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X-ray Studies on Crystalline Complexes Involving Amino Acids and Peptides. XXX. Structural Invariance and Optical Resolution Through Interactions with an Achiral Molecule in the Histidine Complexes of Glycolic Acid

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Abstract

The crystals of DL-histidine glycolate and L-histidine glycolate were prepared and analysed as part of an ongoing programme aimed at studying biologically and evolutionarily important interaction and aggregation patterns. Crystallization experiments involving DL-histidine and glycolic acid yielded, in addition to DL-histidine glycolate, a conglomerate containing crystals of L-histidine glycolate and D-histidine glycolate in an unusual process of chiral separation through interaction with an achiral molecule. The crystal structure of DL-histidine glycolate is made up of alternating layers of unlike molecules as in many other binary complexes involving amino acids. The structure of L-histidine glycolate involves packing of columns containing L-histidine molecules and glycolate ions tightly hydrogen bonded to one another. The arrangement is almost identical to that in the structure of L-histidine acetate, thus providing another example for the invariance of certain aggregation patterns with respect to changes in the molecules involved. The observed aggregation of molecules in the chiral complex also appears to provide a structural rationale for chiral separation of histidine in the presence of glycolic acid.

1. Introduction

Non-covalent interactions are crucial to the structure, function and assembly of proteins. Our laboratory has been involved in the study of these interactions through the X-ray analysis of crystalline complexes of amino acids and peptides among themselves as well as with other molecules. These studies have not only given us a wealth of information regarding biologically relevant non-covalent interactions, but have also led to a detailed understanding of well defined patterns of amino acid and peptide aggregation (Vijayan, 1988; Suresh & Vijayan, 1983*b*, 1985). The aggregation and interaction patterns observed in these crystalline complexes have also been shown to have implications to prebiotic phenomena (Vijayan, 1980, 1988). The present focus of the programme is on complexes of amino acids with simple molecules that are believed to have existed on the primitive earth (Kvenvolden, Lawless & Ponnamperuma, 1971; Miller & Orgel, 1974). Complexes of this type analysed so far involve succinic, acetic and formic acids (Suresh & Vijayan, 1983*a*,*c*; Soman, Rao, Radhakrishnan & Vijayan, 1989; Prasad & Vijayan, 1990, 1991, 1993*a*,*b*; Suresh, Prasad & Vijayan, 1994; Suresh, Padmanabhan & Vijayan, 1994; Suresh & Vijayan, 1994). These analyses have provided useful information on the effect of simple carboxylic acids on amino acid aggregation, amino acid–carboxylic acid interactions and the structural behaviour of the two types of molecules in the presence of each other. In continuation of the study, here we report the crystal structures of the complexes of glycolic acid, the simplest possible hydroxy carboxylic acid with DL- and L-histidine.

2. Methods

The complexes of both L- and DL-histidine with glycolic acid were prepared by the slow diffusion of acetone into aqueous solutions of the components in molar proportions. While some of the crystallization experiments involving DL-histidine and glycolic acid yielded crystals of DL-histidine glycolate, the others yielded, in addition, a conglomerate of L-histidine glycolate and D-histidine glycolate crystals. Several single crystals were picked up and the X-ray diffraction pattern from each was examined photographically. Each crystal was then dissolved in water and the CD spectrum recorded. The spectra were compared with those obtained using the samples of DL-histidine, L-histidine and D-histidine. These experiments showed that the diffraction patterns of the L- and the D-histidine complexes were identical and also indistinguishable from that of the crystals of the complex obtained using optically pure L-histidine. The space groups and unit-cell dimensions of the crystals were determined by Weissenberg photography using nickel-filtered copper radiation and the densities were measured by flotation in mixtures of benzene and carbon tetrachloride. The cell parameters were subsequently refined on a CAD-4 diffractometer, which was also

