X-ray studies on crystalline complexes involving amino acids and peptides. Part XIV: Closed conformation and head-to-tail arrangement in a new crystal form of L-histidine L-aspartate monohydrate

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Abstract. A new form of L-histidine L-aspartate monohydrate crystallizes in space group P2$_1$ with $a = 5 \cdot 131(1)$, $b = 6 \cdot 881(1)$, $c = 18 \cdot 277(2)$ Å, $\beta = 97 \cdot 26(1)°$ and Z = 2. The structure has been solved by the direct methods and refined to an *R* value of 0·044 for 1377 observed reflections. Both the amino acid molecules in the complex assume the energetically least favourable allowed conformation with the side chains staggered between the α-amino and α-carboxylate groups. This results in characteristic distortions in some bond angles. The unlike molecules aggregate into alternating double layers with water molecules sandwiched between the two layers in the aspartate double layer. The molecules in each layer are arranged in a head-to-tail fashion. The aggregation pattern in the complex is fundamentally similar to that in other binary complexes involving commonly occurring L amino acids, although the molecules aggregate into single layers in them. The distribution of crystallographic (and local) symmetry elements in the old form of the complex is very different from that in the new form. So is the conformation of half the histidine molecules. Yet, the basic features of molecular aggregation, particularly the nature and the orientation of head-to-tail sequences, remain the same in both the forms. This supports the thesis that the characteristic aggregation patterns observed in crystal structures represent an intrinsic property of amino acid aggregation.

Keywords. Histidine aspartate complex; crystal structure; amino acid aggregation; amino acid conformation; chemical evolution.

Introduction.

We have been pursuing a programme of X-ray analysis of crystalline complexes involving amino acids and peptides, among themselves as well as with other molecules such as vitamins, in an attempt to elucidate, at atomic resolution, the geometrical details of possible biologically significant interactions important in the structure, assembly and function of proteins (Suresh and Vijayan, 1985b; Salunke and Vijayan, 1984; Suresh *et al.,* 1986). These studies have led to the identification of several specific interactions and characteristic interaction patterns (Salunke and Vijayan, 1983; Vijayan, 1983). They also resulted in a detailed understanding of different well defined patterns of amino acid and peptide aggregation (Suresh and Vijayan, 1983a,b,c, 1985a), in addition to providing useful information on the conformational variability of side chains (Bhat *et al.,* 1980; Bhat and Vijayan, 1977). These patterns have head-to-tail sequences in which the α- or the terminal amino and carboxylate groups are brought into periodic hydrogen-bonded proximity in a polypeptide-like arrangement, as their central feature. They have been suggested to be of probable relevance to chemical evolution (Vijayan, 1980; Vijayan and Suresh, 1985).

 The crystal structures analysed as part of the programme outlined above included that of a hydrated 1:1 complex between L-histidine and L-aspartic acid (Bhat and Vijayan, 1978). The X-ray analysis of this complex was however bedevilled by twinn-

ing, pseudo-symmetry and disorder in the crystals. Consequently, the structural parameters could be determined only inaccurately, leaving even the ionisation states of the two molecules in considerable doubt. We have recently obtained a new, less complicated, crystal form of the same complex in the course of crystallization experiments involving histidine and aspartic acid of different chiralities (Vijayan and Suresh, 1985). The X-ray analysis of this complex and the results obtained are presented here.

Experimental

The crystals of the complex were grown by the slow diffusion of ethanol into an aqueous solution of L-histidine and L-aspartic acid (Sigma Chemical Company, St. Louis, Missouri, USA) in molar proportions. Preliminary investigation showed the space group to be $P2_1$. Unit cell parameters were refined on a CAD-4 computer controlled diffractometer using CuKα radiation employing 25 reflections in the θ range 12–70° and are α = 5·131(1), b = 6·881(1), c = 18·277(2) Å and β = 97·26(1)°. The desnity D_m measured by the method of floatation in a mixture of carbon tetrachloride and benzene (1·57 Mg/m³) is comparable to that calculated (1·58 Mg/m³) for two molecules each of histidine, aspartic acid and water in the unit cell. Data were collected from a crystal of dimensions 0·225 × 0·725 × 0·075 mm³ to a maximum Bragg angle of 75° with h=0–6, k=0–8 and l=–22–22 on a CAD-4 diffractometer using ω/2θ scan and corrected for Lorentz and polarisation factors. Absorption corrections were applied using program SHELX (Sheldrick, 1976). The minimum and the maximum values of the transmission factor were 0·7892 and 0·9228, respectively. Intensities of okl and okl reflections yielded a merging R of 0·037.

