Centrosymmetric Crystals for the Direct Assignment of the Absolute Configuration of Chiral Molecules. Application to the α-Amino Acids by Their Effect on Glycine Crystals†

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Abstract: A new method for the direct assignment of the absolute configuration of chiral molecules is described. The method comprises precipitation of centrosymmetric crystals composed of chiral or prochiral molecules, in the presence of racemic or resolved chiral additives. The additives are occluded via the enantiotopic faces of the centrosymmetric crystals, resulting in changes in morphology and enantiomeric segregation of the additives within the crystal. The absolute configurations of the additives are deduced from these two effects. The structural requirements for host and additive molecules in centrosymmetric triclinic, monoclinic, and orthorhombic space groups are outlined. The method has been successfully applied for determination of the absolute configuration of the α-amino acids by their effect on the centrosymmetric monoclinic crystals of prochiral glycine. The crystal morphology of pure glycine and the changes in its habit are interpreted quantitatively in terms of atom–atom potential energy calculations.

The direct assignment of the absolute configuration of a chiral molecule in a chiral crystal that develops hemihedral faces has long challenged the chemical crystallographer. In 1949 Waser tried to establish the absolute configuration of D-tartaric acid by correlating the relative rates of growth of the hemihedral (hkl) and $(\bar{h}\bar{k}\bar{l})$ faces with the ease of attachment of the "free" molecule at either face in terms of intermolecular distances between the crystal and the to-be-attached molecule.[1] However, in terms of these criteria only, the ease of approach at either face is identical as was pointed out by Turner and Lonsdale[2] one year later. The difference in growth at the opposite hemihedral faces is due to solvent–surface interactions and the difference in structure (e.g., atomic polarizability and conformation) between the surface substrate molecules and the to-be-attached molecules,[3] interactions that are hard to determine on a quantitative level.

Recently we described a new method for the direct assignment of absolute configuration of chiral polar crystals based on changes in crystal habit induced by "tailor-made" additives,[4] whose molecular structures are similar to those of the corresponding substrate molecules comprising the crystals. We proved that the additive may be adsorbed only at those faces in which the part of the absorbate that differs from that of the substrate points away from the crystal interior. Once adsorbed, the additive inhibits the regular deposition of oncoming layers of substrate molecules, so slowing down the growth perpendicular to that face and leading to a concomitant relative increase in its surface area.[5-7]

The absolute configuration of the chiral substrate molecule is assigned by fixing its orientation with reference to the crystal polar axis. This orientation is deduced from differences in crystal habit of the substrate grown from solution in the presence and absence of the additives.

We extend this approach for direct determination of absolute configuration of the chiral additives through their effect on centrosymmetric racemic crystals.[8] We shall demonstrate that these crystals may be composed of prochiral (in dispersed phase) molecules.

Here we have exploited the centrosymmetric monoclinic form of glycine to directly assign the absolute configuration of each of 20 different α-amino acids by the changes they induce in the morphology of glycine crystals and by the enantiomeric segregation of occluded DL-amino acids. The interpretation of the results has been placed on a quantitative level by atom–atom potential energy calculations. We first outline the general crystal symmetry and structural principles on which the method is based.

Assignment of Absolute Configuration Using Centrosymmetric Crystals

Principles. In contrast to chiral crystals the orientations of the constituent molecules in centrosymmetric crystals are unambiguously assigned with respect to the crystal axes. This basic difference is illustrated in Figure 1. Figure 1b depicts a chiral molecule, of configuration R, in a chiral crystal, which is specified mathematically by a set of atomic coordinates x_i, y_i, z_i (i = 1, ..., n, for n atoms) in an axial system a, b, c chosen to be the right-handed one. The enantiomeric crystal structure (Figure 1a) comprising the molecule of configuration S is defined by a set of atoms with coordinates $-x_i$, $-y_i$, $-z_i$ in the original right-handed axial system. The two structures are related by an inversion through the origin. Unless the Bijvoet method[9] is applied to a given chiral crystal, X-ray analysis does not allow one to specify which of the two sets of atomic coordinates describe the actual structure and thus to assign the absolute configuration of the molecule. We note that interchanging the sites of the R and S molecules, i.e., transferring the R molecule at x_i, y_i, z_i (Figure 1b) to the S site would yield a set of atomic coordinates $-x'_i$, $-y'_i$, $-z'_i$ for R (Figure 1c) that cannot be made to coincide with $-x_i$,

† Dedicated to Prof. J. D. Dunitz on the occasion of his 60th birthday.

‡ This work is based, in part, on a doctoral thesis by Isabelle Weissbuch to be submitted to the Feinberg Graduate School.

(1) Waser, J. *J. Chem. Phys.* **1949**, *17*, 498.
(2) Turner, E. E; Lonsdale, K. *J. Chem Phys.* **1950**, *18*, 156.
(3) (a) Kern, R. *Bull. Soc. Fr. Mineral. Crystallogr.* **1953**, *76*, 391. (b) Wells, A. H. *Disc. Faraday Soc.* **1949**, *5*, 197.
(4) Berkovitch-Yellin, Z.; Addadi, L.; Idelson, M.; Leiserowitz, L.; Lahav, M. *Nature (London)* **1982**, *296*, 27.
(5) Addadi, L.; Berkovitch-Yellin, Z.; Domb, N.; Gati, E.; Lahav, M.; Leiserowitz, L. *Nature (London)* **1982**, 296, 21.
(6) (a) Berkovitch-Yellin, Z.; Addadi, L.; Idelson, M.; Lahav, M.; Leiserowitz, L. *Angew. Chem., Suppl.* **1982**, 1336. (b) Berkovitch-Yellin, Z.; Addadi, L.; Idelson, M.; Leiserowitz, L.; Lahav, M., submitted for publication.
(7) This mechanism was invoked by Smythe, Van Hook, and Mantovani to account for the changes in crystal habit induced on sucrose by other oligosaccharides. (a) Smythe, B. M. *Aust. J. Chem.* **1967**, *20*, 1115. (b) Van Hook, A. *Kristallogr. Akad. Nauk SSSR Inst. Krystollogr.* **1968**, *8*, 45. (c) Mantovani, G.; Gilli, G. and Fagioli, F. *C. R. Hebd. Seances Acad. Sci.* XIII Assembly CITS, **1967**, 289.
(8) For a preliminary report on this approach see: Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; Lahav, M.; Leiserowitz, L.; Weinstein, S. *J. Am. Chem. Soc.* **1982**, *104*, 2075.
(9) For a comprehensive discussion of direct methods for the assignement of absolute configuration of chiral molecules see: Dunitz, D. J. "X-ray Analysis and the Structure of Molecules"; Cornell University Press: Ithaca, NY, **1979**; pp 129–148. Mason, S. F. "Molecular Optical Activity and the Chiral Discrimination"; Cambridge University Press: Cambridge, England, 1982.