Table 1. *Experimental details*

	DL-Histidine glycolate	L-Histidine glycolate
Crystal data		
Chemical formula	$C_6H_{10}N_3O_2^+.C_2H_3O_3^-$	$C_6H_{10}N_3O_2^+.C_2H_3O_3^-$
Chemical formula weight	231.21	231.21
Cell setting	Monoclinic	Orthorhombic
Space group	$P2_1/c$	$P2_12_12_1$
a (Å)	5.248 (1)	5.084 (1)
b (Å)	26.485 (4)	11.012 (1)
c (Å)	7.248 (1)	17.899 (3)
β (°)	95.47 (1)	
V (Å^3)	1002.9 (3)	1002.2 (3)
Z	4	4
D_x (Mg m^{-3})	1.531	1.532
D_m (Mg m^{-3})	1.53 (2)	1.54 (2)
Density measured by	Flotation in benzene and carbon tetrachloride	Flotation in benzene and carbon tetrachloride
Radiation type	Mo $K\alpha$	Mo $K\alpha$
Wavelength (Å)	0.71069	0.71069
No. of reflections for cell parameters	25	25
θ range (°)	5–16	5–16
μ (mm^{-1})	0.128	0.128
Temperature (K)	295	295
Crystal form	Chunk	Needle
Crystal size (mm)	0.53 × 0.50 × 0.40	0.58 × 0.25 × 0.13
Crystal colour	Colourless	Colourless
Data collection		
Diffractometer	CAD-4	CAD-4
Data collection method	$\omega/2\theta$ scans	$\omega/2\theta$ scans
Absorption correction	None	None
No. of measured reflections	2655	1432
No. of independent reflections	2411	1432
No. of observed reflections	2265	1358
Criterion for observed reflections	$F > 4\sigma(F)$	$F > 4\sigma(F)$
R_{int}	0.013	–
θ_{max} (°)	28	28
Range of h, k, l	$0 \rightarrow h \rightarrow 6$ $0 \rightarrow k \rightarrow 34$ $-9 \rightarrow l \rightarrow 9$	$0 \rightarrow h \rightarrow 6$ $0 \rightarrow k \rightarrow 14$ $0 \rightarrow l \rightarrow 23$
No. of standard reflections	3	3
Frequency of standard reflections (min)	60	60
Intensity decay (%)	5.1	3.1
Refinement		
Refinement on	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$	0.0368	0.0313
$wR(F^2)$	0.1023	0.0851
S	1.106	1.078
No. of reflections used in refinement	2411	1432
No. of parameters used	197	197
H-atom treatment	All H-atom parameters refined	All H-atom parameters refined
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0466P)^2 + 0.3925P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0561P)^2 + 0.1344P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.104	0.060
$\Delta\rho_{max}$ (e Å^{-3})	0.338	0.198
$\Delta\rho_{min}$ (e Å^{-3})	−0.195	−0.194
Extinction method	None	None
Source of atomic scattering factors	*International Tables for Crystallography* (1992, Vol. C)	*International Tables for Crystallography* (1992, Vol. C)

used to collect the intensity data. The crystal of the L-histidine complex used for data collection was obtained from solutions containing optically pure L-histidine. The experimental details and refinement parameters are given in Table 1.

The structures were solved using the direct methods program *SHELXS*86 (Sheldrick, 1985) and refined by the full-matrix least-squares method, employing a minimization procedure based on F^2 using the program *SHELXL*93 (Sheldrick, 1993). The H atoms were located from difference-Fourier maps using stereochemical considerations. Non-H atoms were refined anisotropically and H atoms isotropically. The positional parameters and equivalent isotropic thermal parameters of the non-H atoms in the two structures are given in Tables 2 and 3.*

* Lists of atomic coordinates, anisotropic displacement parameters and structure factors have been deposited with the IUCr (Reference: LI0226). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Fractional atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for* DL*-histidine glycolate*

