J. Biosci., Vol. 14, Number 2, June 1989, pp. 111–125. © Printed in India.

X-ray studies on crystalline complexes involving amino acids and peptides: Part XVIII. Crystal structure of a new form of L-arginine D-glutamate and a comparative study of amino acid crystal structures containing molecules of the same and mixed chirality

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MS received 7 February 1989; revised 10 April 1989

Abstract. The new form of L-arginine D-glutamate is monoclinic, $P2_1$ with a = 9·941(1), b = 4·668(2), c= 17·307(1) Å, β = 95·27(1)°, and Z = 2. In terms of composition, the new form differs from the old form in that the former is a monohydrate whereas the latter is a trihydrate. The structure has been solved by the direct methods and refined to R = 0·085 for 1012 observed reflections. The conformation of the arginine molecule is the same in both the forms whereas that of the glutamate ion is different. The change in the conformation of the glutamate ion is such that it facilitates extensive pseudosymmetry in the crystals. The molecules arrange themselves in double-layers stabilised by head-to-tail sequences involving main chains, in both the forms. However, considerable differences exist between the two forms in the interface, consisting of side chains and water molecules, between double-layers. A comparative study of the relationship between the crystal structures of L and DL amino acids on the one hand and that between the structures of LL and LD amino acid-amino acid complexes on the other, provides interesting insights into amino acid aggregation and the effect of chirality on it. The crystal structures of most hydrophobic amino acids are made up of double-layers and those of most hydrophilic amino acids contain single layers, irrespective of the chiralities of the amino acids involved. In most cases, the molecules tend to appropriately rearrange themselves to preserve the broad features of aggregation patterns when the chirality of half the molecules is reversed as in the structures of DL amino acids. The basic elements of aggregation in the LL and the LD complexes, are similar to those found in the crystals of L and DL amino acids. However, the differences between the LL and the LD complexes in the distribution of these elements are more pronounced than those between the distributions in the structures of L and DL amino acids.

Keywords. Amino acid complexes; effect of chirality; amino acid aggregation; head-to-tail sequences of amino acids; chemical evolution.

Introduction

We have been pursuing a programme of x-ray studies on crystalline complexes involving amino acids and peptides in order to define, at the atomic resolution, the geometrical features of biologically important non-covalent interactions (Salunke and Vijayan, 1984; Suresh and Vijayan, 1985; Suresh *et al.,* 1986). This work has led, among other things, to the elucidation of specific interactions and characteristic interaction patterns involving functional groups found in proteins (Sudhakar *et al.,* 1980; Sudhakar and Vijayan, 1980; Salunke and Vijayan, 1981, 1983; Vijayan, 1983). It has also resulted in a new approach to prebiotic phenomena based on molecular interactions and aggregation (Vijayan, 1980; Vijayan and Suresh, 1985). The current focus of the programme is on complexes of amino acids of mixed chirality. In this context we have already analysed the crystal structures of 5 LD complexes, namely, L-arginine D-glutamate trihydrate (Suresh *et al.,* 1986), L-arginine D-aspartate

(Suresh *et al.,* 1986), L-lysine D-glutamate (Soman *et al.,* 1988), L-lysine D-aspartate monohydrate (Soman *et al.,* 1988) and L-ornithine D-aspartate monohydrate (Soman and Vijayan, 1988), and two DL-DL complexes, namely, DL-arginine DL-glutamate monohydrate (Soman *et al.,* 1989) and DL-arginine DL-aspartate (Soman *et al.,* 1989). The comparison of these structures with those of the corresponding LL complexes has provided valuable insights into the effect of chirality on amino acid aggregation and its possible relevance to chemical evolution. Here we report the crystal structure of a new form of a complex between L-arginine and D-glutamic acid, which contains only one water molecule per a pair of amino acid molecules instead of 3 in the complex the structure of which has already been analysed.

Having analysed several LL and LD amino acid complexes, it is of obvious interest to investigate if the relation between the two sets of complexes bears any resemblance to that between the structures of L amino acids and the corresponding DL amino acids. Indeed no systematic and comprehensive study of the relationship between the crystal structures of L and DL amino acids appears to have been carried out. In the second part of this paper, this relationship is explored, and the aggregation patterns in the LL and LD complexes are discussed with reference to it.

Materials and methods

X-ray analysis

The complex between L-arginine and D-glutamic acid was originally crystallised from an aqueous solution of the components in molar proportions by the diffusion of acetone into it (Suresh *et al.,* 1986). In addition to the normal crystals the structure of which was reported earlier, the crystallization dish contained a single crystal with a different morphology. Repeated attempts to reproduce more crystals of the latter type were of no avail. Therefore, the one available crystal was used for all subsequent X-ray work. This crystal was needle-like in shape and had dimensions 0·10 × 0·15×0·70 mm. It was found to belong to the monoclinic space group $P2_1$ with a = 9·941(1), b =4·668(2), c= 17·307(1) Å and β = 95·27(1)°. The old form also belonged to the space group $P2_1$ with a=9·968, b= 4·652, c= 19·930 Å and β = 101·20°. Thus the difference in the cell dimensions is primarily in the length of the c axis. The intensity data from the new crystal were collected on a CAD-4 computer controlled diffractometer using nickel-filtered CuKα radiation up to a Bragg angle of 65° with h=0 to 11, k=0 to 5 and l= –19 to 19. The data were corrected for Lorentz and polarisation factors. Intensities of 0kl and 0kT reflections yielded a merging R of 3 %.