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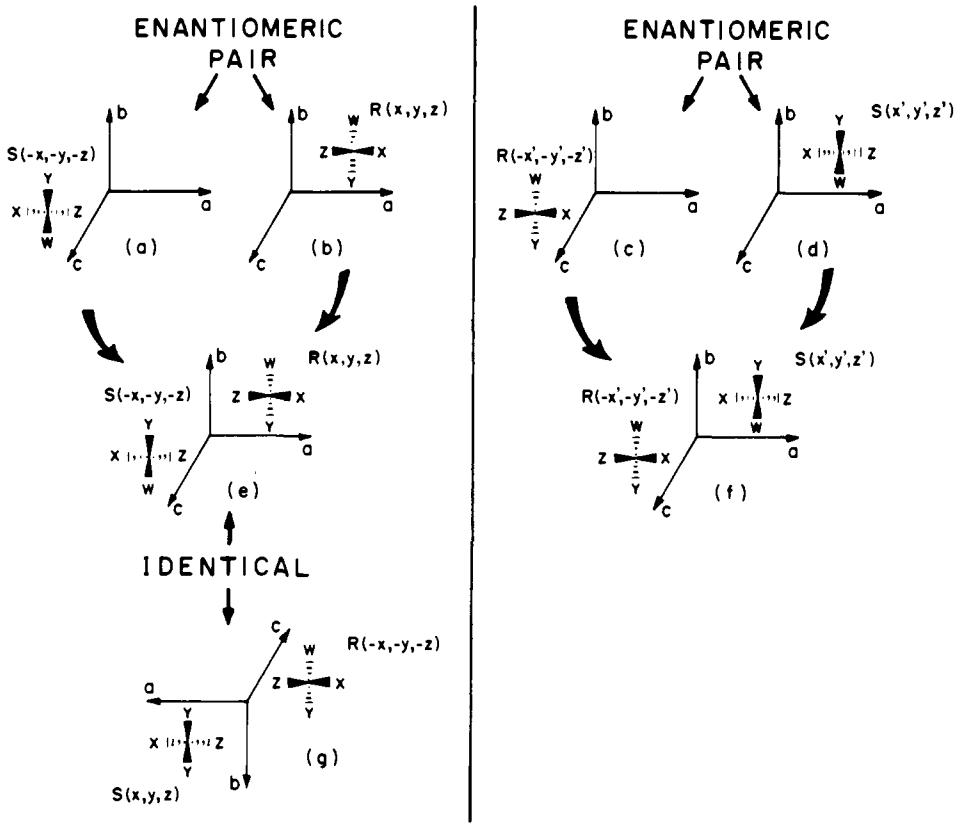


Figure 1. (a) A given chiral S molecule (atomic coordinates $-x_i, -y_i, -z_i$) situated with respect to the right-handed axial system a, b, c. (b) The arrangement enantiomeric to (a) specifying the position and orientation of the R molecule (atomic coordinates x_i, y_i, z_i) with respect to the same axial system as in (a). (c) The $R(x_i, y_i, z_i)$ molecule of (b) transferred to the site of the $S(-x_i, -y_i, -z_i)$ molecule to yield the diastereomeric structure $R(-x'_i, -y'_i, -z'_i)$. (d) The $S(-x_i, -y_i, -z_i)$ molecule of (a) transferred to site at $R(x_i, y_i, z_i)$ to yield the diastereomeric structure $S(x'_i, y'_i, z'_i)$. (e) Centrosymmetric crystal incorporating both molecules $R(x_i, y_i, z_i)$ and $S(-x_i, -y_i, -z_i)$ related to each other by a center of inversion at the origin in the right-handed axial system. (f) Centrosymmetric crystal incorporating both molecules $R(-x'_i, -y'_i, -z'_i)$ and $S(x'_i, y'_i, z'_i)$ diastereomeric to (e). (g) Centrosymmetric crystal of (e) referred to the left-handed axial system a, b, c, obtained by reversing the directions of the axes a, b, c in (e). Molecule R now is specified by $(-x_i, -y_i, -z_i)$ and S by (x_i, y_i, z_i).

$-y_i, -z_i$. This would lead to different (diastereomeric) arrangements that *can* be differentiated by X-ray analysis.

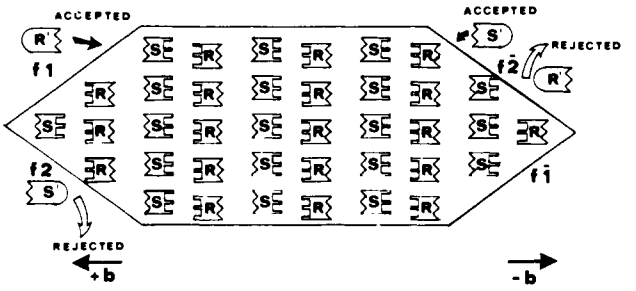
A centrosymmetric crystal structure contains both sets of atoms with coordinates x_i, y_i, z_i and $-x_i$, $-y_i$, $-z_i$, which represent molecules R and S, respectively (Figure 1e), so that their orientations and locations with respect to the right-handed axial system are unambiguously known. We note that, again, interchanging the sites of the R and S molecules results in a diastereomeric structure shown in Figure 1f.

We may describe the centrosymmetric structure in Figure 1e in terms of the alternative, left-handed axial system by choosing the reverse vectors $-a$, $-b$, $-c$ as the axial set as shown in Figure 1g. The whole arrangement remains unchanged, the only difference being that the molecules S and R are now described by coordinate sets x_i, y_i, z_i and $-x_i$, $-y_i$, $-z_i$, respectively.

The known orientation of the two enantiomers in a centrosymmetric crystal can be directly exploited for the assignment of absolute configuration of chiral resolved molecules provided the structural information embedded in the racemic crystal is transferred to a chiral additive molecule. The direct assignment of the absolute configuration of such resolved additives may thus be determined through the morphological changes they induce selectively on the enantiotopic faces of centrosymmetric crystals with appropriate packing features.

