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# X-ray Studies on Crystalline Complexes Involving Amino Acids and Peptides. XIII. Effect of Chirality on Molecular Aggregation: The Crystal Structures of L-Arginine D-Aspartate and L-Arginine D-Glutamate Trihydrate

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## Abstract

The crystal structures of (1) L-arginine D-asparate, $C_6H_{15}N_4O_2^+.C_4H_6NO_4^-$ [triclinic, $P\bar{1}$, $a=5{\cdot}239\,(1)$, $b=9{\cdot}544\,(1)$, $c=14{\cdot}064\,(2)$ Å, $\alpha=85{\cdot}58\,(1)$, $\beta=88{\cdot}73\,(1)$, $\gamma=84{\cdot}35\,(1)°$, $Z=2$] and (2) L-arginine D-glutamate trihydrate, $C_6H_{15}N_4O_2^+.C_5H_8NO_4^-.3H_2O$ [monoclinic, $P2_1$, $a=9{\cdot}968\,(2)$, $b=4{\cdot}652\,(1)$, $c=19{\cdot}930\,(2)$ Å, $\beta=101{\cdot}20\,(1)°$, $Z=2$] have been determined using direct methods. They have been refined to $R=0{\cdot}042$ and 0·048 for 2829 and 2035 unique reflections respectively [$I>2\sigma(I)$]. The conformations of the two arginine molecules in the aspartate complex are different from those observed so far in the crystal structures of arginine, its salts and complexes. In both complexes, the molecules are organized into double layers stacked along the longest axis. The core of each double layer consists of two parallel sheets made up of main-chain atoms, each involving both types of molecules. The hydrogen bonds within each sheet and those that interconnect the two sheets give rise to LL-, DD- and DL-type head-to-tail sequences. Adjacent double layers in (1) are held together by side-chain–side-chain interactions whereas those in (2) are interconnected through an extensive network of water molecules which interact with side-chain guanidyl and carboxylate groups. The aggregation pattern observed in the two LD complexes is fundamentally different from that found in the corresponding LL complexes.

## Introduction

We have been carrying out a programme of preparation and X-ray analysis of crystals involving amino acids and peptides complexed with themselves and with other molecules, in order to elucidate, at atomic resolution, the geometrical details of biologically important non-covalent interactions (Suresh & Vijayan, 1985*a*, and references therein). These studies have led to the identification of some specific interactions and characteristic interaction patterns (Sudhakar & Vijayan, 1980; Sudhakar, Bhat & Vijayan, 1980; Salunke & Vijayan, 1981, 1983, 1984; Vijayan, 1983). It has also been shown that the aggregation of amino acids and peptides in the solid state follows a few well defined patterns based primarily on 'head-to-tail' sequences (Suresh & Vijayan, 1983*a*, *b*, *c*; 1985*b*). It has been suggested that these patterns are of relevance to chemical evolution (Vijayan, 1980; Vijayan & Suresh, 1985).

The results outlined above have been obtained primarily from studies on complexes involving L amino acids only. It is of obvious interest to see if the features observed in them are retained when the complexes contain amino acids of opposite chirality. A systematic study of such complexes might be of relevance to chiral separation and selection during evolution. Apart from its possible evolutionary implications, the effect of chirality on molecular aggregation is of considerable intrinsic interest. Therefore, our studies of crystalline complexes has been extended to include those involving amino acids of opposite chirality as well. The crystal structures of two such complexes, the first of their kind to be prepared and analysed, are reported here.

## Experimental

Crystals of the complexes were grown using the vapour-diffusion technique from aqueous solutions of the respective amino acids (obtained commercially from Sigma Chemical Company, USA) in molar proportions. *n*-Propanol and acetone were used as precipitants for growing L-arginine D-aspartate (1) and L-arginine D-glutamate trihydrate (2). These precipitants were chosen as they were earlier used in the crystallization of the respective LL complexes (Salunke & Vijayan, 1982; Bhat & Vijayan, 1977). The densities of the crystals were measured by flotation in a mixture of benzene and carbon tetrachloride. Unit-cell dimensions were refined on a CAD-4 diffractometer (Cu $K\alpha$ radiation) using 25 reflections in each case [$\theta$ range 11·71 to 46·83° for (1) and 11·01 to 28·90° for (2)]. Intensity data were recorded on the same instrument at 293 K to a maximum Bragg angle of 75°. The data were corrected for Lorentz and polarization factors, and for absorption using *SHELX*76 (Sheldrick, 1976). Other experimental details are given in Table 1 along with additional crystal data and refinement parameters.