The structure solution turned out to be more difficult than anticipated. In retrospect, the extreme pseudosymmetry (see later) appears to have caused the difficulty. The solution obtained using the direct methods program MULTAN (Main *et al.,* 1980) contained what appeared to be peaks corresponding to two arginine molecules instead of an arginine molecule and a glutamate ion. It also contained 4 unexplained peaks. The final correct solution was achieved through several trial calculations, including a few for an averaged structure in space group $P2_1/a$, and examination of several difference Fourier maps. The structure thus

Obtained was refined using the block-diagonal SFLS method. The non-hydrogen atoms were refined anisotropically and the hydrogen atoms, fixed from a difference Fourier map using geometrical considerations, isotropically. Form factors for non-hydrogen atoms were taken from Cromer and Waber (1965) and those for hydrogen atoms from Stewart *et al.* (1965). Refinement was terminated at a *R* factor of 0·085 for 1012 observed reflections with $1 > 2\sigma$ (I). The weighting scheme used had the form $1/(a + bF + cF)$ with $a = 0{\cdot}164$, $b = 0{\cdot}111$ and $c = 0{\cdot}003$. The final difference Fourier map had no positive features >0·48 eÅ^{-3}. Table 1 lists the final positional parameters and the equivalent isotropic temperature factors (Hamilton, 1959) of the non-hydrogen atoms.

Table 1. Positional parameters (X10000) and equivalent isotropic temperature factors of non-hydrogen atoms in L-arginine D-glutamate monohydrate.

Atom	X	Y	Z	Equivalent B
N1	6300(8)	2979	4128(5)	2·0(2)
O1	4348(7)	−848(16)	4379(4)	2·5(2)
O2	2751(7)	2072(17)	3830(5)	2·9(2)
C1	3952(11)	1318(24)	3980(6)	2·3(3)
C2	5037(11)	2968(23)	3595(6)	2·2(3)
C3	5259(10)	1520(22)	2834(5)	1·6(2)
C4	6373(10)	2924(25)	2387(7)	2·5(3)
C5	6777(10)	889(29)	1755(6)	2·5(3)
N6	7816(10)	1931(23)	1324(5)	2·7(2)
C7	7691(9)	3750(29)	708(5)	2·0(3)
N8	6476(9)	4774(24)	469(5)	2·8(2)
N9	8735(10)	4370(28)	356(6)	3·3(3)
N11	1311(7)	−2942(19)	4102(4)	1·5(2)
O11	−662(7)	708(18)	4402(4)	2·3(2)
O12	−2252(7)	−2046(18)	3810(5)	3·0(2)
C11	−1060(9)	−1368(22)	3983(5)	1·1(2)
C12	39(9)	−3015(21)	3605(6)	1·6(2)
C13	229(10)	−1620(25)	2820(6)	2·3(3)
C14	1319(12)	−2640(28)	2362(3)	2·6(3)
C15	1509(10)	−1041(24)	1649(6)	1·9(2)
O16	583(9)	529(23)	1339(5)	3·7(2)
O17	2644(8)	−1217(20)	1368(5)	3·3(2)
W1	3767(9)	3728(20)	887(5)	3·7(2)

Estimated SD are given in parentheses.

Results and discussion

Molecular and crystal structure of the complex

Molecular dimensions: The bond lengths and angles in the zwitterionic positively charged arginine molecules and the zwitterionic negatively charged glutamate ions are by and large normal. There are a few departures from standard values (Vijayan, 1976) but they do not merit special comment in view of the presence of pseudo-symmetry in the crystals and the comparatively high SD. The torsion angles that

define the conformation of the molecules (IUPAC-IUB Commission on Biochemical Nomenclature, 1970) have the following values:

L-Arg: $\psi' = -36{\cdot}8(12)$; $\psi'' = 148{\cdot}6(10)$; $\chi^1 = -58{\cdot}9(11)$
$\chi^2 = 165{\cdot}0(9)$; $\chi^3 = -178{\cdot}4(9)$; $\chi^4 = -82.6(14)$
$\chi^{51} = 1{\cdot}3(17)°$

D-glu: $\psi' = 31{\cdot}2(11)$; $\psi'' = -153{\cdot}7(9)$; $\chi^1 = 53.8(12)$
$\chi^2 = -175.1(9)$; $\chi^{31} = -19{\cdot}2(15)°$

The side chain of the arginine molecule assumes sterically the most favourable orientation (Bhat *et al.,* 1979) in which it is *trans* to the α-carboxylate group. The side chain has an extended conformation except for the guanidyl group which introduces a fold at the terminus of the side chain with X^4 assuming a value close to –90°. The observed conformation is one of the 15 unique conformations of arginine identified so far in crystal structures (Ramachandran *et al.,* 1965; Suresh *et al.,* 1986; Prasad, G. S. and Vijayan, M., unpublished results) and is the same as that found in the original form of the LD complex between arginine and glutamic acid. The glutamate ion in the structure has sterically the most favourable conformation with an all *trans* side chain *trans* to the α-carboxylate group, a conformation observed in several crystal structures (Bhat and Vijayan, 1977; Sequeira *et al.,* 1972; Soman *et al.,* 1988), but not in the original form of the complex. Thus L-arginine has the same conformation in the two forms but the D-glutamate ion has different conformations in them. It is also interesting to note that ψ', χ^1 and χ^2 in the L-arginine molecule and the D-glutamate ion in the present structure have nearly the same magnitudes but opposite signs. The same is true of χ^3 in the arginine molecule and χ^{32} in the glutamate ion. This feature has interesting consequences in terms of pseudosymmetry.