The structure was solved using the direct methods program MULT AN (Germain *et al.,* 1971) and refined, using program SHELX, to a R factor 0·044 (WR = 0·050) for 1377 observed reflections with $I>2\sigma(I)$. Non hydrogen atoms were refined anisotropically and hydrogen atoms isotropically using form factors given in SHELX. Weights applied had the form $W= 1/(\sigma(F)^2 + 0.005681F^2)$. The negative and the positive features in the final difference Fourier map had maximum values of -0·30 and 0·33 eÅ⁻³ respectively. The coordinates and the equivalent isotropic temperature parameters (Hamilton, 1959) of the non-hydrogen atoms are listed in table 1*.

Results and discussion

Molecular dimensions

The amino and the imidazole groups in the structure are protonated and the carboxyl groups deprotonated. The zwitterionic histidine molecule thus carries a net positive charge and the aspartate ion a net negative charge. The torsion angles that

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*Anisotropic thermal parameters of non-hydrogen atoms, hydrogen atom parameters, and the observed and the calculated structure factors can be obtained from the authors on request.

Table 1. Positional parameters (10^4) and equivalent isotropic temperature factors for non-hydrogen atoms. Sigmas are given in parantheses.

Atom	X	Y	Z	Equivalent B
N(1)	1696(5)	2771(6)	2800(1)	1·55(5)
O(1)	−87(4)	4150(6)	4007(1)	2·68(6)
O(2)	−3775(4)	2402(6)	3868(1)	2·11(5)
C(1)	−1609(6)	2967(7)	3683(1)	1·55(6)
C(2)	−851(6)	2016(7)	2980(1)	1·33(6)
C(3)	−799(7)	−193(6)	3019(2)	1·65(7)
C(4)	1120(6)	−1041(7)	3625(2)	1·47(6)
N(5)	3169(6)	−2214(6)	3493(1)	1·88(6)
C(6)	4486(7)	−2775(7)	4128(2)	2·09(7)
N(7)	3361(6)	−1997(6)	4669(1)	2·05(6)
C(8)	1255(6)	−907(7)	4373(2)	1·91(7)
N(11)	2924(5)	−3895	1030(2)	1·60(6)
O(11)	5358(4)	−4257(6)	2394(1)	1·86(5)
O(12)	9360(4)	−3935(6)	2052(1)	1·87(5)
C(11)	6901(5)	−3870(6)	1945(1)	1·37(6)
C(12)	5745(5)	−3323(6)	1151(2)	1·18(6)
C(13)	6178(6)	−1220(7)	944(2)	1·56(6)
C(14)	4509(6)	375(7)	1256(?)	1·67(6)
O(15)	3075(7)	−49(7)	1730(2)	3·59(8)
O(16)	4697(7)	2055(6)	987(2)	3·47(8)
W	−839(6)	2759(7)	347(2)	2·93(7)

define the conformation of the molecules (IUPAC–IUB Commission on Biochemical Nomenclature, 1970) in the structure have the following values

Molecule	ψ'	ψ''	χ^1	χ^{21}	χ^{22}
L-His	-1·2(4)	179·5(3)	64·5(4)	-118·0(4)	63·5(6)
L-Asp	-15·3(4)	162·2(3)	50·5(4)	8·1(6)	-170·7(4).

It is remarkable that in both the molecules the side chain is *gauche* to the α-amino as well as the α-carboxylate groups. This is considered to be sterically the least favourable among the 3 possible staggered orientations of the side chain with respect to the main chain atoms (Bhat *et al.,* 1979). The occurrence of this conformation in the present crystal structure as well as in some others containing histidine or aspartic acid (Bhat and Vijayan, 1978; Suresh *et al.,* 1986) emphasizes the importance of intermolecular interactions in determining molecular conformation. The deviation of χ^{21} in histidine and other aromatic amino acids from the theoretically predicted value of ±90° (Ponnuswamy and Sasisekharan, 1971) in this and several other amino acid, peptide and protein structures (Bhat and Vijayan, 1978; Bhat *et al.,* 1979; Ramani and Boyd, 1981; Janin *et al.,* 1978; Benedetti *el al.,* 1983) is also presumably caused by intermolecular interactions.