$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$

	x	y	z	U_{eq}
N1	0.2408 (2)	0.6080 (1)	0.4420 (2)	0.025 (1)
O1	0.4927 (2)	0.6857 (1)	0.5971 (2)	0.038 (1)
O2	0.8285 (2)	0.6792 (1)	0.4298 (2)	0.036 (1)
C1	0.6118 (2)	0.6661 (1)	0.4772 (2)	0.025 (1)
C2	0.4944 (2)	0.6194 (1)	0.3759 (2)	0.022 (1)
C3	0.4742 (2)	0.6228 (1)	0.1649 (2)	0.026 (1)
C4	0.2872 (2)	0.6613 (1)	0.0842 (2)	0.023 (1)
N5	0.0871 (2)	0.6488 (1)	−0.0459 (1)	0.026 (1)
C6	−0.0501 (3)	0.6897 (1)	−0.0908 (2)	0.030 (1)
N7	0.0521 (2)	0.7283 (1)	0.0055 (2)	0.031 (1)
C8	0.2639 (3)	0.7115 (1)	0.1155 (2)	0.030 (1)
O11	0.1076 (2)	0.4363 (1)	0.2261 (2)	0.043 (1)
O12	0.1443 (2)	0.5191 (1)	0.2623 (2)	0.047 (1)
O13	−0.3903 (2)	0.4372 (1)	0.2169 (1)	0.029 (1)
C11	0.0153 (2)	0.4798 (1)	0.2405 (2)	0.028 (1)
C12	−0.2721 (2)	0.4852 (1)	0.2310 (2)	0.029 (1)

Table 3. *Fractional atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for* L*-histidine glycolate*

$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$

	x	y	z	U_{eq}
N1	0.0045 (3)	0.3058 (1)	0.4221 (1)	0.025 (1)
O1	0.3135 (3)	0.1663 (2)	0.3321 (1)	0.047 (1)
O2	0.2438 (3)	0.2899 (1)	0.2357 (1)	0.034 (1)
C1	0.1954 (3)	0.2490 (2)	0.2994 (1)	0.024 (1)
C2	−0.0454 (3)	0.3020 (1)	0.3397 (1)	0.021 (1)
C3	−0.1381 (3)	0.4257 (2)	0.3115 (1)	0.023 (1)
C4	0.0463 (3)	0.5282 (1)	0.3257 (1)	0.021 (1)
N5	0.0200 (3)	0.6058 (1)	0.3865 (1)	0.025 (1)
C6	0.2068 (4)	0.6900 (1)	0.3826 (1)	0.028 (1)
N7	0.3531 (3)	0.6700 (1)	0.3225 (1)	0.027 (1)
C8	0.2557 (3)	0.5694 (2)	0.2864 (1)	0.024 (1)
O11	0.5386 (3)	0.4103 (1)	0.4733 (1)	0.037 (1)
O12	0.6590 (3)	0.6015 (1)	0.4974 (1)	0.037 (1)
O13	0.1503 (3)	0.4133 (1)	0.5640 (1)	0.044 (1)
C11	0.5176 (4)	0.5100 (1)	0.5075 (1)	0.025 (1)
C12	0.2987 (4)	0.5205 (2)	0.5652 (1)	0.031 (1)

3. Results and discussion

3.1. *Molecular structure*

The histidine molecule is zwitterionic in both the structures, with protonated and positively charged α-amino and imidazole groups and deprotonated and negatively charged α-carbonyl groups. The asymmetric unit in both the structures contains a histidinium cation and a deprotonated and negatively charged glycolate anion (Fig. 1).

The bond lengths and angles in the two structures are normal. The histidine molecule adopts the sterically least favourable closed conformation, g^+, in both the structures (Bhat & Vijayan, 1978; Krause, Baures & Eggleston, 1991), with χ^1 = 58.2 and χ^{21} = −96.8° in L-histidine glycolate and χ^1 = 57.2 and χ^{21} = −123.5° in DL-histidine glycolate (IUPAC-IUB Commission on Biochemical Nomenclature, 1970). The glycolate ion is planar in both the structures, with the hydroxyl group lying in the plane defined by the other four non-H atoms.