Molecular aggregation, hydrogen bonding and pseudosymmetry: The crystal structure of the complex is illustrated in figure 1. The parameters of the hydrogen bonds that stabilise the structure are given in table 2. As in the case of the original complex, the amino acid molecules form double-layers parallel to the *ab* plane in the structure. Each layer in the double-layer is made up of L-arginine molecules and D-glutamate ions and is stabilised by two DL head-to-tail sequences (Suresh and Vijayan, 1983). The two layers in the double-layer are held together by zigzag head-to-tail sequences, one involving L-arginine molecules and the other involving D-glutamate ions. Thus, as in the original form, the core of each double-layer is made up exclusively of main chain atoms interconnected through head-to-tail sequences, and is flanked by side chains. In fact, the structure and the interactions in the core are identical in the two structures.

The original form of the complex contains 3 water molecules and the interactions between double-layers are entirely through water bridges which connect side chains. The new form, however, contains only one water molecule and the interactions between double-layers involve water bridges and side chain-side chain interactions. The interface between the double-layers and indeed side chain-side chain interactions in general, are substantially different in the two structures. In the present structure, the interface contains a straight N–H——O hydrogen bond between side chain guanidyl and carboxylate groups, and a water bridge involving side chains. The water molecule also links side chains of adjacent molecules in the

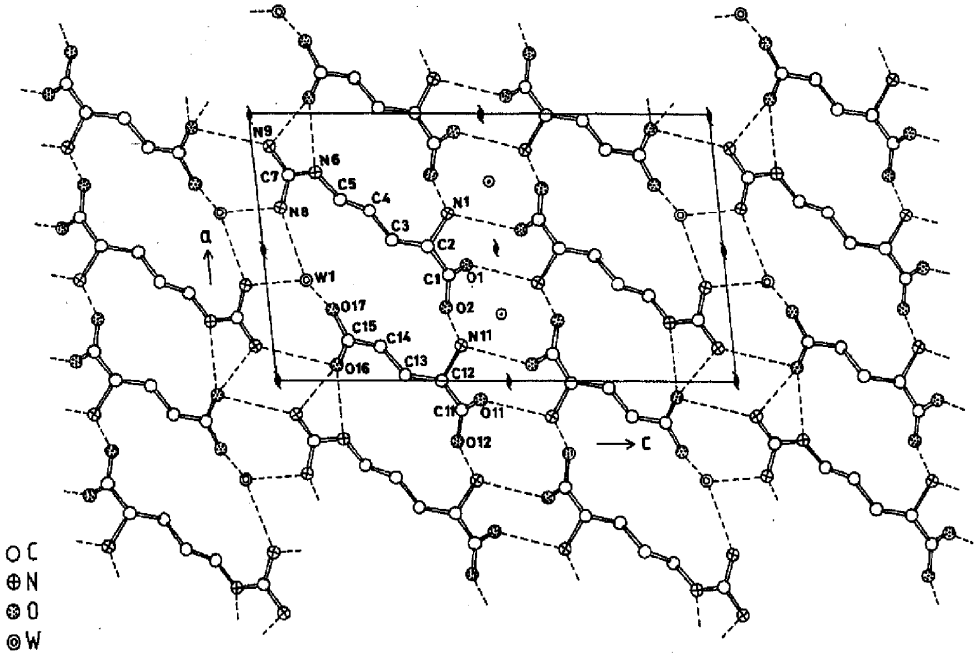

Figure 1. The crystal structure of L-arginine D-glutamate monohydrate as viewed along the *b* axis. Broken lines denote hydrogen bonds. In this and the subsequent figures, hydrogen bonds connecting molecules related by a translation along the direction of projection have been omitted for clarity. The dotted circles represent pseudo inversion centres.

Table 2. Hydrogen bond parameters in L-arginine D-glutamate monohydrate.

A–H . . . B	A . . . B(Å)	H–A . . . B(°)	Symmetry of atom B
N1–H1(N1) . . . 012	2·83(1)	25(8)	x+1, y, z
N1–H2(N1) . . . 012	2·81(1)	23(7)	x+1, y+1, z
N1–H3(N1) . . . 01	2·77(1)	2(10)	−x+1, y+1/2, −z+1
N6–H1(N6) . . . 016	2·83(1)	15(7)	x+1, y, z
N8–H1(N8) . . . W1	2·89(1)	12(7)	x, y, z
N8–H2(N8) . . . W1	2·98(1)	11(8)	−x+1, y+1/2, −z
N9–H1(N9) . . . 016	2·99(1)	33(8)	x+1, y, z
N9–H2(N9) . . . 016	3·12(1)	24(8)	−x+1, y+1/2, −z
N11–H1(N11) . . . 011	2·80(1)	9(6)	−x, y−1/2, −z+1
N11–H2(N11) . . . 02	2·79(1)	28(7)	x, y−1, z
N11–H3(N11) . . . 02	2·81(1)	14(7)	x, y, z
W1–H1(W1) . . . 017	2·73(1)	14(9)	x, y, z
W1–H2(W1) . . . 017	2·77(1)	6(10)	x, y+1, z

Estimated SD are given in parentheses.

same layer. Yet another link between molecules within a layer is a type D specific interaction (Salunke and Vijayan, 1981) between side chain guanidyl and carboxylate groups.

