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High-pressure structural study of L- α -glutamine and the use of Hirshfeld surfaces and graph-set notation to investigate the hydrogen bonding present in the structure up to 4.9 GPa

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The crystal structure of L- α -glutamine has been elucidated at room temperature at pressures between 0 and 4.9 GPa by using single-crystal high-pressure X-ray diffraction techniques. The structure is primarily stabilized by five N—H \cdots O intermolecular interactions, which link molecules in a herringbone-like layer arrangement, giving rise to voids within the solid. The application of pressure on the structure results in a reduction in the size of the voids, as a consequence of the shortening of the N—H \cdots O hydrogen bonds, which compress to minimum N \cdots O distances of around 2.6 Å, without driving the crystal structure to a phase transition. The decrease in the hydrogen-bond distances is due to the necessary stabilization of the structure, which arises from molecules modifying their positions to optimize electrostatic contacts and minimize the occupied space. Hirshfeld surfaces and fingerprint plots have been used to rapidly assess the structural changes that occur on application of pressure.

1. Introduction

L- α -Glutamine is an amino acid found in all forms of life. It is classified as a semi-essential or conditionally essential amino acid. L- α -Glutamine is very versatile, participating in many reactions in the body and, for example, is important in the regulation of acid–base balance. L- α -Glutamine participates in the formation of purine and pyrimidine nucleotides, amino sugars (such as glucosamine and L-glutamate) and other amino acids (*e.g.* nicotinamide, adenine, dinucleotide and glutathione). It also participates in protein synthesis, energy production and the formation of D-glucose and glycogen. Importantly, L- α -glutamine can serve as the primary respiratory substrate for the production of energy in enterocytes and lymphocytes. It is considered to be an immunonutrient and supplementary L- α -glutamine is used in medical foods for such stress situations as trauma, cancer, infections and burns (Skubitz & Anderson, 1996; Anderson *et al.*, 1998).

The first structural studies performed on L- α -glutamine were published by Cochran & Penfold (1952) and Koetzle *et al.* (1973), later followed by the analysis of the charge density and the topology of the crystal structure, reported by Wagner & Luger (2001). The L- α -glutamine structure is formed from five unique and significantly bent N—H \cdots O hydrogen bonds, one for each H atom attached to the nitrogen, which stabilize the structure to form a three-dimensional arrangement of L- α -glutamine molecules linked by a complicated hydrogen-bond network. Owing to the limited description of the crystal structure of L- α -glutamine available in the literature, a more exhaustive study of the molecular packing was performed as part of the work presented here, allowing us to provide a better description of the L- α -glutamine structure.

Table 1
Crystallographic data for L- α -glutamine at increasing pressures.

	0 GPa	0.1 GPa	1.4 GPa	4.9 GPa
Crystal data				
Chemical formula	C ₅ H ₁₀ N ₂ O ₃	C ₅ H ₁₀ N ₂ O ₃	C ₅ H ₁₀ N ₂ O ₃	C ₅ H ₁₀ N ₂ O ₃
M_r	146.15	146.15	146.15	146.15
Cell setting, space group	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$
Temperature (K)	293	293	293	293
Z	4	4	4	4
D_x (Mg m ⁻³)	1.529	1.536	1.616	1.756
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
μ (mm ⁻¹)	0.13	0.13	0.13	0.15
Crystal form, colour	Block, colourless	Block, colourless	Block, colourless	Block, colourless
Data collection				
Diffractometer	Bruker SMART	Bruker SMART	Bruker SMART	Bruker SMART
Data collection method	φ and ω scans	φ and ω scans	φ and ω scans	φ and ω scans
Absorption correction	Multi-scan [†]	Multi-scan [†]	Multi-scan [†]	Multi-scan [†]
T_{\min}	0.407	0.506	0.657	0.722
T_{\max}	1.000	1.000	1.000	1.000
No. of measured, independent and observed reflections	337, 326, 202	336, 324, 205	305, 295, 202	292, 283, 182
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.184	0.224	0.155	0.214
θ_{\max} (°)	23.2	23.3	23.3	23.2
Refinement				
Refinement on	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.105, 0.263, 1.05	0.148, 0.284, 1.16	0.107, 0.243, 1.04	0.114, 0.183, 1.10
No. of reflections	297	267295	330	278
No. of parameters	41	41	41	41
H-atom treatment	Fixed	Not refined	Mixture [‡]	Mixture [‡]
Weighting scheme	$w = q/[\sigma^2(F^*) + (P(1)p)2 + P(2)p + P(4) + P(5)\sin\theta]$; 0.106, 4.74, 0.00, 0.00, 0.00, 0.333	$P = P(6)^*\max(F_o^2, 0) + (1 - P(6))F_c^2$; $w = 1/[\sigma^2(F^*) + (P(1)p)^2 + P(2)p + P(4) + P(5)\sin\theta]$; $P(i)$ are: 0.00, 11.6, 0.00, 0.00, 0.00, 0.333	$w = q/[\sigma^2(F^*) + (P(1)p)2 + P(2)p + P(4) + P(5)\sin\theta]$; 0.897E-01, 4.49, 0.00, 0.00, 0.00, 0.333	$w = q/[\sigma^2(F^*) + (P(1)p)2 + P(2)p + P(4) + P(5)\sin\theta]$; 0.00, 3.26, 0.00, 0.00, 0.00, 0.333
$(\Delta/\sigma)_{\max}$	< 0.0001	< 0.0001	< 0.0001	< 0.0001
$\Delta\rho_{\max}$, $\Delta\rho_{\min}$ (e Å ⁻³)	0.57, -0.54	1.01, -0.76	0.76, -0.55	1.00, -1.05

Computer programs used: SMART (Bruker AXS, 1997–2001), SAINT (Bruker AXS, 2002), SIR92 (Altomare *et al.*, 1993), CRYSTALS (Watkin *et al.*, 1996), CRYSTALS (Betteridge *et al.*, 2003), CAMERON (Watkin *et al.*, 1996). [†] Based on symmetry-related measurements. [‡] Mixture of independent and constrained refinement.

The work presented is one of a number of investigations we are conducting on the effect of pressure on the crystal structures of different organic and biological compounds such as acetone, cyclopropylamine, ethanol, methanol, L-serine and L-cysteine, among others (Lozano-Casal *et al.*, 2005; Allan *et al.*, 1998, 2001; Allen & Clark, 1999; Moggach, Allan *et al.*, 2005; Moggach, Clark & Parsons, 2005). The aim of these studies is to understand how the various inter- and intramolecular interactions respond to pressure, which can often result in the formation of new high-pressure polymorphs. Indeed, the degree to which bond compressibility can be explained and the extent to which a structure can be compressed before a phase transition takes place are profoundly important questions in the field of high-pressure structural chemistry.