3.2. *Crystal structure and hydrogen bonding*

The crystal structures of the DL- and L-histidine complexes are given in Figs. 2 and 3, respectively. The

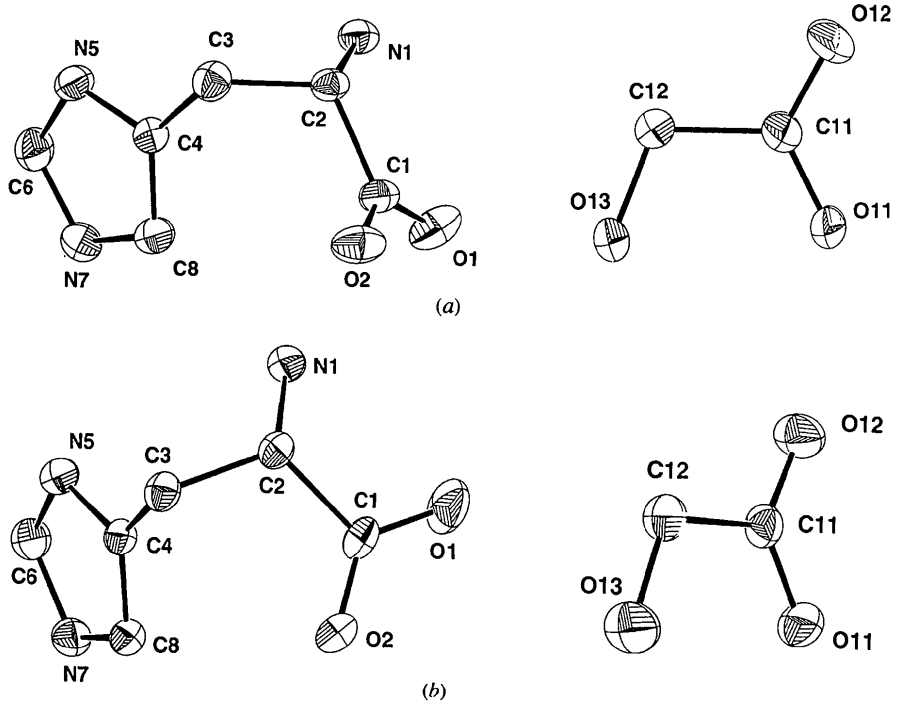

Fig. 1. Perspective view of the molecules in (*a*) DL-histidine glycolate and (*b*) L-histidine glycolate, drawn using *Xtal-GX* (Hall & du Boulay, 1995). The thermal vibration ellipsoids are given at the 50% probability level.

parameters of the hydrogen bonds that stabilize the structures are listed in Tables 4 and 5.

As in many other binary complexes involving amino acids (Vijayan, 1988; Soman, Rao, Radhakrishnan & Vijayan, 1989; Prasad & Vijayan, 1991; Suresh & Vijayan, 1994), the unlike molecules aggregate into separate alternating layers in DL-histidine glycolate. Two sets of hydrogen bonds stabilize the histidine layer parallel to the *ab* plane. One set gives rise to S2-type head-to-tail sequences (Suresh & Vijayan, 1983*b*) made up of a hydrogen bond between N1 of one molecule and O2 of a molecule related by an **a** translation, and its translation equivalents. The other set consists of N7···O2 hydrogen bonds involving glide-related molecules. The adjacent layers of histidine molecules are interconnected by glycolate ions which form columns parallel to **a**, stabilized by hydrogen bonds between the hydroxyl oxygen and a carboxylate oxygen in a translationally related ion. The columns in the glycolate layer do not interact among themselves; they are interconnected by histidine molecules. Histidine–glycolate interactions involve three hydrogen bonds, one between amino nitrogen N1 and carboxylate oxygen O12, another between N1 and the hydroxyl oxygen O13 and the third between the imidazole nitrogen N5 and the carboxylate oxygen O11.