Many complexes containing amino acids of mixed chirality exhibitpseudo symmetry in their crystals (Suresh *et al.*, 1986; Soman *et al.*, 1988, 1989). For

example, in the old form of the L-arginine D-glutamate complex, the main chain atoms in L-arginine and those in D-glutamate are related by a pseudo inversion centre midway between the two 2_1 screw axes in the middle of the double-layer. The pseudo inversion centre and the crystallographic 2_1 screw axis combine to give a pseudo *a* glide perpendicular to *b*. Thus the old form has a pseudosymmetry of $P2_1/a$ when only the main chain atoms are considered. The same pseudosymmetry not only exists but extends to a substantial part of the side chains also in the present structure. The change in the conformation of the glutamate ion is such that the side chain atoms C3, C4, C5 and N6 in the arginine molecule are pseudosymmetrically related to the side chain atoms C13, C14, C15 and O17 of the glutamate ion. Thus 18 out of the 23 non-hydrogen atoms in the structure obey pseudo $P2_1/a$ symmetry.

Comparison of amino acid structures containing molecules of the same and mixed chirality

The relationship between the aggregation patterns in LL amino acid-amino acid complexes and those in the corresponding LD complexes has been investigated in some detail through studies in our laboratory (Suresh *et al.,* 1986; Soman *et al.,* 1988; Soman and Vijayan, 1988). The differences between a LL complex and the corresponding LD complex result from the reversal in the chirality of half the molecules, all of the same type, in the aggregate. The differences between the crystal structure of an L amino acid and that of the corresponding DL amino acid also result from the reversal in the chirality of half the molecules in the aggregate, although in this case both the isomers are of the same type. The two situations are thus somewhat similar. Therefore, a careful comparative study of the aggregation patterns in the crystal structures of L and DL amino acids, and a re-examination of the patterns in the LL and LD complexes on the basis of the results of this study, would be of some value in exploring the effect of chirality on molecular aggregation.

L and DL amino acid crystal structures: Before going into the detailed analysis of aggregation patterns in amino acids and their complexes, a brief introduction to the nomenclature of head-to-tail sequences (Suresh and Vijayan, 1983), in which the α-amino and the α-carboxylate groups of adjacent molecules are brought into hydrogen-bonded proximity in a peptide-like arrangement, used in the following discussion is in order. Most of the sequences can be broadly classified into straight sequences containing molecules related by translation, zigzag sequences generated by 2_1 screw axes, and DL sequences generated by glide planes. A straight sequence is designated as S1 if the acceptor in the hydrogen bond is the carboxylate oxygen *cis* to the amino nitrogen atom and S2 if the acceptor atom is *trans* to it. The zigzag and DL sequences are correspondingly designated Z1 and Z2, and DL1 and DL2.

The amino acids for which the crystal structures of the L isomer as well as the racemate are available are valine, leucine, isoleucine, methionine, alanine, serine, tyrosine, histidine, glutamic acid and aspartic acid, and the analysis outlined below is based on these structures. The crystals of L-glutamic acid exist in two forms designated α and *β*. L-Serine crystallises in the anhydrous as well as the hydrated forms. L-Histidine also crystallises in two forms; one has monoclinic symmetry

while the other is orthorhombic. The only DL amino acid which has two crystal forms is DL-methionine; its dimorphs are designated α and *β*.

All but one of the crystal structures of the amino acids mentioned above can be described in terms of reasonably well-defined planar features resulting from hydrogen bonds involving main chain atoms. The sole exception is the α form of L-glutamic acid (Lehmann and Nunes, 1980) in which the main chain atoms are involved in a truly 3-dimensional network of hydrogen bonds. The 2-dimensional features are essentially of two types, one double-layers and the other, single layers. Crystals of the hydrophobic amino acids L-valine (Torii and Iitaka, 1970), L-leucine (Harding and Howieson, 1976), L-isoleucine (Torii and Iitaka, 1971) and L-methionine (Torii and Iitaka, 1973) are made up of double-layers stacked along the longest crystallographic axis. As an example the crystal structure of L-valine is shown in figure 2. Each layer in the double-layer has molecules held together by a S1 and a S2 sequence. The arrangement of molecules in this layer is schematically illustrated in figure 3. The layers in the double-layer are related by 2_1 screw axes and interconnected by N–H——O hydrogen bonds. Thus the core of a double-layer is made up of two sheets, each belonging to a layer. Each sheet is made up of main chain atoms and is stabilised by head-to-tail sequences. The two sheets are also interconnected by N–H ——O hydrogen bonds. The core is then flanked by side chains.