Strong hydrogen bonds are rare in biological structures since they are very rigid and not easily broken ($D\cdots A$

distance less than 2.5 Å and a $D-H\cdots A$ angle close to 180°, where D and A are the donor and acceptor atoms, respectively), and can hinder processes such as protein folding or unfolding. On the other hand, the salt-bridge intermolecular hydrogen bond, $N^+-H\cdots O=C$, which is present in L- α -glutamine, is one of the two strongest intermolecular interactions that exist in biological compounds, the other being $P-OH\cdots O=P$. It exists in nucleic acids, owing to the strong electrostatic component of the interaction, arising from the charged N and O atoms in the zwitterionic molecules (Steiner, 2002).

This paper is organized as follows. First, we perform a structural analysis of the L- α -glutamine structure by using high-pressure X-ray diffraction techniques to investigate how the crystal packing reacts to increasing pressure. Then the most important structural changes with pressure are studied

and discussed in detail, making use of Hirshfeld surfaces and fingerprints to highlight and visualize any changes taking place in the structure.

2. Experimental study of L- α -glutamine

2.1. Crystal growth

L- α -Glutamine was obtained commercially from the Aldrich Chemical Company. Colourless crystals, in the form of needles, were grown from a 3:1 water–ethanol solution by slow evaporation at ambient temperature. One small crystal of dimensions $0.1 \times 0.1 \times 0.1 \text{ mm}^3$ was selected for the subsequent experiment.

2.2. High-pressure X-ray crystallography

The high-pressure experiments were carried out using a Merrill–Bassett diamond–anvil cell (Merrill & Bassett, 1974), which has a half-opening angle of 40° and was equipped with 600 μm diamond culets and a tungsten gasket. A 250 μm hole was drilled through the gasket in order to accommodate the L- α -glutamine single crystal. A 4:1 mixture of methanol and ethanol was used as a hydrostatic medium. The fluorescence spectrum of a small ruby chip, which was also loaded into the cell, was used to yield all sample pressures. Pressure measurement was carried out by excitation of the ruby R1 and R2 fluorescence line emission using a 632.417 nm line from a He–Ne laser and the resulting ruby fluorescence spectrum was recorded using a Jobin–Yvon LabRam 300 Raman spectrometer.

Diffraction data were collected on a Bruker SMART APEX diffractometer with graphite-monochromated Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). Data collection and processing procedures for the high-pressure experiments were as described by Dawson *et al.* (2004; see also Bruker AXS, 1997–2001, 1999). Data collections were taken in steps from 0 GPa up to a final pressure of 4.9 GPa. Integrations were carried out using the program *SAINT* (Bruker AXS, 2002), resulting in a completeness range of 51.7–58.5%. The absorption corrections were undertaken with the programs *SORTAV* (Blessing, 1987, 1989) and *ABSORB* (Angel, 2004). The unit-cell dimensions were determined to be $a = 16.023$ (3), $b = 7.7678$ (18) and $c = 5.1004$ (13) \AA at 0 GPa and $a = 15.191$ (8), $b = 7.455$ (5) and $c = 4.882$ (4) \AA at 4.9 GPa. The space group was orthorhombic, $P2_12_12_1$. No discontinuities were observed in unit-cell parameters or their first derivatives with pressure, other than what could be expected from a continuous smooth compression and consequently there was no evidence for a structural phase transition.

Refinements were carried out against $|F|^2$ using all data (*CRYSTALS*; Betteridge *et al.*, 2003). In the refinement, since only a limited number of observations are available from the high-pressure experiments, either the number of parameters must be reduced or the number of observations must effectively be increased. In our case, we added observations in the form of geometrical restraints to the 1,2 distances (Engh & Huber, 1991), giving them the values observed in the ambient

pressure structure, so that bond lengths, bond angles and atomic contacts are kept in the ranges typically seen in very high-resolution structures. Additionally, all C, N and O atoms were refined with isotropic displacement parameters.

The L- α -glutamine coordinates of Koetzle *et al.* (1973) were refined against these data to yield conventional R factors in the range 0.077–0.148 for the refinements at the different pressures, from 0 to 4.9 GPa (Table 1). The aim of the zero-pressure experiment was simply to study the effect of the diamond–anvil cell on the quality of the diffracted intensities and the subsequent structural refinement. The resulting crystallographic data can be found in Table 1.¹ It was found that the refined structure obtained with the DAC at 0 GPa is consistent (within standard error) with those of Cochran & Penfold (1952) and Koetzle *et al.* (1973) reported at ambient conditions (*i.e.* with the crystal mounted on a fibre). However, the position of the H atoms differ as they were placed geometrically during the refinement procedure and their positions were not refined due to the limited quality of data, with respect to counting statistics and overall completeness. Nevertheless, the H atoms were allowed to ride on their parent atoms, contributing in this way to the refinement of the model. Consequently, the difficulty of locating the H atoms during the X-ray diffraction analyses led to the identification of hydrogen bonds from the $D \cdots A$ distances ($D = \text{donor}$, $A = \text{acceptor}$) alone and only the distances between the N and O atoms involved in the interactions will be discussed. The values for these distances at 0 GPa agree with those reported by Koetzle *et al.* (1973) at room temperature within experimental uncertainty.

3. Results and discussion

3.1. Analysis of the crystal structure of L- α -glutamine at ambient pressure

L- α -Glutamine crystallizes with one molecule in the asymmetric unit and therefore four molecules in the unit cell. The crystal structure in the solid state is characterized by the formation of molecular layers with the L- α -glutamine molecules adopting a herringbone-like arrangement within each layer (Fig. 1).

L- α -Glutamine exists as a zwitterionic state in the solid state and in aqueous solution; this means that the principal hydrogen-bond donor group, NH_3^+ , cannot, for steric reasons, accept hydrogen bonds, and the principal acceptors, the carboxylate or carbonyl O atoms, have no protons for hydrogen-bond donation. There are five different intermolecular hydrogen bonds present in the L- α -glutamine crystal structure, three formed by the NH_3^+ group and two formed by the NH_2 group (Fig. 2). The distance values for the five intermolecular interactions can be found in Table 2, together with the reference values reported by Koetzle *et al.* (1973). Although, as already mentioned, there are no strong

¹ Supplementary data for this paper are available from the IUCr electronic archives (Reference: BM5054). Services for accessing these data are described at the back of the journal.

intramolecular interactions, the distance between the N1 and the O1 atoms is 2.668 (18) Å and the angle formed by N1—H···O1, taking into account the uncertainty in the position of the H atom, is only around 90° and consequently by the criteria of Steiner (2002) this can still be considered as a weak hydrogen interaction. Consequently, the NH₃⁺ group forms a three-centre or bifurcated hydrogen bond with two different O atoms. The formation of this type of hydrogen-bond network was explained in detail by Jeffrey (1997) and Jeffrey & Mitra (1984).