Effect of Additives on Enantiotopic Faces. A prerequisite for application of this method is that within the centrosymmetric racemic crystal a specific functional group attached to an R molecule (Scheme I) points toward faces f1 and f2 but not toward $f\bar{1}$ and $f\bar{2}$. By symmetry the same functional group attached to an S molecule will emerge at the enantiotopic faces $f\bar{1}$ and $f\bar{2}$ but not at f1 and f2. It is useful here to regard centrosymmetric crystals containing chiral molecules as enantiopolar, comprising two enantiomeric sets of intermeshed polar crystal structures

Scheme I



related to each other by a center of inversion.

Let us consider the crystallization of a racemate of this type, in the presence of a chiral additive R', appropriately designed so that it will fit in the site of an R molecule on the growing crystal faces f1 or f2 (Scheme I) but not at the enantiotopic faces $f\bar{1}$ or $f\bar{2}$. On the basis of the already well-established mechanism of binding and retardation of growth,[4-7] this adsorbed molecule will hinder growth along the $+b$ direction but not along $-b$. It is therefore expected that either the areas of the f1 and f2 faces will increase relative to those of the enantiotopic faces or new faces will develop on the $+b$ side of the crystal. By virtue of symmetry, additive S' will inhibit growth of faces $f\bar{1}$ and $f\bar{2}$, while racemic R',S' will inhibit growth along both directions $+b$ and $-b$. From the morphological changes induced on the R,S crystal coupled with the knowledge of the orientation of the molecules inside the crystal relative to the faces, it is possible to assign the absolute configuration of the additive.

Orientation of Additive and Crystal Symmetry. In principle this method is applicable to any centrosymmetric crystal. In practice, however, triclinic and monoclinic crystals are the most easily

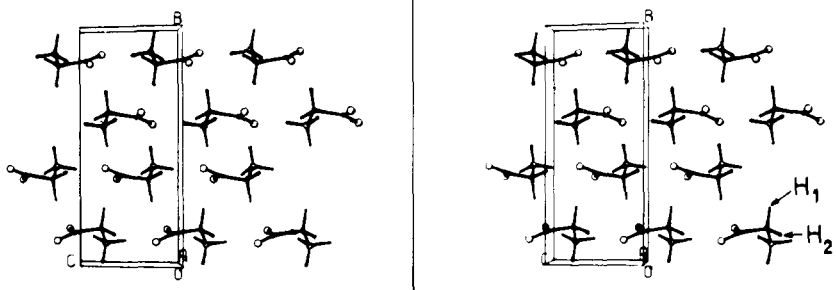
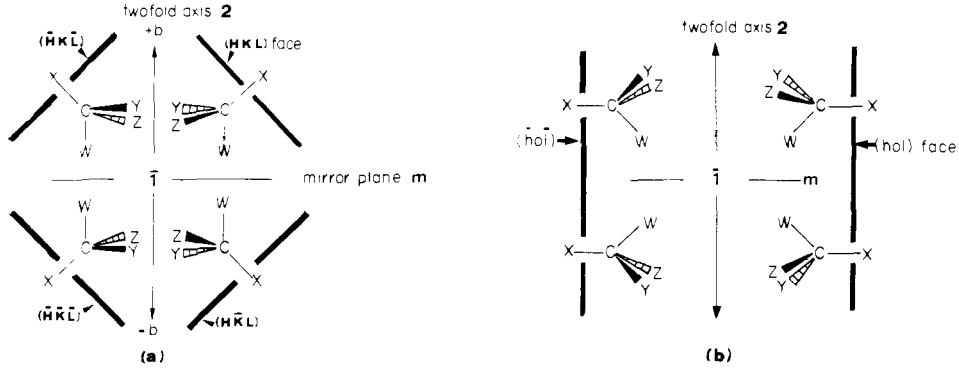


Figure 2. Packing arrangement of α-glycine viewed along the *a* axis. The methylene H atoms are labeled.

Scheme II



amenable to experimental analysis. Centrosymmetric monoclinic crystal structures that exhibit point symmetry $2/m$ in which the twofold axis is parallel to *b* (Scheme II) are appropriate provided the group X, which will be replaced by the additive, emerges from a to-be-affected face (*hkl*), where *k* is nonzero, as illustrated in Scheme IIa. As shown, the faces related to each other by the twofold axis 2 [(*hkl*) and ($\bar{h}\bar{k}l$); ($h\bar{k}l$) and ($\bar{h}k\bar{l}$)] are homotopic; those related by *m* symmetry [e.g., (*hkl*) and ($h\bar{k}l$)] or by a center of symmetry $\bar{1}$ [e.g., (*hkl*) and ($\bar{h}\bar{k}\bar{l}$)] are enantiotopic.[10] Faces for which $k = 0$, i.e., (*h*0*l*) and ($\bar{h}0\bar{l}$), are related both by a center of inversion and by a 2 axis and are thus both enantiotopic and homotopic (Scheme IIb); being identical, these faces would be affected by the resolved chiral additive to the same extent. For the condition to be well met, that the to-be-affected faces be (*hkl*), $k \neq 0$, the functional group of the substrate to be modified, C–X, must be aligned with a pronounced component along the enantiopolar *b* axis (Scheme IIa). Were C–X aligned approximately perpendicular to the *b* axis (Scheme IIb), then the to-be-affected faces would be of the type (*h*0*l*), which is not appropriate.

Centrosymmetric orthorhombic crystals (point symmetry $2/m$ $2/m$ $2/m$) would appear to be appropriate provided the group X emerges at faces of the type (*hkl*) where *h*, *k*, and *l* are all nonzero, because only these faces form enantiotopic pairs, in which they are not related by twofold symmetry. Consequently C–X should be preferentially directed close to a body diagonal of the unit cell (i.e., $a + b + c$), to induce change or formation of new faces (*hkl*), with $h, k, l \neq 0$.

Noncentrosymmetric achiral crystals may also be exploited for the determination of absolute configuration. The symmetry aspects of the monoclinic and orthorhombic point groups are discussed in the Appendix.

The method can be applied as well to centrosymmetric crystals, composed of prochiral molecules $C(X_2YZ)$ [i.e., X = W in Scheme IIa], but with additional limitations regarding the orientations of the two C–X bonds with respect to the enantiopolar axis. These limitations will be clarified in the following application to glycine (X = H).