The bond lengths and angles in the structure, shown in figure 1, are in general comparable to those in similar structures. An interesting feature in this context is the widening of the bond angle at C^β (C(2)–C(3)–C(4) and C(12)–C(13)–C(14)) in both the molecules. This is presumably caused by the steric interaction resulting from the energetically least favourable conformation of the molecules with the side chain staggered between the α-amino and the α-carboxylate groups. It may be noted that this feature has been observed in other structures also when the molecule assumes
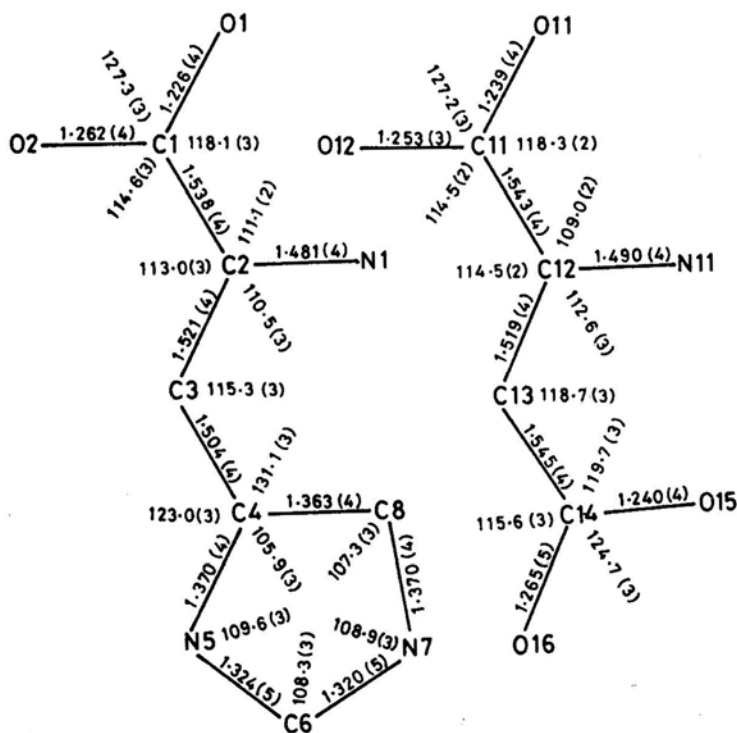
Figure 1. Bond lenguis (Å) and angles (°) involving non-hydrogen atoms. The estimated Standard deviations are given in parentheses.

this closed conformation (Suresh *et al.,* 1986; Oda and Koyama, 1972). Furthermore steric, and possibly electrostatic, interactions apparently leads to the enhancement of C(13)–C(14)–O(15) at the expense of C(13)–C(14)–O(16), a feature observed in the structure of L-arginine D-aspartate also in a similar situation (Suresh *et al.,* 1986). The present structure thus provides a good example of conformation dependent variation of bond angles. It may be mentioned in this context that distortion of bond angles in the α-carboxylate group caused by steric interaction is well known and has been noted as early as about two decades ago (Marsh and Donohue, 1967).

Crystal structure and hydrogen bonding

The crystal structure of the complex is given in figure 2. The parameters of the hydrogen bonds that stabilize the structure are listed in table 2. The two amino nitrogen atoms in the structure are involved in 3 hydrogen bonds each as donors. One of them involving N(l) is a bifurcated hydrogen bond. The nitrogen atoms in the imidazole ring are also involved in one hydrogen bond each as donors. The α-carboxylate oxygens O(2), O(11) and O(12) accept two hydrogen bonds each whereas O(1) accepts none. Among the side chain carboxylate oxygens, O(15) accepts one and O(16) three. The lone water molecule in the structure is a donor in two hydrogen bonds while it is an acceptor in one.
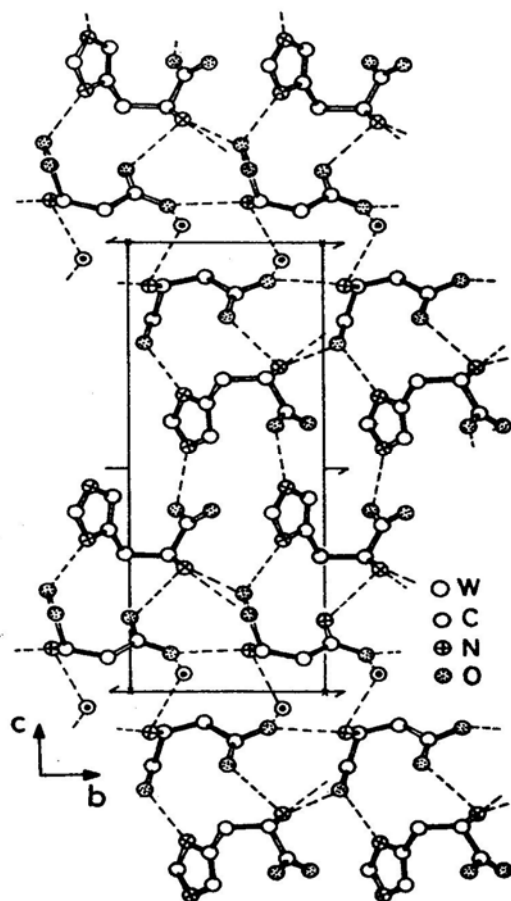
Figure 2. The crystal structure as viewed along *a*. The broken lines in this and the subsequent figures represent hydrogen bonds.