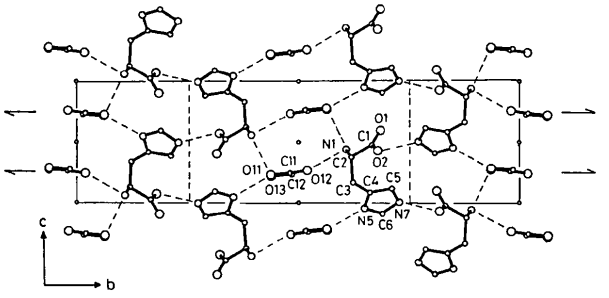

Fig. 2. Crystal structure of DL-histidine glycolate. In this and Figs. 3 and 4, C, N and O atoms are indicated by circles of increasing size and hydrogen bonds by broken lines. Figs. 2–4 were drawn using *PLUTO* (Motherwell & Clegg, 1978).

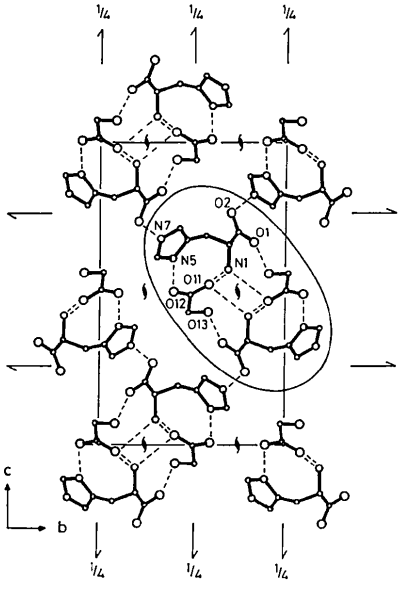

Fig. 3. Crystal structure of L-histidine glycolate. The closely packed column composed of histidine molecules and glycolate ions has been highlighted.

Table 4. *Hydrogen-bond parameters in DL-histidine glycolate*

A—H···*B*	*A*···*B* (Å)	*A*—H···*B* (°)
N1—H1N1···O13^{i}	2.792 (1)	156.6 (2)
N1—H2N1···O12^{ii}	2.715 (2)	163.4 (2)
N1—H3N1···O2^{iii}	2.866 (1)	162.6 (2)
N5—H2N5···O11^{iv}	2.751 (2)	164.9 (2)
N7—H2N7···O2^{v}	2.748 (2)	168.3 (2)
O13—H2O13···O11^{iii}	2.643 (1)	169.7 (2)

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

Table 5. *Hydrogen-bond parameters in L-histidine glycolate*

A—H···*B*	*A*···*B* (Å)	*A*—H···*B* (°)
N1—H1N1···O11^{i}	3.088 (2)	169.6 (2)
N1—H2N1···O11^{ii}	2.788 (2)	155.0 (2)
N1—H3N1···O11^{iii}	3.033 (2)	156.6 (2)
N5—H2N5···O12^{ii}	2.704 (2)	175.5 (2)
N7—H2N7···O2^{iv}	2.651 (2)	173.8 (3)
O13—H2O13···O1^{iii}	2.676 (2)	151.2 (3)

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

The aggregation pattern in the L-histidine complex is fundamentally different from that in the corresponding DL complex. As illustrated in Fig. 3, the basic element of aggregation in the former is a column made up of histidine molecules and glycolate ions centred around a 2_1 screw axis. Of a total of six crystallographically independent hydrogen bonds in the structure, five are used in stabilizing the column, within which the histidine molecules interact exclusively with glycolate ions. Likewise, the glycolate ions in the column (Fig. 4), as indeed in the structure as a whole, do not interact among

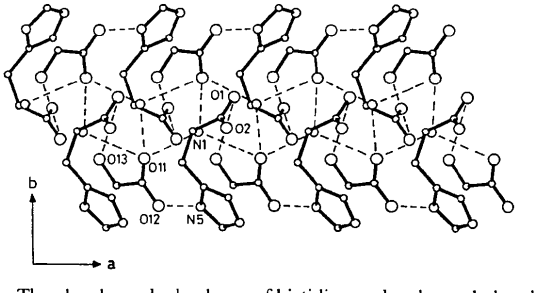

Fig. 4. The closely packed column of histidine molecules and glycolate ions in the crystal structure of L-histidine glycolate.