(10) We are using the terms enantiotopic and homotopic, coined by: Mislow, K.; Raban, M. In "Topics in Stereochemistry"; Allinger, N., Eliel, E. Eds.; Wiley, New York, 1967; Vol. 1, pp 1.

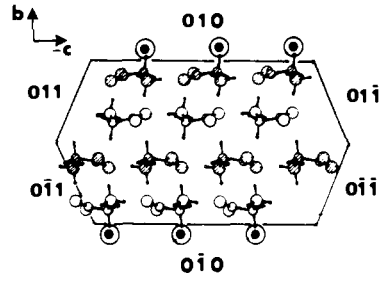


Figure 3. Juxtaposed molecular layers of glycine parallel to the *ac* plane, viewed along *a*. α-Amino acid additives are inserted on (010) and (0$\bar{1}$0) surfaces. At the (010) surface the amino acid residue replaces H1 of glycine (see Figure 2) and so is an additive of *R* configuration.

Interactions between α-Amino Acids and Glycine Crystals

Change in Crystal Habit of Glycine. The stable α-form of glycine[11] is obtained from aqueous solution. Its cell constants are $a = 5.1$, $b = 12.0$, $c = 5.4$ Å, $\beta = 111.6°$, $P2_1/n$, $Z = 4$. The molecules form hydrogen-bonded layers parallel to the *ac* plane, within which molecules are related to each other by translation symmetry only (Figure 2). Each layer is interlinked with its neighbor on one side by N–H··O hydrogen bonds through centers of symmetry and by C–H··O contacts across the *n* glide to its neighbor on the other side. Consequently, juxtaposed layers are related to each other by $\bar{1}$ or glide symmetry and alternate layers by twofold screw symmetry. Of primary relevance to this work is the fact that in the crystal a glycine molecule adopts a conformation such that, of its two C–H bonds (H1–C–H2), the C–H1 bond, which forms the C–H··O contact, is parallel to the *b* axis, whereas the C–H2 bond lies almost in the *ac* plane (Figure 2).

The importance of the molecular conformation and orientation of glycine with respect to the *b* axis is apparent from Figure 3. Here are shown two bounding, (010) and (0$\bar{1}$0), crystal faces. The

(11) Legros, J. P.; Kvick, A. *Acta Crystallogr., Sect. B* **1980**, *B36*, 3052.

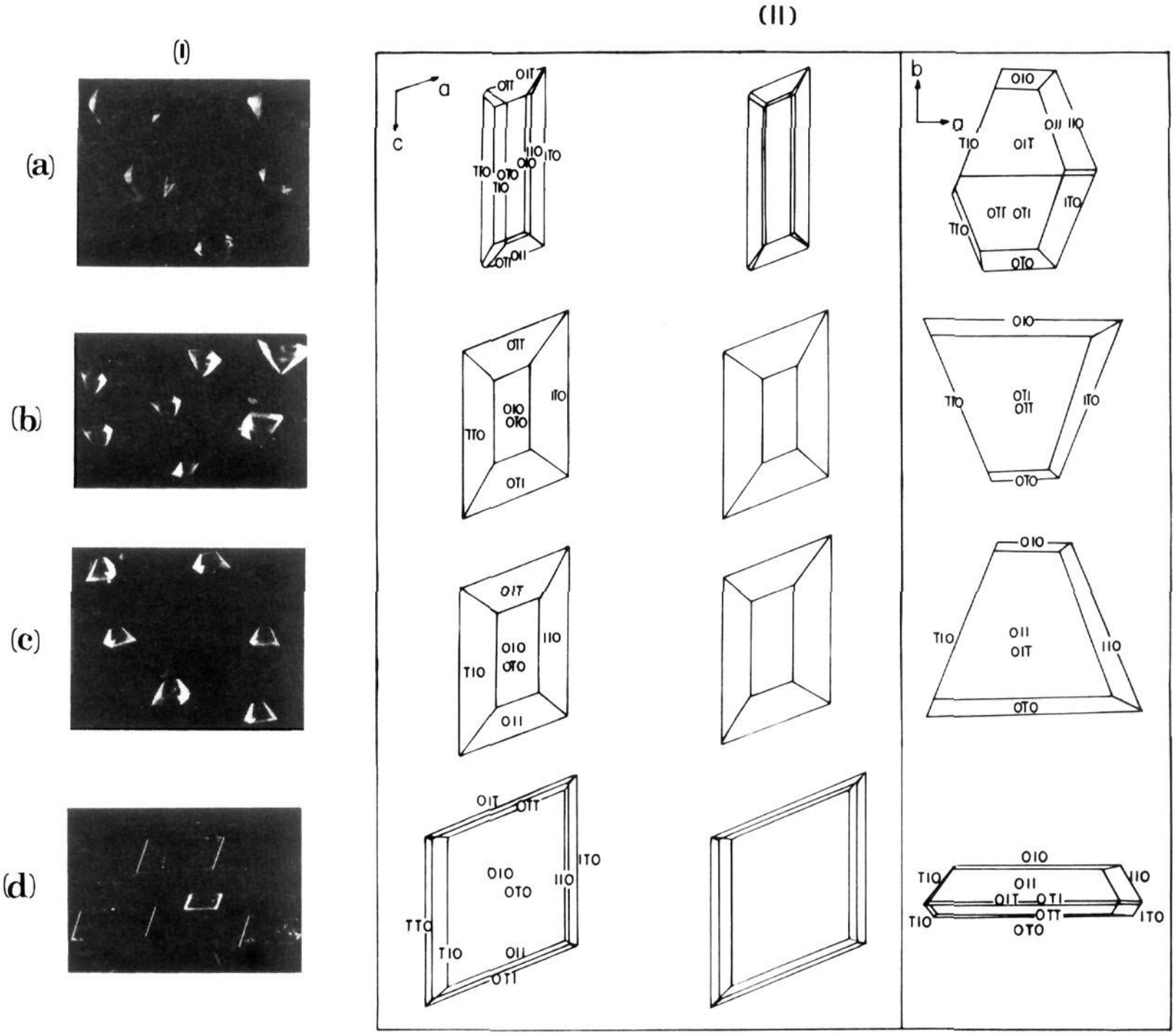


Figure 4. *Comparison between crystals of glycine grown in the presence of additives: (a) pure; (b) + R additive; (c) + S additive; (d) + racemic additive.* (I) Photographs of crystals. (II) Computer-drawn stereo- and monographic pictures of the crystals viewed along two axes. The ratio of crystal dimensions along *a* and *c* in the affected crystals [i.e., (b), (c), (d)] may vary with conditions of crystallization.