Both structures were solved by direct methods using the *MULTAN* system of programs (Germain, Main

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Table 1. *Additional crystal data, experimental information and refinement parameters.*

| | (1) | (2) |
|---|---|---|
| Chemical formula | $C_6H_{15}N_4O_2^+.C_4H_6NO_4^-$ | $C_6H_{15}N_4O_2^+.C_5H_8NO_4^-.3H_2O$ |
| $M_r$ | 307·3 | 375·3 |
| $V$(Å$^3$) | 697·6 (4) | 906·6 (4) |
| $D_m$ (Mg m$^{-3}$) | 1·45 (1) | 1·37 (1) |
| $D_x$ (Mg m$^{-3}$) | 1·46 | 1·38 |
| $\mu$(Cu $K\alpha$) mm$^{-1}$ | 1·04 | 0·92 |
| Crystal size (mm) | 0·80×0·28×0·13 | 0·45×0·80×0·13 |
| $\Delta I$ in standard reflections (%) | 3 | 3 |
| Total unique data | 2867 | 2104 |
| Unique data with $I>2\sigma(I)$ | 2829 | 2035 |
| Ranges of $h$ | 0 to 6 | 0 to 12 |
| $k$ | −11 to 11 | 0 to 5 |
| $l$ | −17 to 17 | −24 to 24 |
| Transmission factor | | |
| max. | 0·89 | 0·90 |
| min. | 0·62 | 0·67 |
| $R$ | 0·042 | 0·048 |
| $wR$ | 0·060 | 0·072 |
| $S$ | 0·492 | 0·559 |
| Maximum shift/$\sigma$ | 0·157 | 0·194 |
| $1/w$ | $1·31-0·155F+0·0217F^2$ | $1·13-0·115F+0·0215F^2$ |
| $(\rho)_{max}$(e Å$^{-3}$) | 0·54 | 0·30 |
| $(\rho)_{min}$(e Å$^{-3}$) | −0·34 | −0·33 |

& Woolfson, 1971). The solution of (2) was straightforward. In the case of (1), the $E$ map corresponding to the most probable set of phases revealed the positions of only 10 non-hydrogen atoms distributed in two fragments. The structure was built up from these fragments through the iterative application of the Karle recyling routine. The structures were refined (against $F$), non-hydrogen atoms anisotropically and hydrogen atoms isotropically, employing the block-diagonal least-squares method (9×9 and 4×4 matrices respectively for non-hydrogen and hydrogen atoms) using the locally modified version of a program originally written by Dr R. Shiono. Form factors were taken from Cromer & Waber (1965) (except hydrogen) and Stewart, Davidson & Simpson (1965). The final positional parameters in the two structures, along with the equivalent isotropic temperature factors (Hamilton, 1959) are given in Tables 2 and 3.*

## Results and discussion

Arginine molecules in both crystal structures exist as positively charged zwitterions with the $\alpha$-amino group and the side-chain guanidyl group protonated and the $\alpha$-carboxylate group deprotonated. Both the aspartic acid and the glutamic acid molecules are negatively charged with deprotonated side-chain carboxylate and $\alpha$-carboxylate groups and a protonated $\alpha$-amino group. The crystal structures of the two complexes are illustrated in Figs. 1 and 2. Tables 4 and 5 list the parameters of the hydrogen bonds.

* Lists of structure factors, anisotropic thermal parameters, hydrogen-atom parameters and bond lengths and angles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42814 (42 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Fractional atomic coordinates* (×10$^4$) *and equivalent isotropic thermal parameters* (Å$^2$) *for non-hydrogen atoms in* (1)

| | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| L-Arg I | | | | |
| N (1) | 3304 (4) | 10216 (2) | 1393 (1) | 2·20 (8) |
| O (1) | 229 (3) | 8214 (2) | 1364 (1) | 2·73 (7) |
| O (2) | 1930 (4) | 6908 (2) | 2605 (1) | 3·25 (8) |
| C (1) | 1802 (4) | 7971 (2) | 2013 (1) | 1·68 (7) |
| C (2) | 3805 (3) | 9010 (2) | 2122 (1) | 1·43 (7) |
| C (3) | 3736 (4) | 9510 (2) | 3131 (2) | 2·08 (8) |
| C (4) | 5943 (4) | 10328 (2) | 3378 (1) | 1·91 (8) |
| C (5) | 5992 (4) | 10506 (3) | 4450 (2) | 2·29 (9) |
| N (6) | 3708 (4) | 11376 (2) | 4739 (1) | 2·20 (7) |
| C (7) | 2833 (4) | 11409 (2) | 5632 (1) | 1·98 (8) |
| N (8) | 4010 (4) | 10618 (3) | 6344 (1) | 2·97 (9) |
| N (9) | 792 (5) | 12274 (3) | 5826 (2) | 3·16 (9) |
| D-Asp I | | | | |
| N (11) | 928 | 4739 | 1490 | 1·63 (6) |
| O (11) | −414 (3) | 2328 (2) | 890 (1) | 2·35 (6) |
| O (12) | −4552 (3) | 2744 (2) | 1296 (1) | 2·37 (6) |
| C (11) | −2252 (3) | 3034 (2) | 1248 (1) | 1·40 (7) |
| C (12) | −1819 (3) | 4451 (2) | 1641 (1) | 1·31 (7) |
| C (13) | −2599 (4) | 4539 (2) | 2687 (1) | 1·51 (7) |
| C (14) | −931 (4) | 3654 (2) | 3429 (1) | 1·85 (7) |
| O (15) | 994 (4) | 2905 (2) | 3171 (1) | 3·30 (8) |
| O (16) | −1676 (4) | 3761 (3) | 4277 (1) | 3·38 (8) |
| L-Arg II | | | | |
| N (21) | 2813 (4) | 3231 (2) | 9305 (1) | 2·07 (7) |
| O (21) | 4147 (3) | 5706 (2) | 9838 (1) | 2·21 (6) |
| O (22) | 8231 (3) | 5229 (2) | 9378 (1) | 2·29 (6) |
| C (21) | 5883 (4) | 5004 (2) | 9417 (1) | 1·54 (7) |
| C (22) | 5149 (4) | 3780 (2) | 8872 (2) | 1·89 (8) |
| C (23) | 4429 (4) | 4299 (3) | 7824 (2) | 2·35 (9) |
| C (24) | 6623 (4) | 4943 (3) | 7271 (2) | 2·77 (10) |
| C (25) | 6060 (5) | 5142 (3) | 6213 (2) | 2·84 (10) |
| N (26) | 3805 (4) | 6152 (2) | 6002 (1) | 2·48 (8) |
| C (27) | 2899 (4) | 6401 (2) | 5124 (1) | 2·15 (8) |
| N (28) | 3987 (5) | 5718 (3) | 4407 (1) | 3·15 (9) |
| N (29) | 892 (5) | 7346 (3) | 4956 (2) | 3·20 (10) |
| D-Asp II | | | | |
| N (31) | 145 (3) | 7825 (2) | 9413 (1) | 1·91 (7) |
| O (31) | 3001 (3) | 9993 (2) | 9420 (1) | 2·51 (6) |
| O (32) | 942 (4) | 11196 (2) | 8208 (1) | 2·92 (7) |
| C (31) | 1193 (4) | 10203 (2) | 8853 (1) | 1·65 (7) |
| C (32) | −946 (3) | 9204 (2) | 8969 (1) | 1·49 (7) |
| C (33) | −2312 (4) | 9047 (2) | 8052 (1) | 1·83 (8) |
| C (34) | −652 (4) | 8449 (2) | 7248 (1) | 1·85 (7) |
| O (35) | 1486 (3) | 7805 (2) | 7456 (1) | 2·66 (7) |
| O (36) | −1568 (4) | 8644 (3) | 6433 (1) | 3·57 (9) |

### *Molecular dimensions*

The bond lengths and angles in the structures are normal. The torsion angles that define the conformation of the molecule (IUPAC–IUB Commission on Biochemical Nomenclature, 1970) in the two structures are given in Table 6. The two crystallographically independent arginine molecules in the aspartate complex have substantially different conformations. Both side chains are appreciably folded. The chain is *trans* to the $\alpha$-carboxylate and *gauche* to the $\alpha$-amino group ($\chi^1\sim-60°$) in molecule I whereas it is *trans* to the $\alpha$-amino group and *gauche* to the $\alpha$-carboxylate group ($\chi^1\sim180°$) in molecule II. It is interesting to note that the variable torsion angles which define the conformation of the side chain ($\chi^2, \chi^3, \chi^4$), as distinct from the torsion angle that define its orientation with respect to the $\alpha$-amino and the $\alpha$-carboxylate groups ($\chi^1$), have equal magnitudes, but opposite signs. The two side chains are thus enantiomorphous to each other with, as will be

Table 3. *Fractional atomic coordinates* ($\times 10^5$) *and equivalent isotropic thermal parameters* ($Å^2$) *for non-hydrogen atoms in* (2)

| | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| L-Arg | | | | |
| N (1) | 32989 (14) | 26420 | 41803 (7) | 1·61 (5) |
| O (1) | 52914 (11) | 66982 (33) | 45108 (6) | 1·96 (5) |
| O (2) | 67956 (12) | 39202 (41) | 42242 (7) | 2·63 (5) |
| C (1) | 56093 (15) | 45831 (39) | 41894 (8) | 1·42 (5) |
| C (2) | 44355 (15) | 27700 (42) | 37915 (7) | 1·40 (5) |
| C (3) | 39626 (17) | 41335 (47) | 30925 (8) | 1·78 (5) |
| C (4) | 28928 (18) | 24252 (47) | 26077 (8) | 1·95 (6) |
| C (5) | 24178 (18) | 40800 (48) | 19465 (8) | 2·03 (6) |
| N (6) | 14169 (14) | 25159 (48) | 14484 (7) | 2·28 (5) |
| C (7) | 17248 (19) | 6659 (49) | 9934 (8) | 2·13 (7) |
| N (8) | 30134 (18) | 1317 (59) | 9494 (9) | 3·11 (7) |
| N (9) | 7265 (18) | −6574 (60) | 5690 (9) | 3·29 (7) |
| D-Glu | | | | |
| N (11) | 83685 (14) | −10175 (41) | 42557 (7) | 1·73 (5) |
| O (11) | 103530 (13) | −50271 (39) | 43773 (6) | 2·36 (5) |
| O (12) | 115904 (11) | −23100 (38) | 38012 (8) | 2·50 (5) |
| C (11) | 105087 (15) | −29697 (43) | 40019 (8) | 1·54 (5) |
| C (12) | 92459 (14) | −11188 (43) | 37281 (8) | 1·45 (5) |
| C (13) | 83684 (16) | −23568 (54) | 30719 (8) | 2·10 (6) |
| C (14) | 91075 (18) | −25742 (71) | 24724 (9) | 2·80 (7) |
| C (15) | 82199 (18) | −39295 (61) | 18409 (9) | 2·41 (7) |
| O (16) | 69822 (13) | −32469 (45) | 17004 (7) | 3·14 (6) |
| O (17) | 87628 (15) | −56624 (65) | 15001 (8) | 4·70 (9) |
| Water oxygens | | | | |
| *W*(1) | 54594 (19) | 72282 (44) | 3904 (8) | 3·77 (7) |
| *W*(2) | 78212 (17) | 12244 (55) | 3664 (8) | 3·82 (7) |
| *W*(3) | 54997 (16) | 16408 (55) | 18173 (11) | 4·48 (8) |

seen later, interesting consequences for the crystal packing. The arginine molecule, with its long and flexible side chain, exhibits considerable conformational variability, and 13 unique conformations have so far been observed in its crystal structures (Bhat & Vijayan, 1977; Salunke & Vijayan, 1982; Sudhakar & Vijayan, 1980; Suresh & Vijayan, 1983*b*). The two conformations observed in the present aspartate complex are different from all of these. The conformation

Table 4. *Hydrogen-bond parameters in structure* (1)

| *A*-H···*B* | *A*···*B*(Å) | H-*A*···*B*(°) | Symmetry of atom *B* |
|---|---|---|---|
| N(1)-H1(N1)···O(11) | 2·725 (2) | 32 (2) | $x, y+1, z$ |
| N(1)-H2(N1)···O(12) | 2·752 (3) | 23 (4) | $x+1, y+1, z$ |
| N(1)-H3(N1)···O(31) | 2·809 (3) | 8 (4) | $x, y, z-1$ |
| N(6)-H(N6)···O(15) | 2·872 (3) | 5 (2) | $x, y+1, z$ |
| N(8)-H1(N8)···O(36) | 2·837 (3) | 23 (3) | $x+1, y, z$ |
| N(8)-H2(N8)···O(32) | 3·103 (3) | 12 (2) | $x, y, z$ |
| N(9)-H1(N9)···O(16) | 2·778 (3) | 7 (3) | $x, y+1, z$ |
| N(11)-H1(N11)···O(21) | 2·983 (2) | 23 (2) | $x, y, z-1$ |
| N(11)-H2(N11)···O(2) | 2·789 (2) | 3 (2) | $x, y, z$ |
| N(11)-H3(N11)···O(12) | 2·910 (1) | 20 (1) | $x+1, y, z$ |
| N(21)-H1(N21)···O(22) | 2·920 (2) | 4 (2) | $x-1, y, z$ |
| N(21)-H2(N21)···O(32) | 2·831 (3) | 10 (2) | $x, y-1, z$ |
| N(21)-H3(N21)···O(12) | 3·132 (3) | 20 (2) | $x+1, y, z+1$ |
| N(26)-H(N26)···O(35) | 2·856 (3) | 9 (2) | $x, y, z$ |
| N(28)-H1(N28)···O(16) | 2·808 (3) | 9 (3) | $x+1, y, z$ |
| N(28)-H2(N28)···O(2) | 2·882 (3) | 12 (4) | $x, y, z$ |
| N(29)-H1(N29)···O(36) | 2·734 (3) | 13 (3) | $x, y, z$ |
| N(31)-H1(N31)···O(1) | 2·799 (3) | 11 (3) | $x, y, z+1$ |
| N(31)-H2(N31)···O(22) | 2·767 (2) | 13 (2) | $x-1, y, z$ |
| N(31)-H3(N31)···O(35) | 2·827 (3) | 31 (2) | $x, y, z$ |