Table 2. Hydrogen bond parameters in the structure.

A–H . . . B	A . . . B(Å)	H–A . . . B(°)	Symmetry of atom B
N(1)–H1(N1) . . . O(2)	2·851(3)	9(4)	$x+1, y, z$
N(1)–H2(N1) . . . O(15)	2·906(5)	21(5)	x, y, z
N(1)–H3(N1) . . . O(12)	2·833(5)	26(5)	$x-1, y+1, z$
N(1)–H3(N1) . . . O(11)	2·935(5)	38(5)	$x, y+1, z$
N(5)–H(N5) . . . O(11)	2·800(4)	20(3)	x, y, z
N(7)–H(N7) . . . O(2)	2·688(3)	1(3)	$-x, \frac{1}{2}+y-1, -z+1$
N(11)–H1(N11) . . . O(12)	2·774(4)	26(3)	$x-1, y, z$
N(11)–H2(N11) . . . W	2·845(5)	6(3)	$-x, \frac{1}{2}+y-1, -z$
N(11)–H3(N11) . . . O(16)	2·936(4)	24(3)	$x, y-1, z$
W–H1(W) . . . O(16)	2·743(5)	8(7)	$x-1, y, z$
W–H2(W) . . . O(16)	2·972(5)	10(6)	x, y, z

Unlike molecules aggregate into alternating double layers in the structure. The water molecules are sandwiched between the layers in the aspartate double layer. A head-to-tail sequence (Suresh and Vijayan, 1983b) exists in each layer. The geometry of these sequences is illustrated in figure 3. Both sequences belong to type S2 (Suresh and Vijayan, 1983b). The two layers in each double layer are related by a 2_1 screw axis. In addition to head-to-tail sequences, the histidine double-layer is stabilized by a hydrogen-bond (and its symmetry equivalents) between the ε nitrogen atom in one layer and a carboxylate oxygen in the other. The two layers in the aspartate double-layer are bridged by water molecules each of which is hydrogen bonded to an amino nitrogen atom in one layer, and to a carboxylate oxygen and its *a* translation equivalent, in the other. Interactions between adjacent double-layers involve main chain as well as side chain atoms; the δ nitrogen atom of histidine is hydrogen bonded to an α-carboxylate oxygen atom of an aspartate ion whereas the α-nitrogen atom of histidine interacts with the α-carboxylate group of one aspartate ion and the side chain carboxylate group of another aspartate ion.
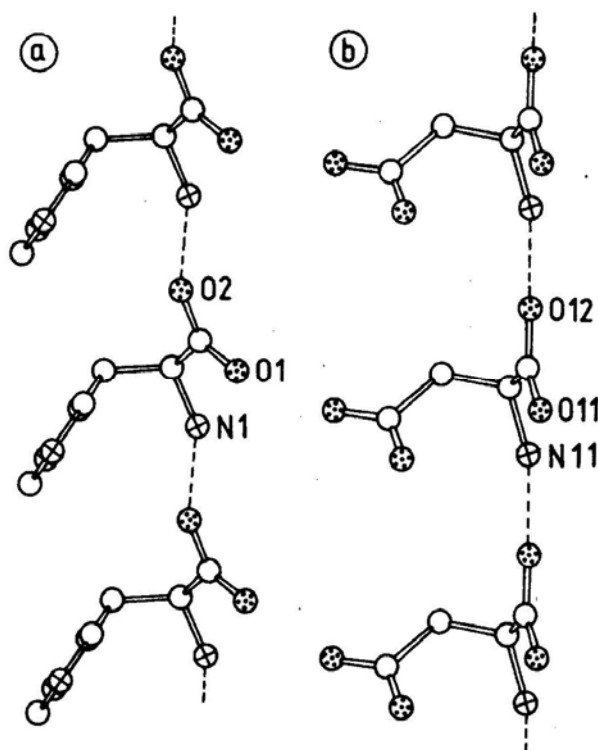

Figure 3. The head-to-tail sequence in (a) the histidine layer and (b) the aspartate layer.