exposed molecules at either face may belong to different *ac* layers. All those molecules on the (010) surface with (Pro-*R*) C–H1 bonds directed toward $+b$ (i.e., with shaded atoms), and thus away from the crystal bulk, form an enantiopolar set and are related to each other by translation or twofold screw symmetry; the remaining molecules on this (010) surface, related to the molecules of the above set by a center of inversion or a glide (i.e., unshaded atoms), belong to the other enantiopolar set and have their (Pro-*S*) C–H1 bonds directed toward $-b$ and thus into the crystal. An α-amino acid additive, of absolute configuration *R*, can replace a glycine molecule on the (010) face only at sites of the enantiopolar set whose (Pro-*R*) C–H1 bonds point away from the crystal. Its side chain, replacing the (Pro-*R*) C–H1 bond of glycine, will thus emerge from the crystal faces as illustrated in Figure 3. Such a glycine site cannot be occupied by an *S* amino acid, as the side chain, which would replace the (Pro-*S*) atom H2, would be almost in the (010) molecular layer (see Figures 2 and 3). Glycine sites on the (010) surface, belonging to the other enantiopolar set, whose (Pro-*S*) C–H1 bonds are directed into the crystal, can neither be replaced by an *S* amino acid, for that would entail penetration of the side chain into the crystal, nor by an *R* amino acid, for that would necessitate replacing the (Pro-*R*) atom H2, by a side chain. Thus, overall, only an *R* amino acid can be adsorbed on the (010) face and only at sites of the first enantiopolar set.

Once adsorbed on the (010) face the *R* additive would hinder the regular deposition of oncoming layers of glycine molecules and so perturb the growth along the $+b$ direction, leading to an increase in the area of the (010) face. By symmetry only an *S* amino acid would be adsorbed on the $(0\bar{1}0)$ face and thus retard growth along the $-b$ direction, leading to an increase in surface area of the $(0\bar{1}0)$ face.

The crystals of pure α-glycine tend to be bipyramidal, with $2/m$ morphological symmetry (Figure 4a), and appear to grow faster along *c* than *a*, sometimes yielding bars. The dominant faces[12] formed are {110} and {011}, and sometimes slightly developed {010} faces are observed. This morphological symmetry is reduced from $2/m$ to 2 when glycine is crystallized in the presence of any resolved α-amino acid, except proline. The relative amount of each different amino acid additive used is listed under Experimental Section. The D-amino acids[13] induce a dramatic increase in the size of the (010) face and reduction in area of the (011), $(01\bar{1})$, (110), and $(\bar{1}10)$ faces (Figure 4b). The corresponding L-amino acids[13] affect the crystals in an enantiomorphous manner (Figure 4c). The racemic additives all yield plates with dominant (010) and $(0\bar{1}0)$ faces, as a result of inhibiting growth along both the $+b$ and $-b$ directions (Figure 4d). According to the analysis presented above, which is exemplified schematically in Figure 5 for an alanine additive, the D-α-amino acids, which induce large (010) faces, must be of *R* configuration and the L acids of *S* configuration.[14] This is in accord with the Bijvoet method.

(12) The symbol {}indicates all symmetry-related faces, ( ) indicates just the given face.

(13) D and L according to the Fischer convention.

(14) But for cysteine where, because of nomenclature, D and L are *S* and *R* configuration, respectively.

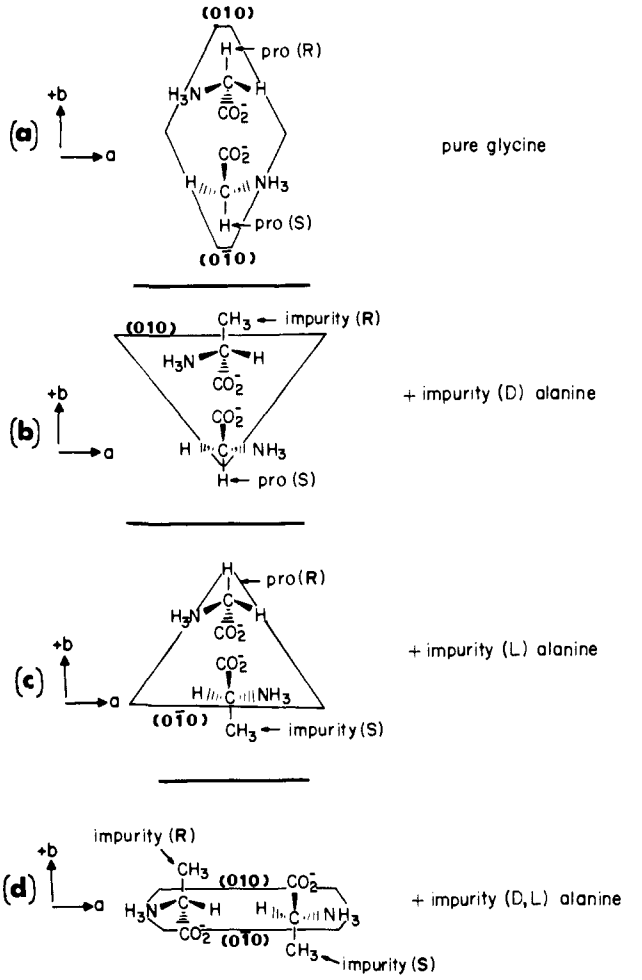


Figure 5. Schematic representations (a)–(d) of the orientation of glycine and additive alanine with respect to the pure and affected crystal faces.

Surface Binding of Additives on Glycine by Energy Calculations. The changes induced in crystal habit of glycine by additives were studied by atom–atom potential energy computations,[15] following the approach by Hartman and Perdok.[16] We shall account first for the morphology of pure glycine. We then compare the ease of adsorption of the *R* and *S* additives at various crystal faces and relative to pure glycine and correlate them with the experimentally observed changes in habit.

A controlling factor in crystal growth is the energy of the interactions between neighboring molecules. The crucial relationship is between the attachment energy[17] (E_a), the energy per molecule released when a new layer is attached to the crystal face, and the layer energy[17] (E_l), which is defined as the energy per molecule released when a new layer is formed. E_l measures the stability of a layer, and E_a controls the growth rate perpendicular to this layer.