Table 5. *Hydrogen-bond parameters in structure* (2)

| *A*-H···*B* | *A*···*B*(Å) | H-*A*···*B* (°) | Symmetry of atom *B* |
|---|---|---|---|
| N(1)-H1(N1)···O(12) | 2·913 (2) | 21 (3) | $x-1, y+1, z$ |
| N(1)-H2(N1)···O(12) | 2·877 (2) | 1 (2) | $x-1, y, z$ |
| N(1)-H3(N1)···O(1) | 2·748 (2) | 9 (2) | $-x+1, \frac{1}{2}+y-1, -z+1$ |
| N(6)-H(N6)···O(17) | 2·799 (2) | 3 (2) | $x-1, y+1, z$ |
| N(8)-H1(N8)···*W*(3) | 2·822 (2) | 13 (2) | $x, y, z$ |
| N(8)-H2(N8)···*W*(2) | 3·166 (3) | 27 (2) | $-x+1, \frac{1}{2}+y-1, -z$ |
| N(9)-H1(N9)···*W*(2) | 2·977 (3) | 12 (3) | $x-1, y, z$ |
| N(9)-H2(N9)···*W*(2) | 2·954 (3) | 19 (3) | $-x+1, \frac{1}{2}+y-1, -z$ |
| N(11)-H1(N11)···O(11) | 2·813 (2) | 15 (2) | $-x+2, \frac{1}{2}+y, -z+1$ |
| N(11)-H2(N11)···O(2) | 2·765 (3) | 22 (2) | $x, y, z$ |
| N(11)-H3(N11)···O(2) | 2·813 (3) | 7 (1) | $x, y-1, z$ |
| *W*(1)-H1(*W*1)···*W*(1) | 2·849 (3) | 3 (3) | $-x+1, \frac{1}{2}+y-1, -z$ |
| *W*(1)-H2(*W*1)···O(16) | 2·762 (2) | 11 (4) | $x, y+1, z$ |
| *W*(2)-H1(*W*2)···O(17) | 2·695 (3) | 12 (3) | $x, y+1, z$ |
| *W*(2)-H2(*W*2)···*W*(1) | 3·008 (3) | 3 (3) | $x, y-1, z$ |
| *W*(3)-H1(*W*3)···O(16) | 2·746 (3) | 10 (4) | $x, y, z$ |
| *W*(3)-H2(*W*3)···O(16) | 2·833 (3) | 10 (4) | $x, y+1, z$ |

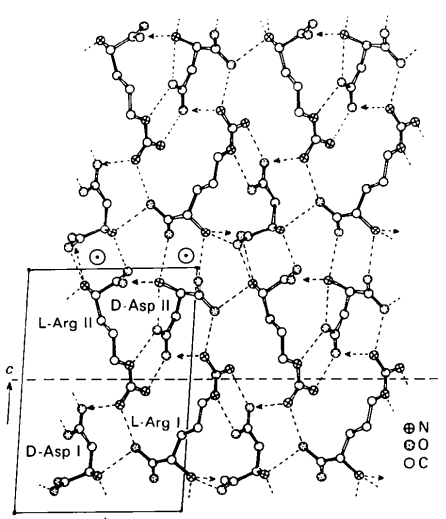


Fig. 1. Crystal structure of L-arginine D-aspartate viewed along **a***. Broken lines connecting atoms here and in Fig. 2 represent hydrogen bonds. Pseudo-inversion centres and the pseudo glide plane in the structure are indicated by dotted circles and a broken line parallel to **b**, respectively.

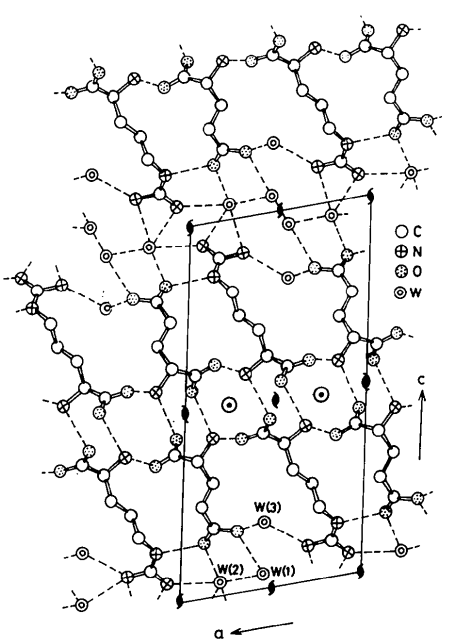


Fig. 2. Crystal structure of L-arginine D-glutamate trihydrate viewed along **b**. Dotted circles represent pseudo-inversion centres.

Table 6. *Torsion angles* (°) *in* L-*arginine* D-*aspartate and* L-*arginine* D-*glutamate trihydrate*