It is instructive to compare the present structure with the complexes between common amino acids analysed so far. Whereas in all such LL complexes, *viz.,* L-lysine L-aspartate (Bhat arid Vijayan, 1976), L-arginine L-glutamate monohydrate (Bhat and Vijayan, 1977) and L-arginine L-aspartate (Salunke and Vijayan, 1982), the molecules aggregate into alternating layers, they aggregate into double layers in the present structure. The other amino acid complexes in which molecules aggregate

into double layers are those between amino acids of opposite chirality (LD complexes) such as L-arginine D-aspartate and L-arginine D-glutamate trihydrate (Suresh *et al.,* 1986). Despite this superficial similarity, the aggregation pattern in L-histidine L-aspartate monohydrate on the one hand and the LD complexes on the other are fundamentally different. In the former, each double layer consists of only one type of molecules whereas it contains both types in the latter. Secondly, and perhaps more importantly, in the LD complexes, the two sheets (one from each layer of the double layer) made up of main chain atoms and head-to-tail sequences, are at the core of the double layer such that they are extensively interconnected through hydrogen bonds. Thus, the arrangement is reminiscent of highly branched polypeptides. In the present structure, however, the adjacent sheets made up of main chain atoms are well separated within the double layer with no hydrogen bonded interactions between them, as is the general case in LL complexes. Thus, despite differences, the aggregation pattern in the present structure is fundamentally close to that in other LL complexes between common amino acids, although the number of head-to-tail sequences in the former is less than that in the latter.

Comparison between the two crystal forms

The unit cell dimensions *a, b, c* of the new crystal form and the dimensions a',' *b', c'* of the old form are approximately related by

$a = a'$
$b = c'/2$
and $c = b'/2$.

The cell volume of the old form is 4 times as much as that of the new form. Both the crystal forms are monoclinic. However, the unique axis *b'* in the old form corresponds to the largest repeat distance whereas the unique axis in the new form corresponds to the intermediate repeat distance. Also the monoclinic angle in the new form is 97° whereas it was 90° in the old form.

The old form, grown by acetone diffusion into an aqueous solution, contained 4 molecules of each type in the asymmetric unit. All the aspartate ions had nearly the same conformations with the side chain carboxylate group staggered between the α-amino and the α-carboxylate groups ($\chi_1 \sim 60_o$). Two of the histidine molecules had a closed geometry with the imidazole group *gauche* to the α -amino as well as the α-carboxylate groups ($\chi^1 \sim 60°$) whereas the imidazole group in the remaining two was *trans* to the α-amino group and *gauche* to the α-carboxylate group ($\chi^1 \sim 180°$). The main chain atoms in these two sets of histidine molecules were related by a pseudo *c'/*2 translation; this pseudo translation was almost exact for the aspartate ions and water molecules. Thus, half the molecules in the unit cell were related to the other half by a pseudo *c'/*2 translation. The two sets of molecules in each half were then related to each other by an almost exact local 2_1 screw axis parallel to *a'*. Furthermore, the crystals were twinned about the *a'* or the *c'* axis leading to an apparent orthorhombic symmetry for the diffraction pattern which also gave evidence for disorder in stacking along *b'*.

The present crystal form contains only one set of molecules in the asymmetric unit. There is no scope therefore for pseudo symmetry. The conformation of the

aspartate ions in the new form is similar to that of the aspartate ions in the old form. The histidine molecule in the former has a conformation similar to half the histidine molecules in the latter. The crystal structure of the new form presents a simpler picture devoid of pseudo translation, local 2_1 screw axes, twinning and disorder.