The energy functions used for the calculation included van der Waals[18] and electrostatic terms. The latter were derived from an experimental deformation electron density distribution of crystalline α-glycine[11] at 120 K, obtained using a method of Hirshfeld.[19] Electrostatic parameters of other polar molecules, derived in a similar manner, have been successfully applied for analyzing packing characteristics.[20]

Table I. (*hkl*) Layer Energies E_l (in kcal/mol), Attachment Energies E_a, and Surface Binding Energies $E_b = E_l + E_a$ of Crystalline Pure Glycine

face	E_l(Gly)	E_a(Gly)	E_b(Gly)
{020}[a]	−40.4	−6.5	−46.9
{110}	−30.0	−11.6	−41.6
{011}	−27.4	−13.0	−40.4
{001}	−25.1	−14.2	−39.3

[a] The {020} layer is specified instead of {010} because the 2_1 axis generates a layer repeat of $b/2$. Note that { } specifies all symmetry-related faces, ( ) only that particular face.

The surface and attachment energies of pure glycine for relevant faces[15] are given in Table I, together with the binding energies ($E_b = E_l + E_a$). These results suggest that the (010) face should be the most well developed, more so than (110) or (011), which is not in keeping with experiment. This inconsistency probably arises from neglect of water–surface interactions. Indeed, the Coulomb potential which was calculated at closest approach distances from the surface of each of the three faces definitely indicates that {110} and {011} are more hydrophilic than {010}, which is compatible with the relative surface areas of these faces from aqueous solutions.

We have calculated the binding energy at a surface site in which glycine is replaced by additive alanine at each of the four symmetry-related glycine positions on the different {*hkl*} surfaces. The results are listed in Table II. Alanine was chosen as a model because its C–CH_3 bond is fixed in space given the geometry of its remaining surface-bound molecular moiety NH_3^+–CH–CO_2^-. The methyl group of alanine may replace either H1 or H2 of glycine (see Figure 3) to yield two different conformers for each enantiomer. Thus adsorbed alanine will be specified both by its chirality (*R* or *S*) and the glycine H atom it replaces (H1 or H2), e.g., *R*(H1)Ala specifies *R* alanine in which the CH_3 group replaces H1 of glycine. We shall consider the effect of adsorbed alanine on the +*b* half of the crystal, namely, on faces (*hkl*), *k* positive. By symmetry, the roles of adsorbed (*R*)- and (*S*)-alanine are interchanged for the enantiotopic faces ($\bar{h}\bar{k}\bar{l}$) at the −*b* half of the crystal.

According to the energy results (Table II) the (010) face is the primary binding surface for alanine; only *R*(H1)Ala can be easily adosrbed on this face, at two of the four different sites (see Figure 6a), with equal binding energies as low as −46 kcal/mol, which is indeed 2 kcal/mol lower than that of the corresponding glycine. The alanine molecules *R*(H2)Ala, *S*(H1)Ala, and *S*(H2)Ala are precluded from binding on the (010) face [see Table II and for *S*(H1)Ala Figure 6b].[21] Alanine cannot be bound at the symmetry-related (110) and ($\bar{1}$10) faces. These results are completely consistent with experiment; crystals affected by D-amino acids yield a large (010) face at the expense in area of the face pair (110) and ($\bar{1}$10).

In general in affected crystals there is an increase in the ratio of the area of face (011) to that of (110). This observation is in agreement with the energy results in Table II; *R*(H1)Ala can be bound at one of the four symmetry-related sites at the (011) face with an energy as low as −32 kcal/mol, which is only 4 kcal/mol more than that of the corresponding glycine molecule. Figure 6c shows that *R*(H1)Ala appears to fit easily on the (011) face at the appropriate site. In contrast alanine cannot be bound at the (110) face. The binding energies of alanine at (001) indicate that *R*(H1)Ala and *S*(H1)Ala could be adsorbed almost as easily on this face as on (011). The (001) face does not, however, supplant (011) in affected crystals, presumably because the latter is more stable in pure glycine.

(15) A comprehensive analysis of the changes in crystal morphology by energy calcualtions will be published in a separate paper.
(16) Hartman, P.; Perdok, W. G. *Acta Crystallogr.* **1955**, *8*, 49. Hartman, P. In "Physics and Chemistry of the Organic Solid State"; Fox, D., Labes, M., Weissberger, A., Eds.; Interscience: New York, 1963, Chapter 6.
(17) The layer energy is the sum of the intermolecular interactions between one molecule and its neighbors inside a slice of width *d*(*hkl*). The attachment energy is the sum of the intermolecular interactions between one molecule and all the molecules that are outside the layer at one half of the crystal.
(18) Hagler, A. P.; Lifson, S. *J. Am. Chem. Soc.* **1979**, *101*, 5111.
(19) Hirshfeld, F. L. *Theor. Chem. Acta*, **1977**, *44*, 129.
(20) Berkovitch-Yellin, Z.; Leiserowitz, L. *J. Am. Chem. Soc.* **1980**, *102*, 7677; **1982**, *104*, 4052.
(21) The positive energies must not be taken at face value because the geometry at the site of the additive was not optimized.