The structure of L-α-glutamine can also be described as corrugated layers of molecules along the *a* axis (Fig. 3). Within each layer, molecules are linked *via* intralayer hydrogen bonds: *via* the two interactions formed by the NH₂ groups (N2···O1 and N2···O2), and the N1···O2, formed by the NH₃⁺ group (Fig. 3). In addition to the intra-layer interactions, there are two inter-layer hydrogen bonds (N1···O3ⁱ and N1···O3ⁱⁱ) present in the crystal structure (Fig. 4). These two N1···O3 interactions, formed by the NH₃⁺ group, link molecules into stacks along the *c* axis. However, owing to the proximity of the molecules between layers, the formation of the two inter-layer hydrogen bonds gives rise to extra inter-layer interactions, such as N2···O1, which are also present within the layers. The *c* cell dimension exhibits a value of around 5.1 Å, which is mainly associated with the formation of two head-to-head hydrogen bonds [N1···O3ⁱ and N1···O3ⁱⁱ], which actively participate in the stacking of layers along this direction. This feature is also present in other amino acids, where molecules are arranged in different chain motifs formed by N—H···OOC interactions. In serine (Benedetti *et al.*, 1973) and asparagine monohydrate (Verbist *et al.*, 1972), for example, the *c* and *a* axes are of 5.615 (2) and 5.593 (5) Å, respectively, which are related with the formation of head-to-tail chains along the *c* and *a* directions.

In terms of graph-set notation (Bernstein *et al.*, 1995) this rather complex hydrogen-bonding scheme can be described in terms of three neighbouring, and coupled, rings (Fig. 5). Thus, the N1···O3ⁱ, N1···O3ⁱⁱ and N2···O1 interactions form $R_3^2(14)$ rings, which link molecules to form the layers running along the *c* direction, whereas the N1···O3ⁱ, N1···O3ⁱⁱ and N2···O2 interactions form $R_3^3(14)$ rings, to link molecules along the *a* and *b* directions. Finally, the five different intermolecular interactions can be combined to form $R_3^3(12)$ rings to form the corrugated layers, which run along the *a* and *c* directions.

Apart from the strong electrostatic interactions already mentioned, the presence of weak C—H···O interactions in biological systems is also fundamental to their structure. The most significant C—H group found in proteins involves the C2 of each amino acid residue. Thus, the large number of such C2—H···O interactions could affect and influence the primary and secondary structures of proteins as well as their functionality. The C2—H···O interaction is the most common type of C—H···O=C interaction found in β sheets, where the C···O distance generally falls in the range 2.91–3.50 Å (with a mean distance of around 3.3 Å; Desiraju & Steiner, 1999). The second class are C—H···O=C contacts in α-helices with some preference for C3—H donors, whereas the third class is

composed of interactions to buried polar-side chains. Finally, the fourth class consists of contacts with buried water molecules. In order to complete this study, we have investigated the possibility of weak C—H···O interactions. Thus, in the crystal structure of L-α-glutamine at 0 GPa presented in this work, there are four C—H···O interactions (where the O atom belongs both to the carboxylic and amide groups; Table 3),

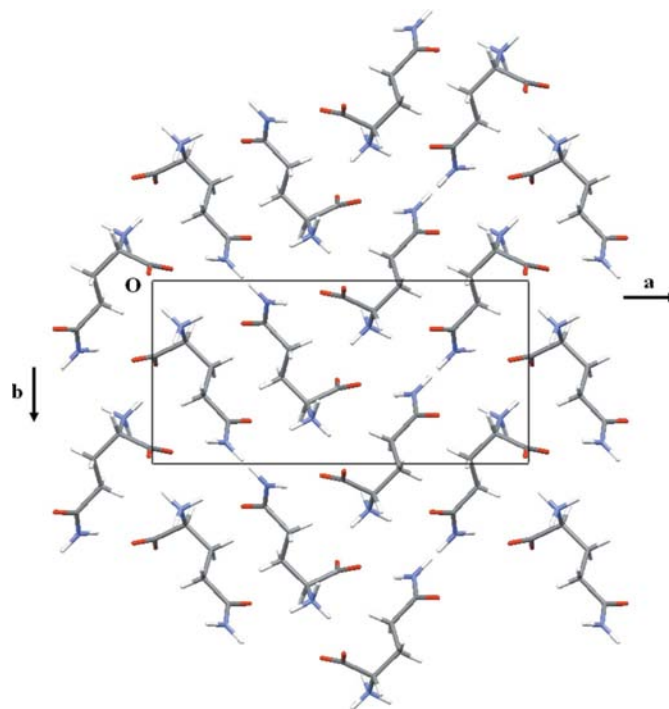


Figure 1
View of the packing along the *c*-crystallographic direction of L-α-glutamine at ambient pressure (0 GPa).

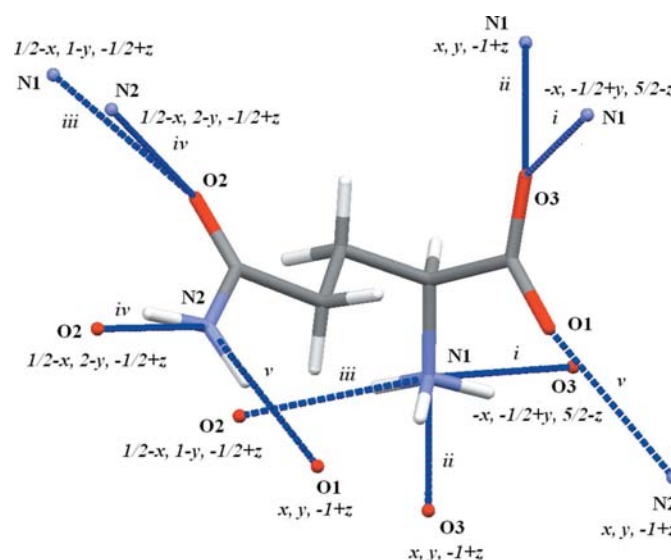


Figure 2
View of the hydrogen-bonding scheme of one molecule in the crystal structure of L-α-glutamine (the interactions are represented with thick blue lines joining the donor and acceptor atoms). This figure is in colour in the electronic version of this paper.

Table 2

Distances (Å) showing the reduction of the hydrogen-bond interactions with pressure for L- α -glutamine inside the diamond–anvil cell, from 0 to 4.9 GPa.

The values are for the intermolecular distance reported by Koetzle *et al.* (1973) at ambient conditions.

