Enantiomorph identification in organic crystals by electron diffraction

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Abstract. The convergent-beam electron diffraction (CBED) method we proposed recently for enantiomorph identification has successfully been applied to some amino acid crystals such as glutamic acid and threonin. Enantiomorph identification (either left-handed or right-handed form) can readily be made within the framework of the proposed method by noting the asymmetric intensity distribution of Bijvoet pairs of reflections in the CBED pattern taken along an appropriate zone-axis orientation. Although the proposed method usually requires only a single CBED pattern, some effort to eliminate the ambiguity of 180°-rotation of the CBED pattern about the incident beam is needed for enantiomorph identification for these organic crystals because of the lack of HOLZ (higher-order Laue zone) reflection disks.

1. Introduction

Objects may have both left-handed and right-handed forms in the absence of improper rotations (rotoinversions and rotoreflections) in the crystallographic description [1]. This is usually called enantiomorphism or chirality. The two members of the pair of enantiomorphic crystals or molecules are mirror-related. Since one of the two enantiomorphic crystals exhibit bioactivities different from another in most cases, distinction of enantiomorph (chirality) of these enantiomorphic crystals is sometimes very important especially when they are used in foods and pharmaceutical drugs [2,3]. Thus, many organic crystals and molecules are sometimes the subject of enantiomorphic investigation and some asymmetric synthesis methods are often adopted to promote the preferential nucleation for one of the two enantiomorphic crystals and molecules [2]. Enantiomorph is often distinguished by polarimetry for many organic molecules and, to the lesser content, organic molecular crystals [2,4], whereas for inorganic crystals and many organic molecular crystals including proteins, this is usually made by X-ray diffraction utilizing anomalous scattering that breaks the Friedel’s law. However, these X-ray diffraction methods generally requires a relatively large-sized single crystals of high quality [5], which are sometimes difficult to prepare. We have recently proposed a new electron diffraction method for enantiomorph identification, in which the intensity asymmetry of Bijvoet pairs of ZOLZ (zero-th order Laue zone) and/or FOLZ (first order Laue zone) reflection disks in a CBED (convergent-beam electron diffraction) pattern taken along an appropriate zone-axis orientation is monitored and compared with the asymmetry in the corresponding computer simulated CBED pattern.
This new method is usually made with a conventional transmission electron microscope (TEM) and allows in principle to identify all possible enantiomorphic crystals that are allowed to exist in crystallography and has indeed been experimentally applied successfully to inorganic crystals belonging to the point groups of 622, 6, 312 and 321 and so on.

Although the proposed method should find out the most fruitful applications in organic crystals, there is an inherent difficulty in observing organic crystals with TEM due mainly to the sublimation of these crystals under electron illumination, which arises from their low thermal and electrical conductivity. However, we have recently applied the proposed method successfully to an organic superconductor \( \kappa-(\text{BEDT-TTF})_2\text{Cu(NCS)}_2 \) by reducing electron illumination damage with the real-time video-recording of CBED patterns. In view of the fact that the organic superconductor contains heavy metal atoms such as copper, the extent of the difficulty in observations is considered to be less significant when compared to other organic crystals consisting only of light elements. Of these organic crystals, those referred to as amino acids (containing at most sulfur) would be a nice example to check the applicability of the proposed electron diffraction method, since they are often the subject of enantiomorph identification for their bioactivity.

2. Amino acids and principles for enantiomorph identification

Of more than 700 amino acids so far identified, 26 have been known to be a constituent of proteins. These 26 amino acids are usually called L-\( \alpha \) amino acids, since they are in general of the L-form in vivo, exhibiting a dextro-rotatory in polarimetry. Many of them crystallize into a form belonging to the space group either P2\(_1\) or P2\(_1\)\(_2\)\(_1\), with some exceptions occurring, for example, in cystine with the space group of P6\(_1\)2\(_2\) [2,3]. This indicates that we need to consider only three different point groups for enantiomorph identification within the framework of the proposed method; the point groups 2, 222 and 622. In the present study, we investigate the point groups 222 and 622, taking glutamic acid (P2\(_1\)2\(_1\)2\(_1\)), threonin (P2\(_1\)2\(_1\)2\(_1\)) and cystine (P6\(_1\)2\(_2\)) as specific examples for these cases.

The proposed method requires to take a CBED pattern along the ‘appropriate’ zone-axis orientation in order to observe Bijvoet pairs of reflections symmetrically with respect to the symmetry line m-m’, as shown in figure 1. Since Bijvoet pairs of reflections are Bragg reflections of space group symmetry equivalents to the two members of a Friedel pair, the appropriate zone-axis orientations to symmetrically observe them vary with crystal point group. The appropriate zone-axis orientations are analyzed to be of the \( \langle u0v0 \rangle \), \( \langle 00w \rangle \) and \( \langle u0w \rangle \)-types for the point groups of 2 and 222, and \( \langle u00 \rangle \), \( \langle u20 \rangle \) and \( \langle uv0 \rangle \)-types for the point group of 622 [6]. Once Bijvoet pairs of reflections are observed symmetrically with respect to the symmetry line m-m’, the phase distribution of these Bijvoet pairs of reflections is not symmetrical with respect to the symmetry line m-m’, since the two members of Bijvoet pair reflections have equal amplitudes (|\( F \)|) but opposite phases.