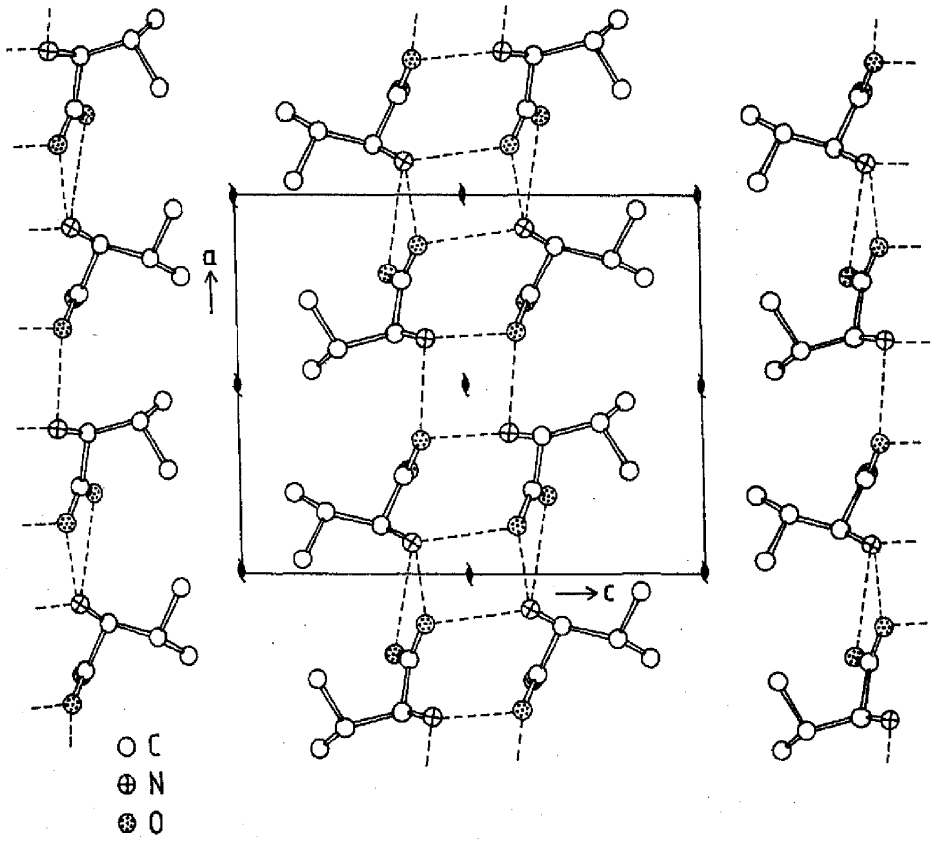

Figure 2. The crystal structure of L-valine as viewed along the b axis (see text for details).

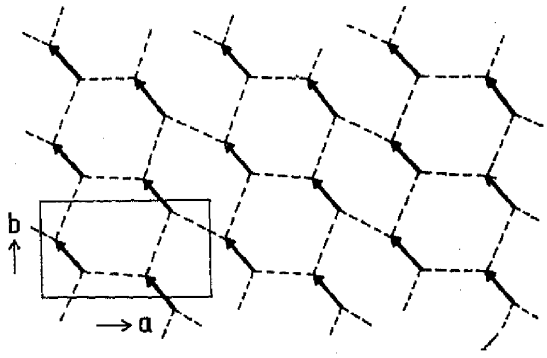

Figure 3. Schematic representation of the S1S2 arrangement in the layers of L-valine. In this and the following schematic diagrams, the head of an arrow represents the α-amino group, and the tail the α-carboxylate group, of an amino acid molecule.

The crystal structures of L-tyrosine (Mostad *et al.,* 1972; Frey *et al.,* 1973a), the two forms of L-histidine (Madden *et al.,* 1972a, b), the *β* form of L-glutamic acid (Hirokawa, 1955), L-aspartic acid (Derissen *et al.,* 1968), L-serine (Kistenmacher *et al.,* 1974), L-serine·H_2 O (Frey *et al.,* 1973b) and L-alanine (Simpson and Marsh, 1966; Lehmann *et al.,* 1972) contain layers stacked along the longest axis. As an example, the structure of the monoclinic form of L-histidine is illustrated in figure 4. It may be noted that most of the amino acids mentioned above are hydrophilic in nature. Each layer consists of a central sheet made up of main chain atoms and stabilised by interactions involving α-amino and α-carboxylate groups. The central sheet is flanked on either side by side chains. Unlike in the case of the arrangement

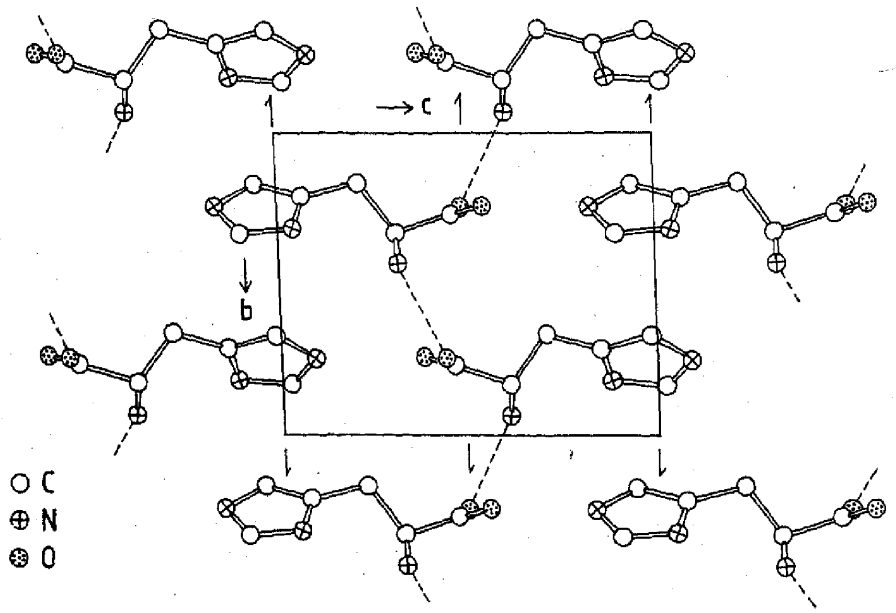

Figure 4. The crystal structure of L-histidine as viewed along the *a* axis (see text for details).

based on double-layers in most of the hydrophobic L amino acids, there are no main chain-main chain interactions between adjacent layers except in the case of L-alanine and L-serine. The small size of the side chains of these two amino acids permits interactions between adjacent sheets. In all the structures except that of L-serine· H_2O, the molecules in each layer are held together by a S2 sequence and a Z2 sequence. This S2Z2 arrangement in a layer is schematically illustrated in figure 5. In the layers of L-serine. H_2O, however, a Z2Z2 arrangement, illustrated schematically in figure 6 exists. Although the above discussion pertains to only those amino acids for which the crystal structures of both the L-isomer and the racemate are available, it must be mentioned that the structures of other amino acids also broadly follow the aggregation pattern outlined above. For example, the crystal structures of the hydrophilic amino acids L-threonine (Shoemaker *et al.,* 1950; Ramanadham *et al.,* 1973) and L-glutamine (Cochran and Penfold, 1952; Koetzle *et al.,* 1973) contain S2Z2 layers. L-Cysteine crystallises in two forms. The aggregation pattern in the orthorhombic form (Kerr and Ashmore, 1973, 1975) is based on layers while the monoclinic form (Harding and Long, 1968) has an aggregation pattern involving double-layers.