| | $\psi'$ | $\psi''$ | $\chi^1$ | $\chi^2/\chi^{21}$ | $\chi^3/\chi^{31}/\chi^{22}$ | $\chi^4/\chi^{32}$ | $\chi^{51}$ | $\chi^{52}$ |
|---|---|---|---|---|---|---|---|---|
| L-Arginine D-aspartate | | | | | | | | |
| L-Arg I | 2·8 (2) | −177·9 (2) | −69·9 (2) | −168·2 (2) | −65·0 (2) | 160·7 (2) | −0·5 (3) | 177·6 (2) |
| L-Arg II | −27·0 (3) | 154·5 (2) | 178·5 (2) | 167·8 (2) | 63·7 (3) | −175·5 (2) | 1·3 (3) | −178·4 (2) |
| D-Asp I | 1·0 (2) | −176·2 (1) | −53·0 (2) | 0·5 (3) | −179·5 (2) | | | |
| D-Asp II | −27·1 (2) | 154·9 (2) | −63·0 (2) | 17·9 (2) | −162·4 (2) | | | |
| L-Arginine D-glutamate trihydrate | | | | | | | | |
| L-Arg | −35·8 (2) | 148·9 (1) | −65·4 (2) | 175·8 (1) | 178·2 (2) | −87·3 (2) | −1·4 (3) | 179·3 (2) |
| D-Glu | 33·4 (2) | −149·2 (2) | 179·2 (2) | 177·6 (2) | 40·0 (3) | −139·4 (2) | | |

of arginine observed in the glutamate complex, however, is similar to that of one of the two crystallographically independent molecules in L-Arg.HCl.$H_2O$ (Dow, Jensen, Mazumdar, Srinivasan & Ramachandran, 1970) and L-Arg.HBr.$H_2O$ (Mazumdar & Srinivasan, 1966).

Both crystallographically independent aspartate ions in L-arginine D-aspartate assume the sterically least favourable conformation (Bhat, Sasisekharan & Vijayan, 1979) with the side-chain carboxylate group staggered between the $\alpha$-amino and the $\alpha$-carboxylate groups. L-Histidine L-aspartic acid monohydrate is the only other structure, other than metal complexes, in which such a sterically least favourable arrangement has been observed (Bhat & Vijayan, 1978). Although the two independent molecules have essentially the same conformations, there are some differences in the magnitudes of the torsion angles. These differences are such as to permit the formation of an internal hydrogen bond between the $\alpha$-amino group and the side-chain carboxylate in one, but not in the other (Table 5).

The glutamate ion in L-arginine D-glutamate trihydrate assumes the sterically most favourable conformation (Bhat, Sasisekharan & Vijayan, 1979) with an all-*trans* side chain *trans* to the $\alpha$-carboxylate group.

*Pseudosymmetry in the crystal structures*

The two complexes present interesting examples of local non-crystallographic symmetry. As indicated in Fig. 1, the main-chain atoms (the $\alpha$-carbon atom and the atoms belonging to the $\alpha$-amino and $\alpha$-carboxylate groups) in L-Arg I and those in D-Asp II in the aspartate complex are related by a local pseudo-inversion centre; so are the main-chain atoms in L-Arg II and D-Asp I. Also, a pseudo *b* glide nearly perpendicular to the *c* axis relates the side chains of the two sets of crystallographically independent molecules. The fact, noted earlier, that the side chains of chemically identical, but crystallographically independent, molecules in the structure are mirror images of each other, has been neatly made use of in crystal packing.

The glutamate complex contains only one set of amino acid molecules in the asymmetric unit. Thus, there is no scope for pseudosymmetry involving side chains. However, the main-chain atoms in L-arginine and those in D-glutamate are related by a pseudo-inversion centre at $x\sim\frac{1}{4}, y\sim\frac{1}{3}, z\sim\frac{1}{2}$, and its equivalents (Fig. 2). This inversion centre combined with the crystallographic $2_1$ screw axis leads to a pseudo *a* glide perpendicular to **b**. Thus, the structure has approximate $P2_1/a$ symmetry if only the main-chain atoms are considered.

*Double layers and head-to-tail sequences*

The aggregations of the amino acids in the two complexes exhibit remarkable similarities along with considerable differences in detail. In both cases, the crystal structure is made up of double layers, stacked along the longest crystallographic axis. The core of each double layer consists of two parallel sheets, each involving the main-chain atoms of both types of molecules. The two sheets are stabilized and are interconnected exclusively through interactions involving $\alpha$-amino and $\alpha$-carboxylate groups. Most of these interactions give rise to infinite head-to-tail sequences.

In the aspartate complex, L-Arg II and its equivalents generated by *a* translation give rise to a straight head-to-tail sequence of type *S*2 (Suresh & Vijayan, 1983*a*). The same is true for D-Asp I and its translation equivalents, but not for the two remaining molecules. However, if the main-chain atoms in L-Arg I and D-Asp I, and those in L-Arg II and D-Asp II are considered as structurally equivalent (Suresh & Vijayan, 1983*a*), two additional straight head-to-tail sequences of type DL1 and DL2 involving alternating L-arginine and D-aspartate are obtained. Thus each sheet in the arginine aspartate double layer contains one LL head-to-tail sequence, one DD head-to-tail sequence and two DL head-to-tail sequences. As can be seen from Fig. 1, the interactions between the two sheets mainly involve N–H···O hydrogen bonds between unlike molecules, whose main-chain atoms are related by pseudo-inversion centres. These interactions give rise to closed hydrogen-bonded loops involving $\alpha$-amino and $\alpha$-carboxylate groups. Thus, peptide-like and diketopiperazine-like arrangements coexist in each double layer.