Pressure (GPa)	Koetzle <i>et al.</i> (1973)	0	0.1	0.8	1.4	2.7	3.6	4.9
$D(N1 \cdots O3)^i$	2.772 (3)	2.782 (19)	2.759 (24)	2.747 (18)	2.686 (18)	2.672 (14)	2.684 (16)	2.654 (19)
$D(N1 \cdots O3)^{ii}$	2.866 (3)	2.824 (22)	2.831 (28)	2.847 (21)	2.814 (20)	2.746 (16)	2.736 (19)	2.679 (20)
$D(N1 \cdots O2)^{iii}$	2.948 (3)	2.927 (18)	2.946 (24)	2.888 (17)	2.824 (18)	2.801 (13)	2.743 (15)	2.683 (19)
$D(N2 \cdots O2)^{iv}$	2.937 (3)	2.911 (15)	2.921 (20)	2.873 (15)	2.840 (15)	2.810 (12)	2.814 (14)	2.788 (16)
$D(N2 \cdots O1)^v$	2.911 (3)	2.864 (21)	2.854 (27)	2.846 (19)	2.799 (20)	2.765 (14)	2.754 (16)	2.710 (19)
$D(N1 \cdots O1)$	2.689	2.668 (18)	2.643 (25)	2.697 (18)	2.691 (17)	2.682 (13)	2.688 (15)	2.744 (18)

Symmetry codes: (i) $-x, -\frac{1}{2} + y, \frac{5}{2} - z$; (ii) $x, y, -1 + z$; (iii) $\frac{1}{2} - x, 1 - y, -\frac{1}{2} + z$; (iv) $\frac{1}{2} - x, 2 - y, -\frac{1}{2} + z$; (v) $-x, \frac{1}{2} + y, \frac{3}{2} - z$.

which arise from buried polar side chains; one formed by the C2, one formed by the C3 and two formed by the C4 of each L- α -glutamine molecule.

3.2. Effect of pressure on the intramolecular bond distances and bond angles

During the crystal structure refinement restraints were applied to the 1,2 distances. Our results show that the intramolecular bond lengths do not change significantly with increasing pressure. However, there are some changes in the N1–C2–C1–O3 dihedral angle, which changes progressively from 168.76 (14) to 170.52 (15)°, respectively, as the pressure increases from 0 to 4.9 GPa. This suggests that the molecules tend to become slightly more planar with increasing pressure.

3.3. Effect of pressure on hydrogen bonding

The variation of the intermolecular distances with pressure is shown in Table 2. On increasing the pressure to 4.9 GPa, all the intermolecular distances are shortened monotonically. The two intra-layer N2···O2 and N2···O1 interactions shorten by 4.2%, respectively, whereas the N1···O2 interaction is reduced by 8.3%. As these hydrogen bonds lie approximately parallel to the *a* axis, they will closely correlate to the *a*-axis compression. The inter-layer hydrogen bonds, N1···O3ⁱ and N1···O3ⁱⁱ, shorten by 5.1 and 4.6% from their values at 0 GPa.

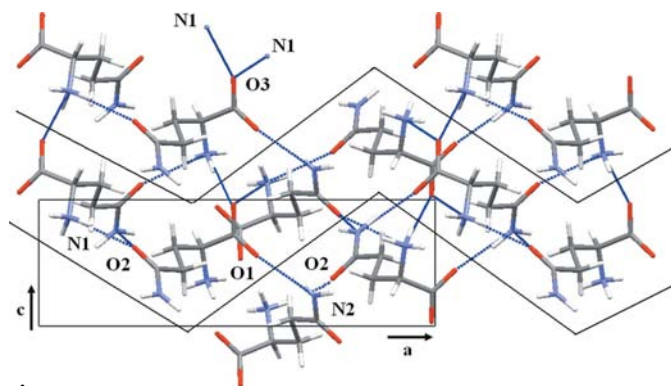


Figure 3

View along the *b* axis of two corrugated layers where molecules of L- α -glutamine are linked *via* the intra-layer hydrogen bonds, which are represented with thick blue lines. This figure is in colour in the electronic version of this paper.

The reduction of these inter-layer interactions with pressure contribute significantly to the *c*-axis compression.

As can be anticipated, there is a clear correlation between the strength of the hydrogen bonds and their compressibility. A good example of this is the 8.3% reduction in the length of the N1···O2 interaction, which is also the weakest in terms of hydrogen-bond geometry. Similar results were found for other amino acids, such as L- α -serine, where one of the N···O distances changed from 2.887 (4) to 2.691 (13) Å (6.8%) over 4.8 GPa (Moggach, Allan *et al.*, 2005). Finally, the weak intramolecular N1···O1 interaction increased its distance by 2.8% on compression from 0 to 4.9 GPa. This was an unexpected result, which shows how the molecule needs to slightly change its conformation in order to accommodate all the changes induced in the crystal structure by increasing pressure.

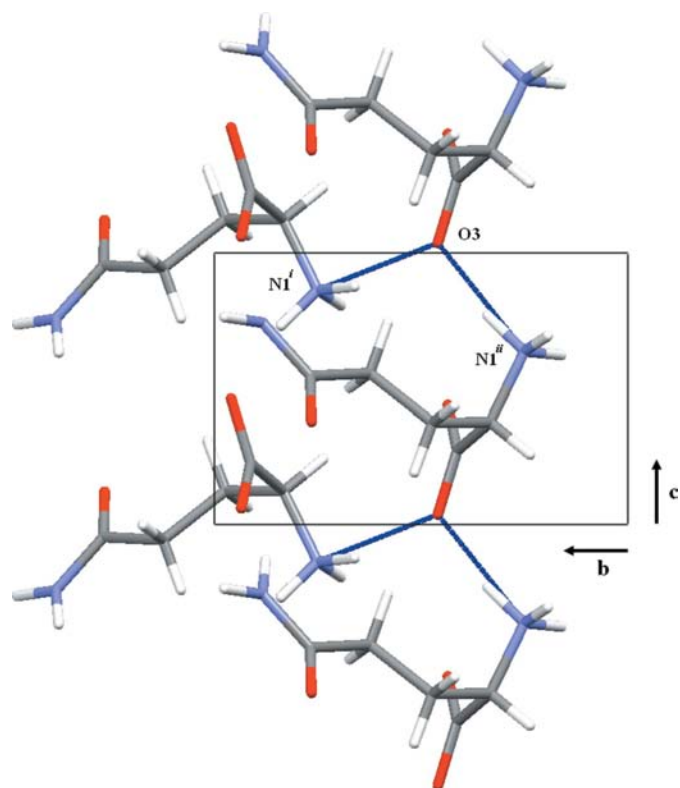


Figure 4

View along the *a* axis of the inter-layer hydrogen bonds (represented by thick blue lines), which stack the corrugated layers of L- α -glutamine molecules along the *c* direction.