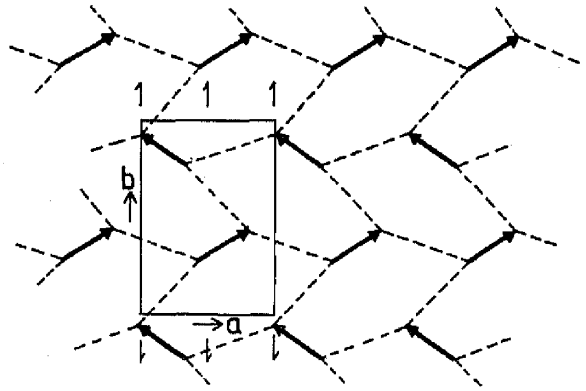

Figure 5. Schematic representation of a typical S2Z2 arrangement in the layers of most hydrophilic amino acids.

The reversal of chirality of half the molecules produces only minimal changes in the aggregation patterns of the hydrophobic amino acids valine, leucine and isoleucine as seen by the examination of the crystal structures of DL-valine (Mallikurjan and Thyagaraja Rao, 1969), DL-leucine (DiBlasio *et al.,* 1975) and DL- isoleucine (Benedetti *et al.,* 1973). The molecules still aggregate into double-layers stacked along the longest crystallographic axis. In the L amino acid crystal structures, both layers in the double-layer obviously contain the L isomer, and they are related to each other by screw axes. In the DL amino acid crystal structures, one layer contains only the L molecules and the other, the D molecules. Both the layers continue to have the S1S2 arrangement of head-to-tail sequences. The two layers in the double-layer are then related by inversion centres. The molecules in the crystals of both α- and *ß*-DL-methionine (Mathieson 1952) also aggregate into double-layers as in the crystals of the other DL hydrophobic amino acids. However, in the structures of α-and *ß* -DL-methionine, each layer in the double-layer contains

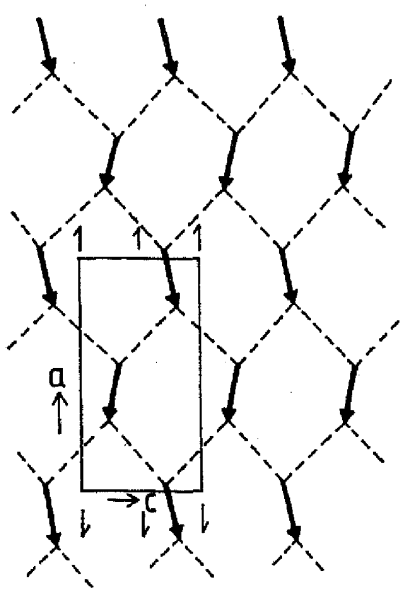

Figure 6. Schematic representation of the Z2Z2 arrangement in the layers of L-serine· H_2O.

L as well as D molecules (related by glide planes). The crystal structure of α-DL-methionine is shown in figure 7. The two layers in the double-layer are related to each other by screw axes as well as inversion centres. In each layer, the molecules are held together by two DL2 head-to-tail sequences, as schematically illustrated in figure 8.

A greater variety exists in the aggregation patterns observed in the crystal structures of DL-tyrosine (Mostad and Romming, 1973), DL-histidine (Edington and Harding, 1974), DL-glutamic acid (Ciunik and Glowiak, 1983), DL-aspartic acid (Rao, 1973), DL-serine (Kistenmacher *et al.,* 1974) and DL-alanine (Donohue, 1950). All of them contain single layers as do the crystals of the corresponding L isomers. The molecular arrangement in the L amino acid and the DL amino acid crystals are very similar in the case of alanine and tyrosine. Each layer in the DL amino acid crystals contains D as well as L molecules. The molecules in the layer are held together by a S2 head-to-tail sequence and a DL2 sequence. As far as the main chain atoms are concerned, the geometry of the DL2 sequence is close to the Z2 sequence in the crystals of the L isomers. Thus the S2Z2 arrangement in the L amino acid crystals and S2DL2 arrangements in the DL amino acid crystals are essentially similar. Consequently, the reversal in the chirality of half the molecules brings about only marginal changes in the essential features of the aggregation patterns in the case of tyrosine and alanine. A close relationship exists between the aggregation patterns in the crystals of L-serine-H_2O and in those of DL-serine. As in the case of L-serine· H_2O, a Z2Z2 pattern exists in the molecular layers in the crystals of DL-serine except that the alternating layers are of opposite chirality in the latter. The arrangement of molecules in the remaining 3 DL amino acid crystals, namely, those of DL-histidine, DL-aspartic acid and DL-glutamic acid, do not bear any relationship with one another, with those in other DL amino acid crystals or