Figure 1. Schematic illustration of zone-axis CBED patterns of (a) right-handed and (b) left-handed enantiomorphic crystals, in which Bijvoet pairs of reflections are observed symmetrically with respect to the symmetry line m-m’. The nomenclatures a, b, c, … indicated at reflection disk positions schematically depict phase angles of the corresponding reflection disks.
(\(\varphi\)) in the expression of structure factors for any hkl reflection as in the case of a Friedel pair. Then, the intensity distribution of Bijvoet pairs of reflections in the CBED pattern is expected to be asymmetrical with respect to the symmetry line m-m', as a result of multiple scattering among reflections in the zone-axis CBED pattern. Since the right-handed crystal can be converted to the left-handed one by changing the coordinates of atom positions from \((x,y,z)\) to \((-x,-y,-z)\), the asymmetrical phase distribution with respect to the symmetry line m-m' in the zone-axis CBED pattern for one of the two members of the enantiomorphic crystal is antisymmetric with that for the other (Figures 1(a) and (b)). Then, the asymmetric intensity distribution in the zone-axis CBED pattern with respect to m-m' is also expected to be antisymmetric for the two members of the enantiomorphic crystal, by which enantiomorph identification is made.

3. Experimental Procedures
L-\(\alpha\) glutamic acid and L-threonin were purchased in the form of powders from Osaka Asahi Metal. Co. Ltd. These powder samples were crushed in liquid nitrogen in order to obtain very thin samples so as to minimize the temperature rise of specimen during TEM observations. They are then mounted on a microgrid for TEM observations. CBED experiments were made with the JEM-2000FX TEM operated at 100 kV at ambient temperature and with the JEM-3010 TEM operated at 100 kV in the temperature range from 10 K to ambient temperature. CBED patterns were recorded in real-time video-recording to minimize the recording time. Calculation of CBED patterns was made with a Win HREM software [16] with the atomic coordinates tabulated in [17] for the L-forms of glutamic acid, threonin and cystine, respectively.

4. Results
L-\(\alpha\) cystine is formulated to be \(C_6H_{12}N_2O_4S_2\) (molecular weight: 240.30) and is reported to belong to the space group of P\(6_122\) in the L-form. Since enantiomorphism in this case is related to the screw axis parallel to the hexagonal c-axis, the space group of the D-form is changed to P\(6_522\). Of the appropriate

Figure 2. Calculated CBED patterns of L-\(\alpha\) cystine taken along (a),(e) \([1\overline{2}1]\), (b),(f) \([1\overline{2}3]\), (c),(g) \([\overline{1}0\overline{1}]\) and (d),(h) \([\overline{1}0\overline{2}]\) zone-axis orientations. The space groups assumed in the calculation are P\(6_122\) for (a)-(d) and P\(6_522\) for (e)-(h). The accelerating voltage and crystal thickness used in the calculation are 100 kV and 22.4 nm, respectively.
zone-axis orientations allowed for the point group of 622, we choose [11\bar{2}1], [11\bar{2}3], [1\bar{1}01] and [1\bar{1}02] to calculate zone-axis CBED patterns to see whether the expected intensity asymmetry for Bijvoet pairs of reflection disks appears or not. As seen in figure 2, the intensity distribution is asymmetric with respect to the m-m’ line for each of these CBED patterns. The intensity asymmetry for Bijvoet pairs of reflection disks occurs for both ZOLZ and FOLZ reflection disks for all these CBED patterns. When the L-form is changed to the D form (and vise versa), the asymmetric intensity distribution with respect to the m-m’ line is reversed, by which enantiomorph identification is readily made. This indicates that enantiomorph identification can readily be made with only such a CBED pattern even for organic crystals consisting only of light elements, once the CBED pattern is taken along an appropriate zone-axis orientation.

This was indeed confirmed by experiments made for glutamic acid and threonin. L-β glutamic acid is formulated to be C_{5}H_{9}NO_{4} (molecular weight: 147.13) and belongs to the space group of P2_{1}2_{1}2_{1}. The L-form is converted to the D-form by changing the sign of the atomic coordinates within the same space group. An experimental CBED pattern taken along [101], which belongs to the appropriate zone-axis orientations of the \(\langle u0w\rangle\)-type, is shown in figure 3(a). The intensity asymmetry with respect to the m-m’ line is clearly observed not only for a Bijvoet pair of 020-020 but also for those of \(15\bar{1}T\)-1\bar{3}T and \(T51\)-T\bar{3}1. However, unlike in figure 1, the CBED pattern of figure 3(a) exhibits only Bijvoet pairs of ZOLZ reflections and lacks the intensity for Bijvoet pairs of FOLZ reflections. The absence of the intensity for Bijvoet pairs of FOLZ reflections was similarly confirmed even at 10 K. This causes an ambiguity of 180°-rotation of the CBED pattern of figure 3(a) about the incident beam. In the present case, the existence of the [100] zone-axis upward and [001] zone-axis downward was confirmed upon tilting the specimen about [010]. Then, Bijvoet pairs of ZOLZ reflections are all unambiguously indexed as in figure 3(b). Figures 3(b) and (c) are calculated [10\bar{1}] CBED patterns of

![Figure 3](image)

Figure 3. (a) Experimental and calculated [10\bar{1}] zone-axis CBED patterns for β glutamic acid of (b) the L- and (c) D-forms. In the calculation, the value of crystal thickness was assumed to be 20.2 nm.

