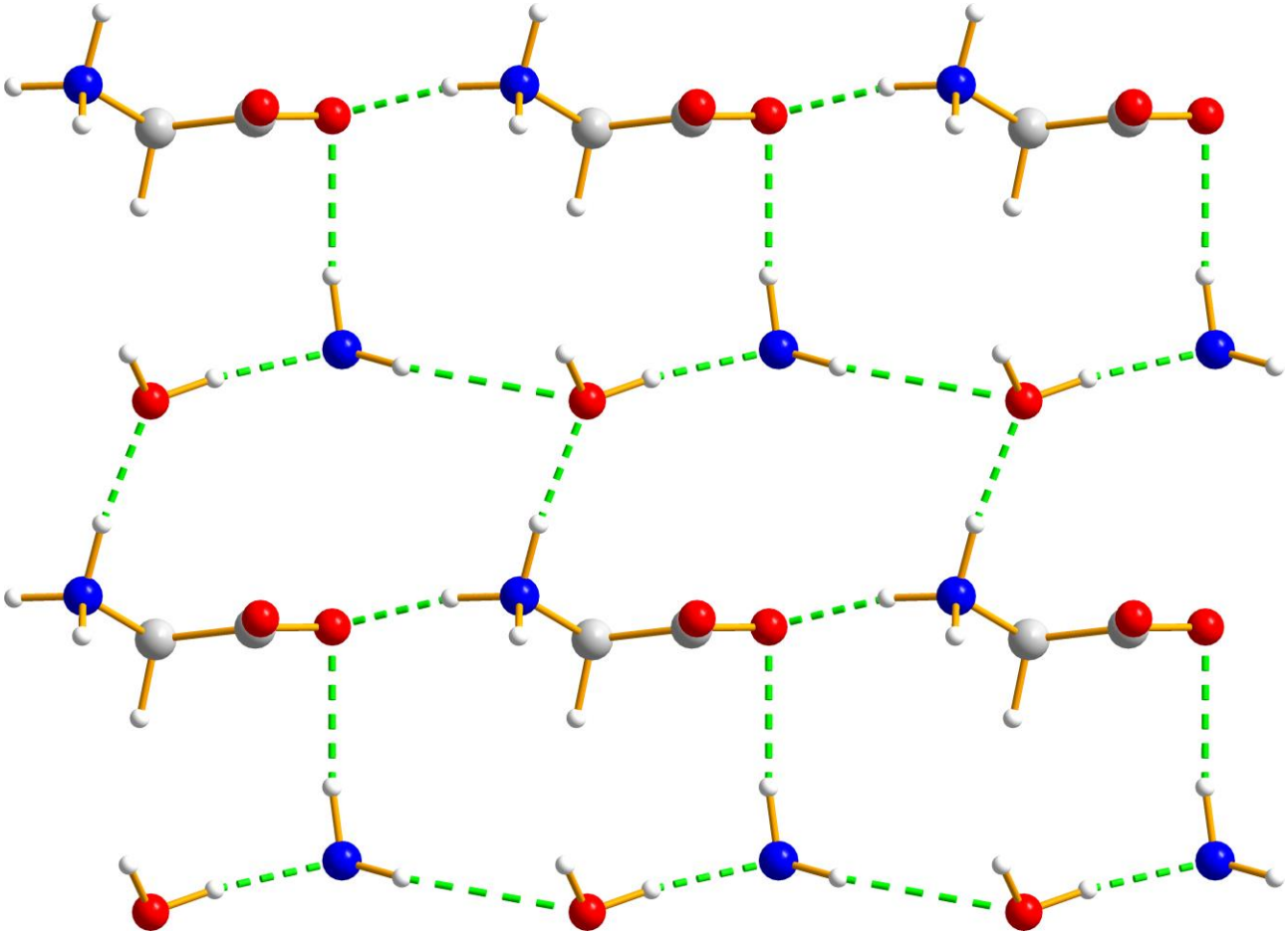

Figure 10: Single layer of hydrogen bonding between head groups, side-chain amine groups and water molecules in the crystal structure of L-lysine monohydrate.

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