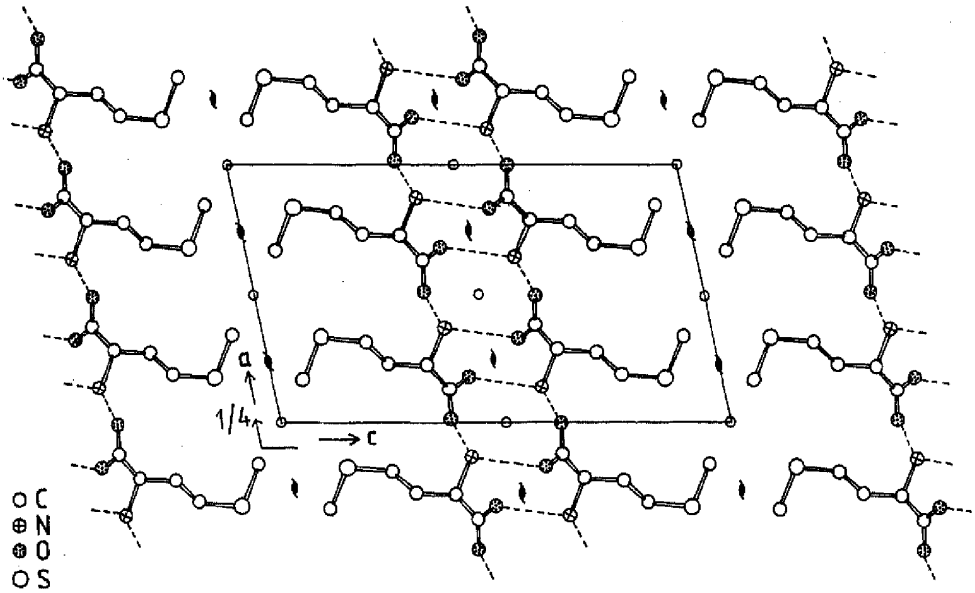

Figure 7. The crystal structure of α-DL-methionine as viewed along the *b* axis (see text for details).

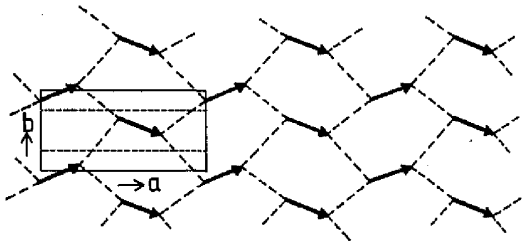

Figure 8. Schematic representation of the DL2DL2 arrangement in the layers of α-DL-methionine.

With those in the crystals of the corresponding L amino acids. The 3 arrangements are schematically illustrated in figure 9. A DL2 sequence is present in each of them. The DL-histidine layer consists of inversion centres but no other head-to-tail sequences. The DL-aspartic acid layers and the DL-glutamic acid layers contain in addition a Z2 sequence and a DL1 sequence respectively.

The broad basic feature of aggregation in most amino acid crystals is a layer or a double-layer. Mostly the same feature is found in a L amino acid crystal and the corresponding DL amino acid crystal. The arrangement of molecules in a layer in a L amino acid crystal and that in the corresponding DL amino acid crystal are found to be the same or closely related in a majority of the cases. These arrangements are substantially different only in 3 cases. Thus when all the amino acid molecules in a crystal are of the same chemical type, the general patterns of molecular aggregation tend to be retained even when the chirality of half the molecules is reversed. It however turns out that the packing efficiency in the crystals