![Figure 4](image)

Figure 4. (a) Experimental and calculated [11\bar{1}] zone-axis CBED patterns for threonin of (b) the L- and (c) D-forms. In the calculation, the value of crystal thickness was assumed to be 33.0 nm.
the L- and D-forms of glutamic acid with the assumption of the value of crystal thickness of 20.2 nm. In the experimental pattern (figure 3(a)), the intensity of the 020 ZOLZ reflection disk is stronger than that of the 020 disk, indicating that glutamic acid investigated corresponds to the L-form.

L-threonin is formulated to be $\text{C}_4\text{H}_9\text{NO}_3$ (molecular weight: 119.12) and belongs to the space group of $\text{P2}_1\text{2}_1\text{2}_1$ and the L-form is converted to the D-form by changing the sign of the atomic coordinates within the same space group. Enantiomorph identification is thus made for threonin as in the case of glutamic acid. A CBED pattern experimentally taken along the [111] zone-axis orientation and the corresponding calculated patterns assuming the L- and D-forms are shown in Figures 4(a)-(c), respectively. Since the [111] zone-axis orientation does not belong to any of the appropriate zone-axis orientations, the symmetry line m-m’ cannot be defined and the intensity distribution of all reflection disks in the experimental pattern should be compared with the calculated patterns. As in the case of glutamic acid, the experimental [111] CBED pattern of threonin also lacks the intensity of FOLZ reflections, giving rise to the ambiguity of 180°-rotation of the CBED pattern about the incident beam. To eliminate this ambiguity, the specimen was tilted about $[\text{1} \text{1} \text{0}]$ so as to determine which direction (upper-right or lower-left) of the CBED pattern corresponds to the way to reach the [110] or [001] zone-axis orientations. Then, Bijvoet pairs of ZOLZ reflections are all unambiguously indexed as in figure 4(b). When the intensity for the Friedel pair of 011-011 is compared with each other in the calculated CBED patterns, the intensity of the former disk is stronger than that of the latter disk for the right-handed form (figure 4(b)), whereas the opposite is true for the left-handed form (figure 4(c)). The intensity asymmetry in the experimental pattern (figure 4(a)) clearly indicates that threonin investigated corresponds to the L-form.

5. Discussion
Enantiomorph identification by electron diffraction was successfully made for amino acids consisting only of light elements for the first time. This was made at ambient temperature without cooling the specimen by noting the asymmetric intensity distribution for Bijvoet pairs of ZOLZ reflection disks arranged symmetrically with respect to the m-m’ line in a CBED pattern taken along the appropriate zone-axis orientation (figure 1). In spite of the difficulty that the intensity for Bijvoet pairs of FOLZ reflections is too low to be detected in the CBED pattern, enantiomorph identification could be made successfully within the framework of the method we proposed recently [6] only by taking an additional SAED pattern to eliminate the ambiguity of 180°-rotation of the CBED pattern about the incident beam. The difficulty in observing FOLZ reflections in organic crystals may arise from the following factors [14]: (i) the atomic numbers of constituent atoms are in general low, (ii) thermal vibration of constituent atoms is high at ambient temperature and (iii) electron illumination damage quickly occurs introducing short-range disorder. Since the visibility for the intensity of Bijvoet pairs of FOLZ reflections remained almost identical even at 10 K, the factor (ii) may not make the main contribution. The factor (iii) can also be effectively avoided if real-time video recording is employed. Indeed, the intensity of FOLZ reflections was clearly visible for a while (a few seconds) in some CBED patterns with a smaller radius for the FOLZ ring. We thus believe that enantiomorph identification for organic crystals is also possible only with a single CBED pattern (as in many inorganic crystals) if an appropriate zone-axis orientation, where Bijvoet pairs of ZOLZ and FOLZ reflection disks are arranged symmetrically with respect to the m-m’ line and the radius for the FOLZ ring is small in the CBED pattern, is properly chosen.

6. Conclusions
The CBED method we proposed recently for enantiomorph identification has successfully been applied to some amino acids such as glutamic acid and threonin. Enantiomorph identification is made at ambient temperature by making real-time video recording without cooling the specimens, which is generally believed to be necessary for reducing electron illumination damage for organic crystals. Although the proposed method usually requires only a single CBED pattern, an additional diffraction pattern is needed for enantiomorph identification of amino acids in order to exactly determine the
crystallographic geometry of the relevant CBED pattern. This is due to the fact that the intensity of high-angle scattering from the organic crystal, which appears as HOLZ reflection disks, is too low to be imaged in the relevant CBED pattern even the specimen is cooled down to 10 K.

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