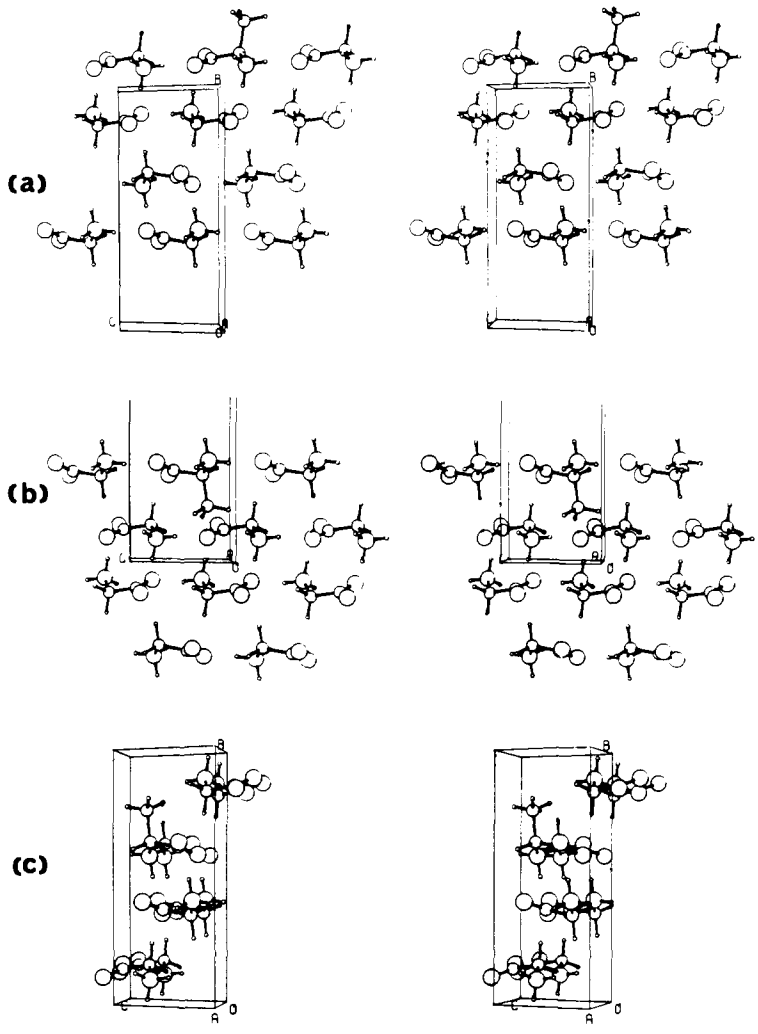


Figure 6. Alanine inserted at various glycine sites on crystal surfaces: (a) *R*(H1)Ala inserted on (010) face; (b) *S*(H1)Ala inserted on (010) face; (c) *R*(H1)Ala inserted at one of the four different sites of (011) forming favorable intermolecular contacts.

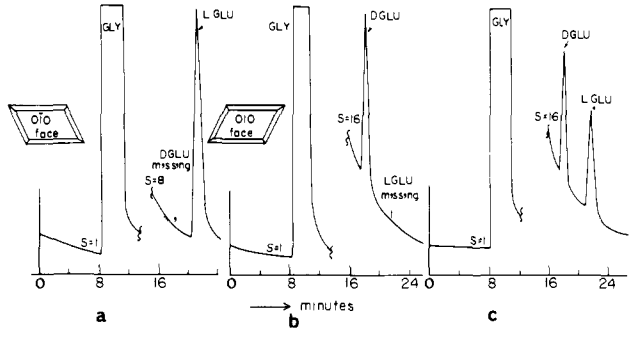


Figure 7. Enantiomeric distribution of occluded DL-glutamic acid in the {010} platelike crystals of glycine by HPLC (*S* denotes arbitrary sensitivity scale 1, 8, 16). (a) Only additive L-Glu is present in material shaved from the (0$\bar{1}$0) face of the crystal; (b) only additive D-Glu is present in material shaved from the (010) face; (c) relative distribution of DL-Glu in the remaining whole crystal.

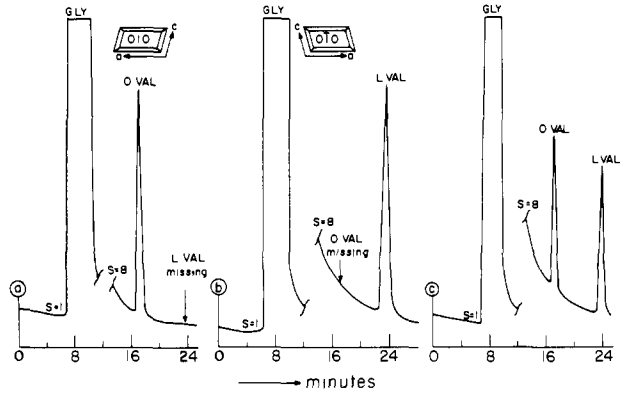


Figure 8. Enantiomeric distribution of occluded DL-valine in the {010} platelike crystals of glycine. (a) Only additive D-Val is present from the (010) face; (b) only additive L-Val is present from the (0$\bar{1}$0) face; (c) relative distribution of DL-Val in whole crystal.

Enantiomeric Segregation of Racemic Additives in Glycine Crystals. The observed changes in crystal habit and the calculated energies indicate that occluded (*R*)- and (*S*)-amino acid mixtures that induce {010} plates should be enantioselectively segregated along the *b* axis during crystal growth; occluded *R* additives should prevail at the $+b$ half of the crystal plate whereas the *S* additives should prevail at the $-b$ half. This prediction was experimentally confirmed by HPLC analysis of glycine crystals grown in solutions each containing one of ten different α-amino acids as additives; the results on two of these are illustrated in Figures 7 and 8. The enantiomeric segregation at the opposite sides of each plate in all the systems analyzed proved to be total within detection limits of not less than 1% enantiomeric purity; the content of occluded additive ranged from 0.02% to 0.2% of glycine. Evidence for enantiomeric segregation was also provided visually using $N\epsilon$-(2,4-dinitrophenyl)-L-lysine as additive; when a platelike crystal of glycine was allowed to continue growing in the presence of this yellow compound, the material that was added on the $-b$ side was yellow whereas the $+b$ side remained colorless. These measurements provide another independent means for the assignment of absolute configuration of the occluded additives.

Table II. Binding Energies E_b (kcal/mol) of Impurity Alanine Inserted at Each of the Four Different Surface Sites[a] on Various (*hkl*) Faces, *k* Positive

face[b]	site	alanine impurity	E_b(Ala)	E_b(Ala) − E_b(Gly)	face	site	alanine impurity	E_b(Ala)	E_b(Ala) − E_b(Gly)[c]
(010)	1	$R(H_1)$	−45.8	−2.3	(110)	1	$R(H_1)$	>1000	
	2	$R(H_1)$			or	2	$R(H_1)$	113	
	3	$S(H_1)$	>1000		$(\bar{1}10)$	3	$S(H_1)$	>1000	
	4	$S(H_1)$				4	$S(H_1)$	125	
	1	$S(H_2)$	53			1	$S(H_2)$	63	
	2	$S(H_2)$				2	$S(H_2)$	35	
	3	$R(H_2)$	14			3	$R(H_2)$	120	
	4	$R(H_2)$				4	$R(H_2)$	−9	
(011)	1	$R(H_1)$	>1000		(002)	1	$R(H_1)$	>1000	
or	2	$R(H_1)$	−32.1	+4.2	or	2	$R(H_1)$	−29.6	+5.7
$(01\bar{1})$	3	$S(H_1)$	>1000		$(00\bar{2})$	3	$S(H_1)$	>1000	
	4	$S(H_1)$	>1000			4	$S(H_1)$	−29.6	+5.7
	1	$S(H_2)$	73			1	$S(H_2)$	42	
	2	$S(H_2)$	20			2	$S(H_2)$	23	
	3	$R(H_2)$	46			3	$R(H_2)$	42	
	4	$R(H_2)$	15			4	$R(H_2)$	23	