From our previous work on amino acids (Moggach, Allan *et al.*, 2005) we generally find that the N—H···O hydrogen-bond distances tend to compress to a value no less than approximately 2.65 Å before a structural phase transition takes place which relieves the strain on the bonds. This ‘minimum’ value coincides with the shortest N···O hydrogen-bond distance reported in the Cambridge Structural Database [CSD;

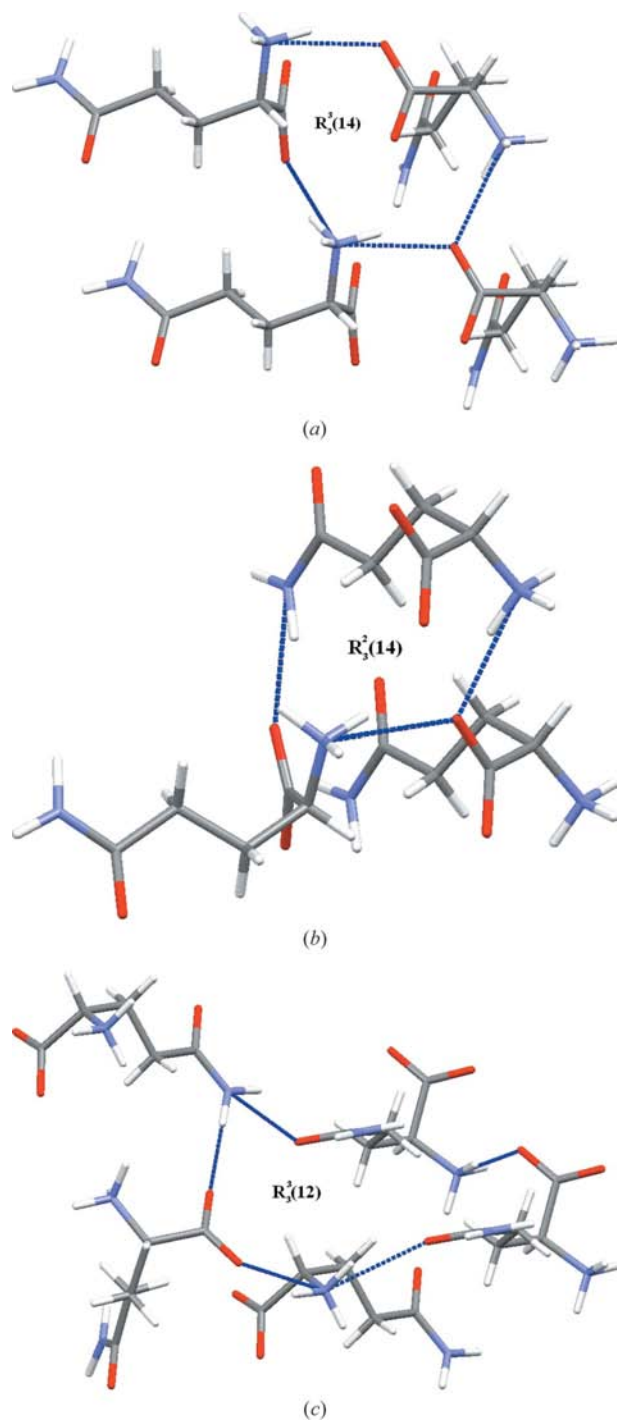


Figure 5
View of the three different ring motifs formed by the combination of (a) two (N1···O3ⁱ and N1···O3ⁱⁱ), (b) three (N1···O3ⁱ, N1···O3ⁱⁱ and N2···O1) and (c) all five intermolecular interactions in the crystal structure of L- α -glutamine at ambient conditions. All intermolecular interactions are represented by thick blue lines.

2.651 Å, (1*S*,2*R*)-*cis*-1-ammonioindan-2-ol (*R*)-2-phenylbutyrate, KAPWAZ; Kinbara *et al.*, 2000]. An example of this behaviour is exhibited by L-serine (Moggach, Allan *et al.*, 2005), which undergoes a phase transition at 5.4 GPa as one of the hydrogen-bond distances approaches 2.691 (13) Å. However, for the current study of L- α -glutamine, no phase transition was observed up to 4.9 GPa, although the various intermolecular interactions shortened considerably, from values of around 2.7–2.8 Å to values close to 2.65 Å. The lengths of these intermolecular hydrogen bonds converge towards essentially the same distance as that exhibited by the single N—H···O intramolecular interaction in the structure. This bond shows very little variation with pressure, save for a very slight increase in length.

The effect of pressure on the four different C—H···O weak interactions was studied, revealing that the different C···O distances reduced their values by different amounts when pressure was increased up to 4.9 GPa (Table 3). Thus, the C α 2···O1 interaction reduced its length by 4.5% and the C β 3···O2 interaction decreased its distance by 10.9%, whereas the C γ 4···O3 interaction decreased its distance by 6.2%. Finally, the C γ 4···O3 interaction shortened by 7.5%. From these results it can be seen how soft these C—H···O interactions are and they are thus inferred to be much weaker than the N—H···O interactions present in the structure.

3.4. Effect of pressure on the lattice parameters

The variation of the unit-cell parameters and unit-cell volume with pressure are shown in Table 4. As expected, due to the compression of the intra-layer hydrogen bonds, the *a* axis has the largest change (5%), whereas both the *b* and *c* axes are changed by around 4% at 4.9 GPa, mainly due to changes in the inter-layer interactions.

The relative compressibilities of the unit-cell edges and the unit-cell volume are similar to those observed previously for other amino acids. For example, in L- α -serine (Moggach, Allan *et al.*, 2005), the largest change in the cell parameters is along

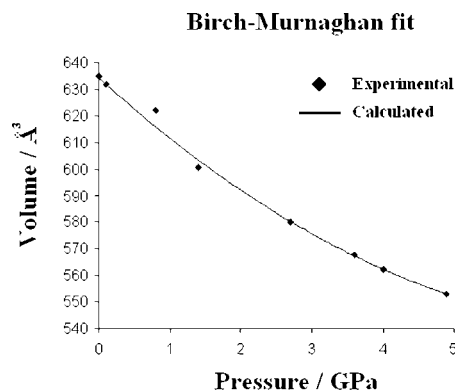


Figure 6
Plot of the experimental values and calculated values for the unit-cell volume (Å³) of L- α -glutamine. The line is a polynomial fit through the calculated values which have been obtained after a Birch-Murnaghan fit of the experimental data against pressure (GPa; refining only the values of V_0 and K_0).

Table 3

Donor-to-acceptor atom distances (Å) for the four most significant C...O intermolecular interactions present in the crystal structure of L-α-glutamine.

These values arose from the data collected on a crystal inside the diamond-anvil cell, at 0 GPa.

Pressure (GPa)	0	4.9
(C...O) interaction	<i>D</i> (C...O) (Å)	
Cα(C2)...O1	3.54 (2)	3.38 (2)
Cβ(C3)...O2	3.44 (3)	3.07 (3)
Cγ(C4)...O3 ⁱ	3.43 (3)	3.22 (2)
Cγ(C4)...O3 ⁱⁱ	3.518 (18)	3.254 (18)

Symmetry codes: (i) $-x, -\frac{1}{2} + y, \frac{5}{2} - z$; (ii) $x, y, -1 + z$.

the *b* direction (6.2%), which suffers three times the change along the *a* and *c* directions (2.6 and 2.1%). The corresponding change in the unit-cell volume was of 11% over 4.8 GPa, which is similar to the 13% reduction in unit-cell volume found here for L-α-glutamine over approximately the same pressure range.

Finally, the program *EOSFIT5.2* (Angel, 2002) was used to fit the pressure dependence of the unit-cell volume of L-α-glutamine with the Birch–Murnaghan equation-of-state.

Table 4

Lattice parameters obtained in the L-α-glutamine X-ray diffraction experiments between ambient pressure and 4.9 GPa.