Each sheet in the glutamate complex contains two DL head-to-tail sequences involving both types of molecules, if the main-chain atoms in the adjacent arginine molecules and glutamate ions are considered

to be structurally equivalent. Both are of type DL2. In addition, the interaction between the two sheets gives rise to an LL sequence containing arginine molecules and a DD sequence containing glutamate ions.

*Side-chain interactions*

In each double layer, the core of hydrogen-bonded $\alpha$-amino and $\alpha$-carboxylate groups is flanked by side chains which also interact among themselves. In the aspartate complex, the adjacent double layers are interconnected primarily through side-chain–side-chain interactions. The double layers in L-arginine D-glutamate trihydrate are interconnected through an extensive network of water molecules which interact with side-chain guanidyl and carboxylate groups.

In contrast to many other structures containing arginine (Salunke & Vijayan, 1981; Suresh & Vijayan, 1983*b*), the potential of the guanidyl groups to form specific interactions is not fully realized in the present complexes. While *a priori* each guanidyl group is capable of taking part in two specific interactions, here they are involved in only one specific interaction each. In the aspartate complex, each is involved in a type *B* specific interaction (Salunke & Vijayan, 1981) forming two parallel hydrogen bonds, to a side-chain carboxylate group. The guanidyl group in the glutamate complex takes part in a type *C* interaction involving two convergent hydrogen bonds, to a water molecule. It is interesting to note that the two terminal nitrogen atoms of the arginyl side chain in this complex interact with water molecules only.

*Water structure in the glutamate complex*

L-Arginine D-glutamate is the most heavily hydrated among the binary complexes of amino acids analysed so far. As in the dipeptide–dipeptide complex reported earlier (Suresh & Vijayan, 1985*a*), the number of water molecules per amino acid molecule in the glutamate complex is comparable to the number of ordered water molecules per amino acid residue observed in the crystal structures of lysozyme (Blake, Pulford & Artymiuk, 1983). Likewise the interaction patterns of water molecules in the complex are reminiscent of those observed in protein structures. Thus the water structure in the complex also appears to provide a model, at the atomic resolution, for that in proteins.

Of the three water molecules in the structure, $W(1)$ is involved in four hydrogen bonds, two as a donor and two as an acceptor. Of these four hydrogen bonds, only one involves an atom belonging to an amino acid molecule. $W(1)$ accepts a proton from one of its two screw-related neighbours while it donates a proton to the other. Thus $W(1)$ and its equivalents form a hydrogen-bonded zigzag arrangement parallel to the *b* axis. The fourth hydrogen bond involving $W(1)$ is with $W(2)$ which takes part in a total of five hydrogen bonds. $W(2)$ donates a proton each to $W(1)$ and O(15). It accepts three hydrogen bonds, all from guanidyl groups. Two of these form the specific interaction of type *C*, referred to earlier, and point to one of the lone pairs of the water oxygen while the remaining one points to the other lone pair. $W(3)$ takes part in only three hydrogen bonds, two as a donor and one as an acceptor, all to atoms of amino acid molecules.

In terms of the crystal structure, the role of $W(3)$ appears to be somewhat different from that of $W(1)$ and $W(2)$. $W(3)$ is involved in hydrogen bonds among amino acid molecules in the same layer, bridging the side chain of arginine to that of an adjacent glutamate. It also bridges adjacent glutamate side chains related by a *b* translation. $W(1)$ and $W(2)$, on the other hand, interconnect adjacent double layers.

Interestingly the main-chain atoms do not interact with water molecules. Among the eight crystallographically independent side-chain–water hydrogen bonds, four involve the side chain of arginine and the remaining four the side chain of the glutamate. Thus the two side chains share the water molecules equally and hence, on average, each side chain is associated with 1·5 water molecules, a number similar to the average number of water molecules associated with the arginyl and the glutamyl side chains in the crystal structures of lysozyme (Blake, Pulford & Artymiuk, 1983).

It is interesting to note that $W(1)$, $W(2)$ and their symmetry equivalents form an uninterrupted water sheet in the *ab* plane. Each such water sheet separates two adjacent double layers made up of amino acid molecules.