themselves; they interact through histidine molecules. The hydrogen bonds which stabilize the column include two involved in a specific interaction between N1 and N5 of histidine and the carboxylate group of the glycolate ion. As noted earlier (Suresh, Prasad & Vijayan, 1994), the formation of such a specific interaction is facilitated by a histidine conformation with $\chi^1 \simeq 60$ and $\chi^{21} \simeq -90°$, as in the present structure. The columns are held together in the crystal by a N7···O2 hydrogen bond and its symmetry equivalents. It is interesting to note that each column in the crystal is surrounded by six other columns, as is expected in a close-packed arrangement.

3.3. *Conserved structure*

The crystal structure of L-histidine glycolate is remarkably similar to that of one form of L-histidine acetate (Suresh, Prasad & Vijayan, 1994). The space groups of the two structures are the same and the cell parameters of the glycolate complex (Table 1) are very close to those of this form of L-histidine acetate. The conformations of the molecules in the two structures are also almost the same and the 15 non-H atoms common to the two structures can be superposed with a r.m.s. deviation of 0.153 Å. All the hydrogen bonds of L-histidine acetate are retained, while the extra hydroxyl group in the glycolate complex participates as a donor in another hydrogen bond with the α-carboxylate oxygen (O1) of histidine. Thus, the structure of L-histidine glycolate can tolerate the removal of the hydroxyl group, while at the same time preserving, with hardly any distortion, the aggregation pattern and crystal structure. The glycolate complex of DL-histidine could not be compared with the corresponding acetate complex as the structure of the latter is not available. However, it may be mentioned that the relative invariance of aggregation patterns with respect to changes in the size and nature of molecules present in the system is a recurring theme in the study of crystalline complexes involving amino acids (Suresh & Vijayan, 1983*b*; Soman, Rao, Radhakrishnan & Vijayan, 1989; Suresh & Vijayan, 1994). Such patterns involving hydrogen bonding have been a feature of aggregation of other organic molecules as well (Bernstein, Davis, Shimoni & Chang, 1995).

3.4. *Chiral separation*

As indicated earlier, an interesting result obtained during crystallization experiments was the separation of the L and D isomers of histidine from the racemate through interactions with glycolic acid. It has been shown that in the case of most amino acids, including histidine, the racemate is more stable than either of the chiral forms (Matsumoto & Amaya, 1980, 1983). Not surprisingly, chiral separation can be achieved through interactions with other chiral molecules (Vijayan, 1988; Jacques, Collet & Wilen, 1981; Kitaigorodsky, 1973). However, the optical resolution of DL-histidine in the form of L- and D-histidine glycolate provides a rare example, if not the only one, of chiral separation of amino acids through interactions with an achiral molecule. Therefore, an attempt is made here to find a structural rationale for this separation on the basis of the known structures of DL- and L-histidine glycolate.

The decrease in entropy accompanying chiral separation should be overcome by enthalpic factors for spontaneous optical resolution to occur (Jacques, Collet & Wilen, 1981). It has been suggested that compactness and symmetry tend to lower the free energy (Kitaigorodsky, 1973). In the present case, DL-histidine glycolate and L-histidine glycolate have the same degree of symmetry, with both having four equivalent units in the unit cell. The two structures have comparable densities (Table 1) and are, therefore, equally compact. The number of hydrogen bonds is also the same in the two structures. Therefore, the gross features of the two structures do not provide a ready explanation for the observed optical resolution.

The packing of molecules in L-histidine glycolate, however, appears to provide a possible rationale for chiral separation. It is characterized by the presence of columns involving both L-histidine molecules and glycolate ions. In fact, among the six hydrogen bonds in the structure, five are between unlike molecules within the column. Thus, the histidine–glycolate interactions are much stronger than histidine–histidine interactions, while glycolate–glycolate interactions are non-existent. This points to the high propensity for the occurrence of this type of aggregation involving chiral molecules in association with glycolate ions even in a solution containing molecules of both chiralities. Such aggregates might co-exist in high concentration with those involving both L- and D-histidine molecules and glycolate ions, despite the entropic advantages the latter might have. The crystals obtained from the solution could then be those of L-histidine glycolate, D-histidine glycolate or DL-histidine glycolate, often leading to chiral separation.