The values obtained by Koetzle *et al.* (1973) are also given for comparison.

Pressure (GPa)	<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	<i>V</i> (Å ³)
Koetzle <i>et al.</i> (1973)	16.020 (10)	7.762 (6)	5.119 (4)	636.5
0	16.023 (3)	7.7678 (18)	5.1004 (3)	634.8 (2)
0.1	15.992 (2)	7.7558 (12)	5.0941 (9)	631.83 (17)
0.8	15.879 (6)	7.705 (3)	5.084 (2)	622.0 (4)
1.4	15.679 (11)	7.628 (6)	5.023 (5)	600.8 (9)
2.7	15.450 (8)	7.55 (6)	4.972 (5)	580.0 (7)
3.6	15.328 (7)	7.497 (5)	4.941 (4)	567.8 (6)
4.9	15.191 (8)	7.455 (5)	4.882 (14)	552.8 (6)

Given the relatively low number of observations, *k'* was fixed to a value of 4.0 and *k''* was set at -0.01498 in order that the higher-order terms of the equation were eliminated. The refined values for *V*₀ and *k*₀ are 635 (2) Å³ and 260 (11) GPa, respectively. The value of *V*₀ obtained from this least-squares refinement is in good agreement with the measured ambient pressure value [634.8 (2) Å³] and the value of *k*₀ is very similar to that observed in other amino acid systems [e.g. L-α-asparagine monohydrate, 176 (9) GPa]. Finally, a plot of the unit-cell volume *versus* pressure, including the Birch–Murnaghan fit, is shown in Fig. 6.