[a] The four symmetry sites 1–4 are (1) *x*, *y*, *z*; (2) $^1/_2 - x$, $^1/_2 + y$, $^1/_2 - z$, (3) $\bar{x}$, $\bar{y}$, $\bar{z}$, and (4) $^1/_2 + x$, $^1/_2 - y$, $^1/_2 + z$. [b] For enantiotopic faces (*hkl*) *k* is negative; alanine of opposite chirality applies. [c] The difference in binding energy E_b(Ala) − E_b(Gly) is listed for favorable cases.

Conclusion

We may conclude that this simple and direct method of assigning absolute configuration is not limited but may be applied to a variety of molecular systems and has proven to be self-consistent. It has been used to determine the absolute configurations of the following: (a) resolved lysine and cinnamoyl alanine as substrate molecules in chiral polar crystal;[4] (b) resolved threonine as additive by its effect on the centrosymmetric racemate DL-serine;[8] and (c) resolved α-amino acids as additives by their effect on the centrosymmetric crystals of prochiral glycine. Studies with other molecules such as peptides, sugars, and steroids are under way. The method would be particularly useful for molecules not easily amenable to the Bijvoet analysis, for example chiral hydrocarbons.

The enantiomeric segregation of occluded racemic additives in a centrosymmetric crystal appears to be general.[8] Its possible relevance to the generation and amplification of optical activity under prebiotic conditions is under study.

Experimental Section

All amino acids were commercial analytical grade materials; their purity was checked in the limits of the sensitivity of HPLC method ($\sim 10^{-6}$ mg), and they were used without further purification.

Crystallization Experiments. In a typical experiment 1 g of glycine was dissolved together with the appropriate amount of additive in 3 mL of distilled water (4.4 M). After spontaneous cooling to room temperature, crystallization of the supersaturated solutions occurred in the range of 24–48 h. 20% crystalline material was recovered by filtration, and from this at least five sample crystals with well-developed faces were selected for crystallographic and HPLC measurements from each batch. In every case the chosen crystals were representative of the morphology of the whole batch. When available, both enantiomers of each amino acid were tested separately. The amount of the chiral α-amino acids used as additives (in percent (w/w) of glycine) was the following: alanine (0.5), α-aminobutyric acid (0.5), arginine (1), aspartic acid (1), asparagine (0.5), cysteine (0.5), glutamic acid (1), histidine (0.5), isoleucine (0.5), leucine (1), lysine (0.5), methionine (0.5), ornithine (0.5), phenylalanine (0.5), phenylglycine (0.5), serine (1), threonine (1), tryptophane (0.5), tyrosine (0.5), valine (1). The amount of the racemic amino acids used as additives was twice that of the corresponding chiral one.

HPLC Analyses. Samples (20 μL) were injected onto a reversed-phase column (25 cm × 4.6 mm) self-packed with 5 μm Nucleosil C18 (Macherey Nagel), with a mobile-phase composition of an aqueous solution of cupric acetate (4×10^{-3} M) and *N*,*N*-di-*n*-propyl-(*S*)-alanine (8×10^{-3} M) at pH 5.3–5.5.[22]

The samples were prepared by shaving away with a blade small amounts of material from the middle surface of the (010) or $(0\bar{1}0)$ faces of the crystals and dissolving it in an appropriate amount of the mobile phase.

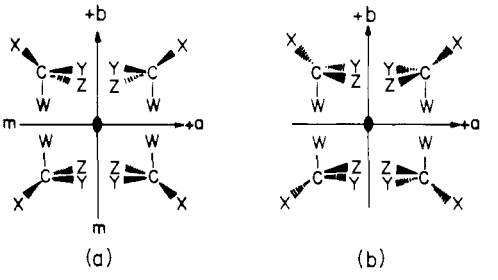
Crystallographic Measurements. The crystal dimensions, including the areas of all faces, were determined on a Siemens diffractometer by measuring the perpendicular distance of each face from a convenient reference point.[5]

Acknowledgment. We thank R. Azhari for assistance with the energy computations. We are grateful to Prof. Mendel Cohen for a critical reading of the manuscript. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the US/Israel Binational Science Foundation, Jerusalem, for financial support.

Appendix

Noncentrosymmetric achiral monoclinic and orthorhombic crystals, of point symmetry *m* and *mm*2, respectively, are appropriate for determination of absolute configuration of chiral resolved additives. For the point group *m*, only the left or right half of Scheme II (see main text) is relevant. In such an arrangement the directions of *R* and *S* molecules are unambiguously assigned with respect to the unique *b* axis (although not with respect to the *a* and *c* axes), allowing assignment of the absolute configuration of the chiral resolved additive by the affected faces (*hkl*) $k \neq 0$. The absolute sense of the *a* and *c* axes may also be fixed from the affected faces provided they are of the type (*hkl*), *h* and/or $l \neq 0$, $k \neq 0$.

In the orthorhombic point group *mm*2 there is an ambiguity in the sense of the polar axis *c*. Conventional X-ray diffraction does not allow one to differentiate, with respect to a chosen coordinate system, between the *mm*2 structures a and b.



Nevertheless by determining which polar end of a given crystal (e.g., face *hkl* or $hk\bar{l}$) is affected by the chiral (resolved or racemic) additive, it is possible to fix the absolute sense of the polar *c* axis; the absolute configuration of the chiral resolved additive is assigned by which face of the enantiotopic pair (e..g., *hkl* or $hk\bar{l}$) is affected.

Registry No. Glycine, 56-40-6; D-alanine, 338-69-2; L-alanine, 56-41-7; DL-alanine, 302-72-7.

(22) Weinstein, S. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 218. Weinstein, S.; Engel, M. H.; Hare, P. E. *Anal. Biochem.* **1982**, *121*, 370.