In spite of the differences between the two forms outlined above, there are remarkable similarities in molecular aggregation in them. In both the forms, unlike molecules aggregate into alternating double layers, with the water molecules sandwiched between the two layers in the aspartate double layer. The structure of the aspartate double layer, along with the water molecules, is nearly the same in the two forms. In the old form, the two layers in the histidine double layers are related to each other by local 2_1 screw axes parallel to a'; in the new form, however, they are related by a crystallographic 2_1 screw axis parallel to b. Consequently, in the old form the direction of the C^α—C^β bonds in one layer is opposite to that of the C^α–C^β bonds in the other; in the new form, the two directions are parallel to each other. Thus, unlike in the case of the aspartate double-layer, the structures of the histidine double-layer in the two forms are somewhat dissimilar. However, the interactions between the two double-layers are the same in the two forms. It also turns out that the same type of head-to-tail sequences exist in both the forms.

It is noteworthy that, despite the differences in the distribution of symmetry elements, the basic features of molecular aggregation in the two crystal forms are remarkably similar. In particular, the nature and the orientation of the head-to-tail sequences remain unaltered. This appears to further strengthen our thesis, developed in the earlier papers of this series, that the characteristic aggregation patterns observed in crystal structures represent an intrinsic property of amino acid aggregation.

Acknowledgement

References

Benedetti, E., Morelli, G., Nemethy, G. and Scheraga, H. A. (1983) *Int. J. Peptide Protein Res.s* **22,** 1.

Bhat, T. N., Sasisekharan, V. and Vijayan, M. (1979) *Int. J. Peptide Protein Res.,* **13,** 170.

Bhat, T. N., Sudhakar, V. and Vijayan, M. (1980) in *Biomolecular structure, conformation, function and evolution* (*ed.'R.* Srinivasan) (Oxford, New York: Pergamon Press) vol. 1, p. 633.

Bhat, T. N. and Vijayan, M. (1976) *Acta Crystallogr.,* **B32,** 891.

Bhat, T. N. and Vijayan, M. (1977) *Acta Crystallogr.,* **B33,** 1754.

Bhat, T. N. and Vijayan, M. (1978) *Acta Crystallogr.,* **B34,** 2556.

Germain, G., Main, P. and Woolfson, M. M. (4971) *Acta Crystallogr.,* **A27,** 368.

Hamilton, W. C. (1959) *Acta Crystallogr.,* **12,** 609.

IUPAC-IUB Commission on Biochemical Nomenclature (1970) *J. Mol. Biol.,* **52,** 1.

Janin, J., Wodak, S., Levitt, M. and Maigret, B. (1978) *J. Mol. Biol.,* **125,** 357.

Marsh, R. E. and Donohue, J. (1967) in *Advances in Protein Chemistry* (eds C.'B. Anfinsen Jr., M. L. Anson, J. T. Edsall and F. M. Richards) (New York, London: Academic Press) vol. **22,** p. 235.

Oda, K. and Koyama, H. (1972) *Acta Crystallogr.,* **B28,** 639.

Ponnuswamy, P. K. and Sasisekharan, V. (1971) *Int. J, Protein Res.,* **3,** 9.

Ramani, R. and Boyd, R. J. (1981) *Can. J. Chem.,* **59,** 3232.

Salunke, D. M. and Vijayan, M. (1982) *Acta Crystallogr.,* **B38,** 1328.

Salunke, D. M. and Vijayan, M. (1983) *Int. J. Peptide Protein Res.,* **22,** 154.

Salunke, D. M. and Vijayan, M. (1984) *Biochim. Biophys. Acta,* **798,** 175.

Sheldrick, G. M. (1976) *SHELX 76. Program for crystal structure determination and refinement,* University of Cambridge, England.

Suresh, C. G., Jayanthi Ramaswamy and Vijayan, M. (1986) *Acta Crystallogr.,* **B42,** 473.

Suresh, C. G. and Vijayan, M. (1983a) *Int. J. Peptide Protein Res.,* **21,** 223.

Suresh, C. G. and Vijayan, M. (1983b) *Int. J. Peptide Protein Res.,* **22,** 129.

Suresh, C. G. and Vijayan, M. (1983c) *Int. J. Peptide Protein Res.,* **22,** 617.

Suresh, C. G. and Vijayan, M. (1985a) *Int. J. Peptide Protein Res.,* **26,** 311.

Suresh, C. G. and Vijayan, M. (1985b) *Int. J. Peptide Protein Res.,* **26,** 329.

Vijayan, M. (1980) *FEBS Lett.,* **112,** 135.

Vijayan, M. (1983) in *Conformation in biology* (eds R. Srinivasan and R. H. Sarma) (New York: Adenine Press) p. 175.

Vijayan, M. and Suresh, C. G. (1985) *Curr. Sci.,* **54,** 771.