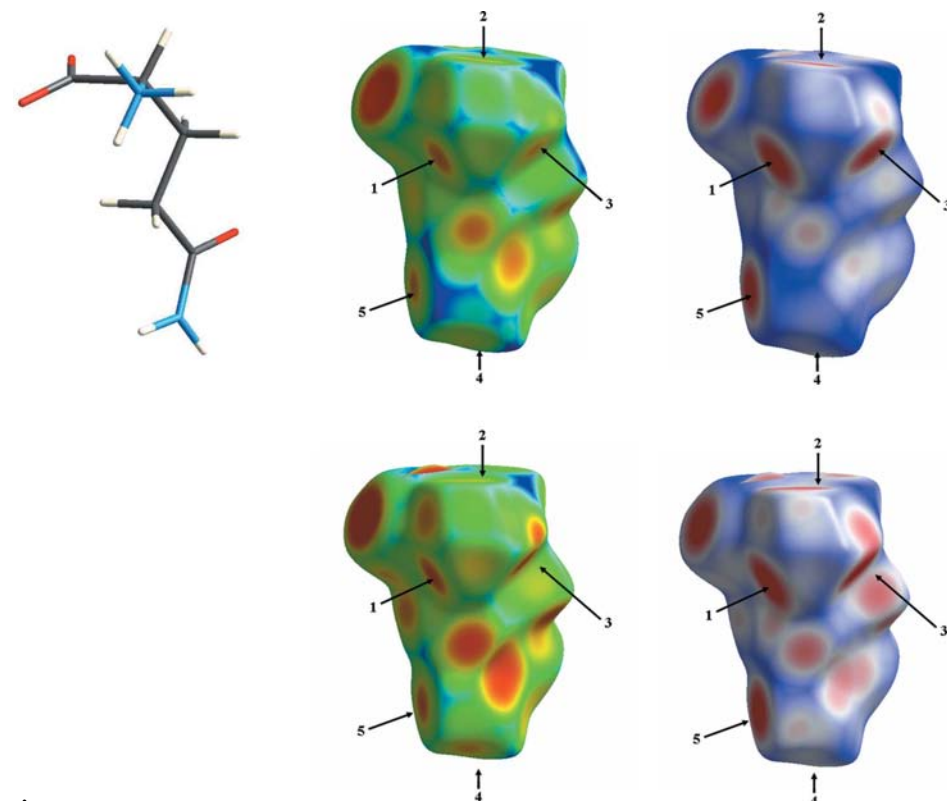


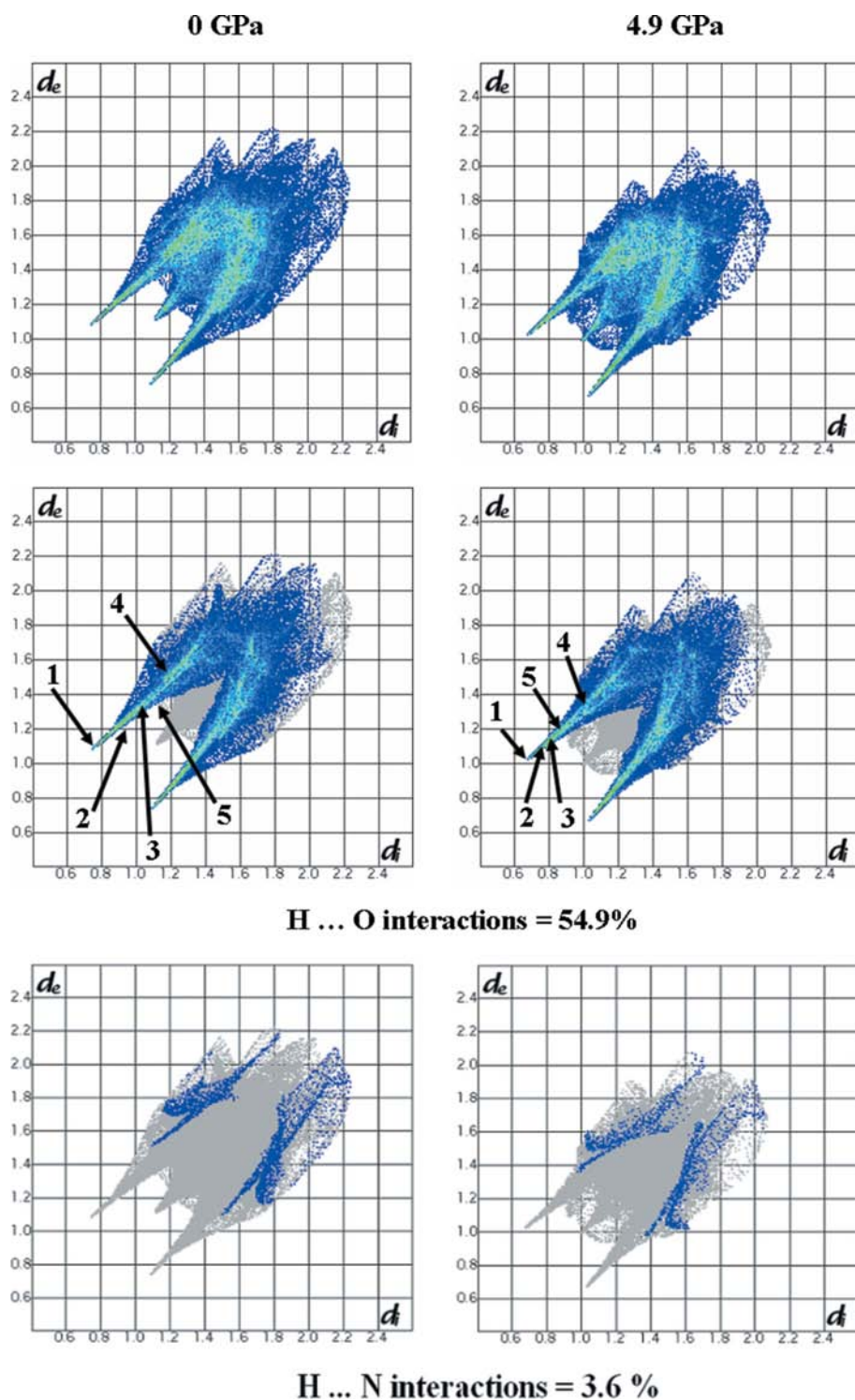
Figure 7

Hirshfeld surfaces for the ambient pressure (top) and high-pressure (bottom) structures of L-α-glutamine. Each molecule is shown with the Hirshfeld surface mapped with *d_c* [left; for this series mapped between 1.0 (red) and 2.0 Å (blue)] and *d_{norm}* [(mapped between -0.66 (red) and -0.89 (blue)], where *d_c* is the distance to the nearest atom centre exterior to the surface and *d_{norm}* is the normalized contact distance, which takes into account the van der Waals radius of the atoms. (The different interactions are labelled 1–5, as shown in the text: (1) N1...O3ⁱ, (2) N1...O3ⁱⁱ, (3) N1...O2, (4) N2...O2, (5) N2...O1.

3.5. Comparison of the ambient pressure and high-pressure crystal structures of L-α-glutamine: Hirshfeld surfaces

The program *CrystalExplorer* (Wolff *et al.*, 2005) is a recently developed tool that allows the use of Hirshfeld surfaces to partition crystal space in order to explore packing modes and intermolecular interactions in molecular crystals (McKinnon *et al.*, 2004). We have used this program to visualize the structure of L-α-glutamine at ambient pressure (*i.e.* 0 GPa) and at high pressure (*i.e.* 4.9 GPa) in order to make a more detailed comparison between them.

Hirshfeld surfaces (McKinnon *et al.*, 2004) are shown for the structure of L-α-glutamine at ambient pressure and high pressure in Fig. 7. It can be seen how the three H atoms of the NH₃⁺ group and the two H atoms of the NH₂ group actively participate in the formation of hydrogen bonding. This is shown by the orange–red region on the surface adjacent to the O atoms, which act as acceptors. From Fig. 7 it is possible to see


Figure 8

Two-dimensional fingerprint plots for the ambient pressure (left) and high-pressure (right) structures of *L*- α -glutamine, showing (top) the overall intermolecular interactions present in the crystal structures, (middle) the N–H \cdots O interactions and (bottom) the N–H \cdots N contacts in coloured areas, but keeping the rest of the interactions in grey as contrast. These plots provide a visual summary of the frequency of each combination of d_i (distance to the nearest atom centre interior to the surface) and d_e (distance to the nearest atom centre exterior to the surface) across the surface of the molecule, so they show not only which intermolecular interactions are present (N–H \cdots O interactions are labelled 1–5, as used in Fig. 7), but the relative area of the surface corresponding to each kind of interaction. Whereas the N–H \cdots O interactions constitute 54.9% of the molecule surface at 0 GPa decreasing up to 52.6% at 4.9 GPa, the N–H \cdots N interactions only account for 3.6% of the total at 0 GPa and 3.3% at 4.9 GPa.

significant differences in the hydrogen bonding between the ambient and high-pressure structures. One of the main differences is the shortening of the hydrogen bonds at high pressure, which is illustrated by an increase in the redness of the contact areas (yellow–orange) in the d_e surface as well as the formation of other intermolecular interactions (extra red regions in high-pressure surfaces), which only become significant when they shorten. In addition to this, the voids ‘close up’ on pressure increase. This can be seen by comparing the blue areas in the d_e surface, which are much larger in the ambient pressure structure than those at high pressure. Additionally, Fig. 7 illustrates a second type of surface, known as the d_{norm} surface, which includes the van der Waals (vdW) radius of the internal and external atoms involved in the contact. In these surfaces, red areas highlight shorter contacts, white represent contacts around the vdW separation and blue is for longer contacts. In contrast to d_e surfaces, the d_{norm} surface highlights both donor and acceptor equally. These differences are also shown by the total and partial fingerprint plots (McKinnon *et al.*, 2007) in Fig. 8.

One of the main differences between the plots is that the voids (upper region of the plots) are more compact at 4.9 GPa than they are at 0 GPa, indicating a more effective packing. The N–H \cdots O intermolecular interactions (see partial fingerprint in Fig. 8), which comprise 54.9 and 52.6% of the total interactions at ambient and high pressure, respectively, are shown as spikes in the plots; these spikes appear at shorter distances at 4.9 GPa owing to the shortening of the intermolecular interactions (note also the decrease in the amount of contacts observed). The N–H \cdots N interactions only account for 3.6% of the total contacts at 0 GPa (decreasing to 3.3% at 4.9 GPa) and so any changes with pressure on these will not have a strong influence on the *L*- α -glutamine crystal structure (see Fig. 8). The area

between the spikes corresponds to C—H···O interactions and the spike within the middle area is representative of short H···H contacts. From the plots it is possible to see how the weak C—H···O interactions shorten when pressure is applied to the L- α -glutamine crystal structure. This can be seen from the increment of the contacts in the area characteristic for C—H···O contacts (*i.e.* area between the spikes). In addition to this, the spikes characteristic of the N—H···O interactions shortened slightly owing to the decrease in the hydrogen-bond distances.

4. Conclusions

High-pressure X-ray diffraction techniques were used to study the compressibility of the L- α -glutamine crystal structure, from 0 to 4.9 GPa. Pressure induces the different intra- and inter-layer hydrogen-bond distances to shorten by varying amounts and changes ranging between 8.3 and 4.2% were observed. The hydrogen bond which is reduced the most (8.3%) is N1···O2, followed by changes in N1···O3ⁱ (5.1%), N1···O3ⁱⁱ (4.6%), N2···O2 and N2···O1 (4.2%). Consequently, the molecules within layers are pushed together and the layers are compressed along the *c* axis. The reduction in the hydrogen-bond distances can also be described using the Hirshfeld surfaces and fingerprint plots presented in this work. Additionally, significant changes occurred on the weak C—H···O interactions, with C···O distances shortened by diverse amounts, from 4.5 to 10.9% of their values at 0 GPa, therefore giving an insight of their soft nature.

The unit-cell volume data of L- α -glutamine, from 0 to 4.9 GPa, were fitted to the Birch–Murnaghan equation-of-state. Thus, values for the V_0 and k_0 were found to be 635 (2) Å³ and 260 (11), respectively. The value of k_0 is similar to the value obtained from a high-pressure study of L- α -asparagine monohydrate and falls broadly within the range of other hydrogen-bonded systems.