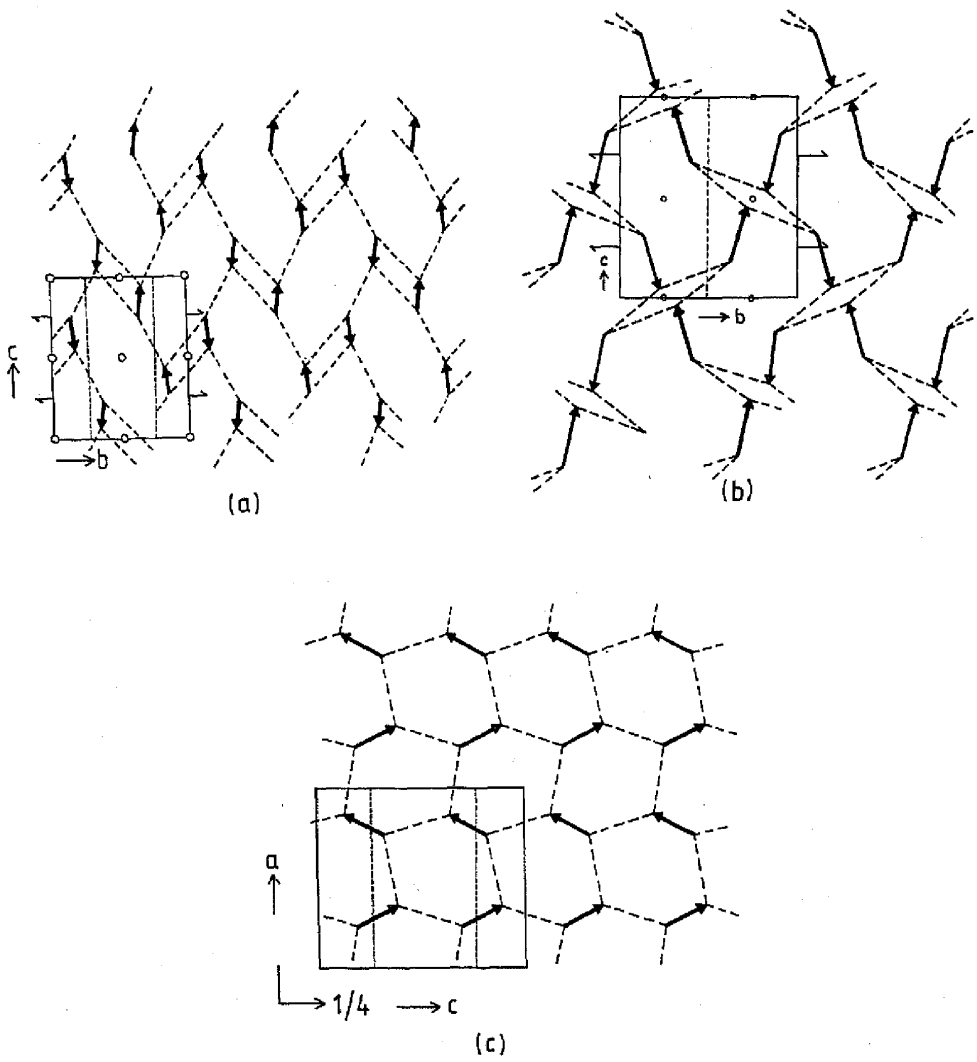

Figure 9. Schematic representation of (a) the arrangement consisting of a DL2 sequence and inversion centres in the layers of DL-histidine, (b) the DL2Z2 arrangement in the layers of DL-aspartic acid and (c) the DL1DL2 arrangement in the layers of DL-glutamic acid.

of DL amino acids is in general slightly higher than that in the crystals of L amino acids. In all but one case, the unit cell volume per molecule is slightly lower in the former than in the latter. The percentage difference in this volume ranges between 0·1 and 5·9.

LL and LD complexes: There are 4 amino acid-amino acid complexes for which the LL and LD type crystal structures are available. They are lysine aspartate (Bhat and Vijayan, 1976; Soman *et al.,* 1988), arginine glutamate (Bhat and Vijayan, 1977; Suresh *et al.,* 1986), arginine aspartate (Salunke and Vijayan, 1982; Suresh *et al.,*

1986) and ornithine aspartate (Salunke and Vijayan, 1983; Soman and Vijayan, 1988). Among the LL complexes, L-ornithine L-aspartate hemihydrate cannot be explained in terms of layers. In the 3 remaining LL complexes, the crystal structures may be considered as made up of alternating layers of basic and acidic molecules. The molecules in the basic layer in L-lysine L-aspartate and in L-arginine L-aspartate have a S1Z2 arrangement which is similar to that found in the layers in crystals of most hydrophilic amino acids (figure 5). A Z2Z2 arrangement similar to that found in the crystals of L-serine· H_2O exists in the basic layer of L-arginine L-glutamate monohydrate. In the acidic layers in the LL complexes, head-to-tail sequences by themselves do not define a plane. Other interactions are also necessary for doing so. The aspartate layer in L-lysine L-aspartate and L-arginine L-aspartate contains just one Z1 type head-to-tail sequence parallel to the shortest repeat distance. The glutamate layer in L-arginine L-glutamate contains two Z 1 sequences parallel to the shortest repeat distance, but they also stabilise only a column of molecules. Other interactions are needed to define the layer.

The greatest differences between the LL and the corresponding LD complexes exist in the case of the complexes involving arginine. In L-arginine D-glutamate trihydrate and L-arginine D-glutamate monohydrate (reported in this paper) the molecules arrange themselves in double-layers in a fashion similar to that found in the structure of α-DL-methionine illustrated in figure 7. Incidentally, a similar arrangement exists in the structure of L-lysine D-glutamate also. However, as L-lysine L-glutamate could not be crystallised so far, this arrangement cannot be compared with that in the corresponding LL complex. An arrangement based on double-layers exists in the crystal structure of L-arginine D-aspartate also. Each layer in the double layer contains two S1, DL1 and DL2 sequences. In contrast to the situation encountered in L-arginine D-glutamate and L-arginine D-aspartate (and L-lysine D-glutamate), unlike molecules aggregate into separate alternating layers in the structure of L-lysine D-aspartate monohydrate. The arrangement of molecules in the lysine layer is the same as that found in L-lysine L-aspartate. The arrangement of molecules in the aspartate layer, however, is different from that in the LL complex. In fact no head-to-tail sequence exists in this layer. The crystal structure of L-ornithine D-aspartate monohydrate is also based on alternating layers. The arrangement in the ornithine layer is similar to that found in the lysine layers in the lysine aspartate complexes. However, the arrangement in the aspartate layer, which involves a S2 head-to-tail sequence, is different from any observed so far in crystal structures containing aspartic acid or aspartate ions. It must also be mentioned that the aggregation pattern in L-ornithine D-aspartate monohydrate bears no resemblance to that in the corresponding LL complex.

It is clear from the above discussion that the basic elements of molecular aggregation found in the complexes are by and large similar to those observed in the crystals of L and DL amino acids. However, the changes brought about in the nature and the distribution of these elements when going from LL to the corresponding LD complexes are often more drastic than the differences between the crystals of L amino acids and those of the corresponding DL amino acids. For example, the LL complexes involving arginine have alternating layers of unlike molecules as the basic pattern of aggregation whereas the corresponding LD complexes are made up of double-layers. In the case of lysine aspartate, both the LL and the LD complexes contain alternating layers of basic and acidic molecules.

The aggregation of molecules in the lysine layer is similar in the two complexes, but it is entirely different in the aspartate layer. As far as ornithine aspartate is concerned, the LL and the LD complexes have fundamentally different aggregation patterns. Thus while the reversal of chirality of half the molecules in crystals made up of amino acids of the same type leads only to comparatively small differences in the aggregation pattern in most cases, profound changes result when the chirality of one type of molecules is reversed in crystals composed of equal number of basic and acidic amino acid molecules. These changes could, however, be of many types.

Acknowledgement

The authors thank the SERC, Department of Science and Technology, New Delhi for the financial support.

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