*Comparison with* LL *complexes*

The aggregation of molecules in the complexes of L-arginine with D-aspartic acid and D-glutamic acid, reported here, is fundamentally different from that in

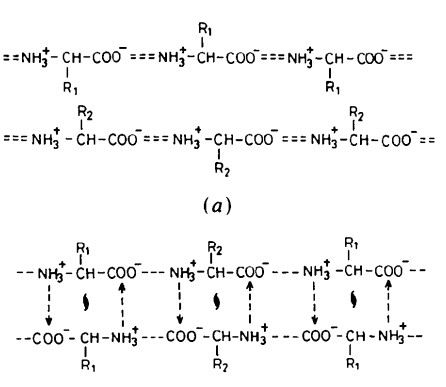

Fig. 3. Schematic representation of amino acid aggregation in the complexes of L-arginine with (*a*) L-glutamate and (*b*) D-glutamate.

its complexes with L-aspartic acid and L-glutamic acid (Salunke & Vijayan, 1982; Bhat & Vijayan, 1977). The difference is most striking in the case of the complexes with glutamic acid. A schematic representation of the complexes of L-arginine with L- and D-glutamic acid is given in Fig. 3. The unlike molecules aggregate into separate alternating layers in the LL complex, held together in head-to-tail sequences by hydrogen bonds. The molecules, however, form double layers in the LD complex, with each layer containing both types of molecules in LL and DL types of head-to-tail sequences. Similar differences are found between L-arginine L-aspartate and L-arginine D-aspartate. As indicated earlier, each LL complex and the corresponding LD complex were crystallized under identical conditions. Therefore, the differences between the LL and LD crystal structures are unlikely to have resulted from environmental effects. They must represent the different packing requirements arising out of the reversal in the chirality of one of the components.

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### References

BHAT, T. N., SASISEKHARAN, V. & VIJAYAN, M. (1979). *Int. J. Pept. Protein Res.* **13**, 170-184.
BHAT, T. N. & VIJAYAN, M. (1977). *Acta Cryst.* B**33**, 1754-1759.
BHAT, T. N. & VIJAYAN, M. (1978). *Acta Cryst.* B**34**, 2556-2565.
BLAKE, C. C. F., PULFORD, W. C. A. & ARTYMIUK, P. J. (1983). *J. Mol. Biol.* **167**, 693-723.
CROMER, D. T. & WABER, J. T. (1965). *Acta Cryst.* **18**, 104-109.
DOW, J., JENSEN, H., MAZUMDAR, S. K., SRINIVASAN, R. & RAMACHANDRAN, G. N. (1970). *Acta Cryst.* B**26**, 1662-1671.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368-376.
HAMILTON, W. C. (1959). *Acta Cryst.* **12**, 609-610.
IUPAC-IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1970). *J. Mol. Biol.* **52**, 1-17.
MAZUMDAR, S. K. & SRINIVASAN, R. (1966). *Z. Kristallogr.* **123**, 186-205.
SALUNKE, D. M. & VIJAYAN, M. (1981). *Int. J. Pept. Protein Res.* **18**, 348-351.
SALUNKE, D. M. & VIJAYAN, M. (1982). *Acta Cryst.* B**38**, 1328-1330.
SALUNKE, D. M. & VIJAYAN, M. (1983). *Int. J. Pept. Protein Res.* **22**, 154-160.
SALUNKE, D. M. & VIJAYAN, M. (1984). *Biochim. Biophys. Acta*, **798**, 175-179.
SHELDRICK, G. M. (1976). *SHELX*76. Program for crystal structure determination. Univ. of Cambridge, England.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175-3187.
SUDHAKAR, V., BHAT, T. N. & VIJAYAN, M. (1980). *Acta Cryst.* B**36**, 125-128.
SUDHAKAR, V. & VIJAYAN, M. (1980). *Acta Cryst.* B**36**, 120-125.
SURESH, C. G. & VIJAYAN, M. (1983*a*). *Int. J. Pept. Protein Res.* **22**, 129-143.
SURESH, C. G. & VIJAYAN, M. (1983*b*). *Int. J. Pept. Protein Res.* **21**, 223-226.
SURESH, C. G. & VIJAYAN, M. (1983*c*). *Int. J. Pept. Protein Res.* **22**, 617-621.
SURESH, C. G. & VIJAYAN, M. (1985*a*). *Int. J. Pept. Protein Res.* **26**, 329-336.
SURESH, C. G. & VIJAYAN, M. (1985*b*). *Int J. Pept. Protein Res.* **26**, 311-328.
VIJAYAN, M. (1980). *FEBS Lett.* **112**, 135-137.
VIJAYAN, M. (1983). *Conformation in Biology*, edited by R. SRINIVASAN & R. H. SARMA. New York: Adenine Press.
VIJAYAN, M. & SURESH, C. G. (1985). *Curr. Sci.* **54**, 771-780.

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# The $\alpha_2$-Adrenoceptor Agonists B-HT 920, B-HT 922 and B-HT 958,* a Comparative X-ray and Molecular-Mechanics Study

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### Abstract

The crystal structures of the $\alpha_2$-adrenoceptor agonists B-HT 920, B-HT 922 and B-HT 958 were determined by X-ray analysis. In addition, molecular-mechanics calculations were performed for B-HT 920 and B-HT 922 to reveal any conformational differences tentatively being responsible for the additional dopaminergic effects observed with B-HT 920. The X-ray studies showed that B-HT 920 and B-HT 922 prefer a chair conformation in the crystalline state,

* Compound codes, used particularly in the medicinal-chemistry and pharmacological literature instead of the full chemical names.
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