L- α -Glutamine does not undergo a phase transition up to a pressure of 4.9 GPa, as was also found to be the case for L- α -aspartic acid up to a similar pressure (Lozano-Casal, 2006). However, the much simpler amino acid, L-serine, does undergo a phase transition at about 4.8 GPa, to a previously unobserved polymorph (Moggach, Allan *et al.*, 2005). A reason for this can be found in the shape and size of the molecules. For example, the smallest amino acid, glycine, which forms simple hydrogen-bond networks, presents several phase transitions (Boldyreva, 2003, 2004; Boldyreva *et al.*, 2004; Dawson *et al.*, 2005). However, for larger amino acids such as L- α -glutamine and L- α -aspartic acid, this is not found to be the case. Owing to the flexibility of the molecules, the presence of several hydrogen donor and acceptor atoms, and the necessity to form multiple hydrogen bonds to hold the crystal structure together, these larger molecules have difficulty achieving a minimum in their packing energy in order to go through a phase transition, probably due to kinetic impediments. Nevertheless, in the compression of the structure of L- α -glutamine, the 'limit' distance was reached but no phase transition took place. This question of how much pressure is

required to drive the structure to a new polymorph is thus still open.

References

- Allan, D. R. & Clark, S. J. (1999). *Phys. Rev. B*, **60**, 6328–6334.
- Allan, D. R., Clark, S. J., Brugmans, M. J. P., Ackland, G. J. & Vos, W. L. (1998). *Phys. Rev. B*, **58**, R11809–R11812.
- Allan, D. R., Parsons, S. & Teat, S. J. (2001). *J. Synchrotron Rad.* **8**, 10–17.
- Altomare, A., Cascarano, G., Giacovazzo, C. & Guagliardi, A. (1993). *J. Appl. Cryst.* **26**, 343–350.
- Anderson, P. M., Ramsay, N. K., Shu, X. O., Rydholm, N., Rogosheske, J., Nicklow, R., Weisdorf, D. J. & Skubitz, K. M. (1998). *Bone Marrow Transplant.* **22**, 339–344.
- Angel, R. J. (2002). *EOSFIT5.2*. Virginia Tech, Blacksburg, USA.
- Angel, R. J. (2004). *J. Appl. Cryst.* **37**, 486–492.
- Benedetti, E., Pedone, C. & Sirigu, A. (1973). *Gazz. Chim. Ital.* **103**, 555–561.
- Bernstein, J., Davis, R. E., Shimoni, L. & Chang, N. L. (1995). *Angew. Chem. Int. Ed. Engl.* **34**, 1555–1573.
- Betteridge, P. W., Carruthers, J. R., Cooper, R. I., Prout, K. & Watkin, D. J. (2003). *J. Appl. Cryst.* **36**, 1487.
- Blessing, R. H. (1987). *Cryst. Rev.* **1**, 3–58.
- Blessing, R. H. (1989). *J. Appl. Cryst.* **22**, 396–397.
- Boldyreva, E. V. (2003). *J. Mol. Struct.* **647**, 159–179.
- Boldyreva, E. V. (2004). *Cryst. Eng.* **6**, 235–254.
- Boldyreva, E. V., Ivashevskaya, S. N., Sowa, H., Ashbaha, H. & Weber, H. P. (2004). *Dokl. Phys. Chem.* **396**, 111–114.
- Bruker AXS (1997–2001). *SMART*, Version 5.049–5.059. Bruker-AXS, Madison, Wisconsin, USA.
- Bruker AXS (1999). *GEMINI*, Version 1.01. Bruker-AXS, Madison, Wisconsin, USA.
- Bruker AXS (2002). *SAINT*, Version 6. Bruker-AXS, Madison, Wisconsin, USA.
- Cochran, W. & Penfold, B. R. (1952). *Acta Cryst.* **5**, 644–653.
- Dawson, A., Allan, D. R., Belmonte, S. A., Clark, S. J., David, W. I. F., McGregor, P. A., Parsons, S., Pulham, C. R. & Sawyer, L. (2005). *Cryst. Growth Des.* **5**, 1415–1427.
- Dawson, A., Allan, D. R., Parsons, S. & Ruf, M. (2004). *J. Appl. Cryst.* **37**, 410–416.
- Desiraju, G. R. & Steiner, T. (1999). *The Weak Hydrogen Bond in Structural Chemistry and Biology*, pp. 350–363. New York: Oxford University Press.
- Engh, R. A. & Huber, R. (1991). *Acta Cryst.* **A47**, 392–400.
- Jeffrey, G. A. (1997). *An Introduction to Hydrogen Bonding*. New York: Oxford University Press.
- Jeffrey, G. A. & Mitra, J. (1984). *J. Am. Chem. Soc.* **106**, 5546–5553.
- Kinbara, K., Kobayashi, Y. & Saigo, K. (2000). *J. Chem. Soc. Perkin Trans.* **2**, 111–119.
- Koetzle, T. F., Frey, M. N., Lehmann, M. S. & Hamilton, W. C. (1973). *Acta Cryst.* **B29**, 2571–2575.
- Lozano-Casal, P. (2006). PhD thesis. The University of Edinburgh.
- Lozano-Casal, P., Allan, D. R. & Parsons, S. (2005). *Acta Cryst.* **B61**, 717–723.
- McKinnon, J. J., Jayatilaka, D. & Spackman, M. A. (2007). *Chem. Commun.* pp. 3814–3816.
- McKinnon, J. J., Spackman, M. A. & Mitchell, A. S. (2004). *Acta Cryst.* **B60**, 627–668.
- Merrill, L. & Bassett, W. A. (1974). *Rev. Sci. Instrum.* **45**, 290–294.
- Moggach, S. A., Allan, D. R., Morrison, C. A., Parsons, S. & Sawyer, L. (2005). *Acta Cryst.* **B61**, 58–68.
- Moggach, S. A., Clark, S. J. & Parsons, S. (2005). *Acta Cryst.* **E61**, o2739–o2742.

- Skubitz, K. M. & Anderson, P. M. (1996). *J. Lab. Clin. Med.* **127**, 223–228.
- Steiner, T. (2002). *Angew. Chem. Int. Ed.* **41**, 48–76.
- Verbist, J. J., Lehmann, M. S., Koetzle, T. F. & Hamilton, W. C. (1972). *Acta Cryst.* **B28**, 3006–3013.
- Wagner, A. & Luger, P. (2001). *J. Mol. Struct.* **595**, 39–49.
- Watkin, D. J., Prout, C. K., Carruthers, J. R. & Betteridge, P. W. (1996). *CRYSTALS*, Issue 10. Chemical Crystallography Laboratory, Oxford, England.
- Wolff, S. K., Grimwood, D., McKinnon, J., Jayatilaka, D. & Spackman, M. (2005). *CrystalExplorer*, Version 1.5. University of Western Australia, Australia.