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References

Bernstein, J., Davis, R. E., Shimoni, L. & Chang N.-L. (1995). *Angew. Chem. Int. Ed. Engl.* **34**, 1555–1573.

Bhat, T. N. & Vijayan, M. (1978). *Acta Cryst.* B**34**, 2556–2565.

Hall, S. R. & du Boulay, D. (1995). *Xtal-GX. Crystallographic Package*. University of Western Australia, Australia.

IUPAC-IUB Commission on Biochemical Nomenclature (1970). *J. Mol. Biol.* **52**, 1–17.

Jacques, J., Collet, A. & Wilen, S. M. (1981). *Enantiomers, Racemates and Resolutions*. New York: John Wiley and Sons.

Kitaigorodsky, A. I. (1973). *Molecular Crystals*. New York: Academic Press.
Krause, J. A., Baures, P. W. & Eggleston, D. S. (1991). *Acta Cryst.* B**47**, 506–511.
Kvenvolden, K. A., Lawless, J. G. & Ponnamperuma, C. (1971). *Proc. Natl. Acad. Sci. USA*, **68**, 486–490.
Matsumoto, M. & Amaya, K. (1980). *Bull. Chem. Soc. Jpn*, **53**, 3510–3512.
Matsumoto, M. & Amaya, K. (1983). *Bull. Chem. Soc. Jpn*, **56**, 2521–2522.
Miller, S. L. & Orgel, L. E. (1974). *The Origins of Life on Earth*. Prentice-Hall, New Jersey.
Motherwell, W. D. S. & Clegg, W. (1978). *PLUTO. Program for Plotting Molecular and Crystal Structures*. University of Cambridge, England.
Prasad, G. S. & Vijayan, M. (1990). *Int. J. Peptide Protein Res.* **35**, 357–364.
Prasad, G. S. & Vijayan, M. (1991). *Acta Cryst.* B**47**, 927–935.
Prasad, G. S. & Vijayan, M. (1993*a*). *Acta Cryst.* B**49**, 348–356.
Prasad, G. S. & Vijayan, M. (1993*b*). *Biopolymers*, **33**, 283–292.
Sheldrick, G. M. (1985). *SHELXS86. Program for the Solution of Crystal Structures*. University of Göttingen, Germany.
Sheldrick, G. M. (1993). *SHELXL93. Program for the Refinement of Crystal Structures*. University of Göttingen, Germany.
Soman, J., Rao, T., Radhakrishnan, R. & Vijayan, M. (1989). *J. Biomol. Struct. Dyn.* **7**, 269–277.
Suresh, S., Padmanabhan, S. & Vijayan, M. (1994). *J. Biomol. Struct. Dyn.* **6**, 1425–1435.
Suresh, S., Prasad, G. S. & Vijayan, M. (1994). *Int. J. Peptide Protein Res.* **43**, 139–145.
Suresh, S. & Vijayan, M. (1994). *Acta Cryst.* B**51**, 353–358.
Suresh, C. G. & Vijayan, M. (1983*a*). *Int. J. Peptide Protein Res.* **21**, 223–226.
Suresh, C. G. & Vijayan, M. (1983*b*). *Int. J. Peptide Protein Res.* **22**, 129–143.
Suresh, C. G. & Vijayan, M. (1983*c*). *Int. J. Peptide Protein Res.* **22**, 617–621.
Suresh, C. G. & Vijayan, M. (1985). *Int. J. Peptide Protein Res.* **26**, 311–328.
Vijayan, M. (1980). *FEBS Lett.* **112**, 135–137.
Vijayan, M. (1988). *Prog. Biophys. Mol. Biol.* **52**, 71–99.