Treatment of Diffraction Data from Protein Crystals Twinned by Merohedry

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Abstract

Crystals of the protein B-phycoerythrin from the red alga *Porphyridium cruentum* undergo twinning by merohedry in which the two members of the twin are related by rotation about (1,1,−1,0), a symmetry operation of the lattice but not of the crystals. Several methods are compared for estimation of the volume fractions of the two members of the twin so that measured data can be corrected for this twinning. The effect of these corrections on the final electron-density map is analyzed. Results show that when the volume fraction of the smaller crystal in a twinned specimen used for structure determination by multiple isomorphous replacement is 0–0·1, correction of diffraction data for twinning results in a small but significant improvement in the accuracy of the electron-density map.

Introduction

Crystals of the light-harvesting protein B-phycoerythrin from the marine red alga *Porphyridium cruentum* form in space group R3 with $a = b = 189$ and $c = 60$ Å in the hexagonal setting (Sweet, Fuchs, Fisher & Glazer, 1977). Virtually all crystals experience some twinning, ranging from a negligible amount to nearly equal fractions of the two individual crystals in the twin. Although distinct twin domains can sometimes be distinguished in the polarizing microscope, the twinning is of the twin-lattice-symmetry type (Donnay & Donnay, 1974) in which reflection $hkil$ of crystal I is superimposed onto reflection $khil$ of crystal II. This sort of twinning, where the reciprocal lattices of both twinned individuals coincide and where the twinning operation, here rotation by 180° about (1,1,−1,0), involves a symmetry element of the lattice alone but not of the crystal, was recognized by Bravais who called it twinning by merohedry

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(Friedel, 1926; Buerger, 1960). Diffraction patterns from a single crystal and from a twin differ only in the distribution of intensities [Fig. 1(a) and (b), respectively].

In a twin by merohedry, the measured diffraction intensities I_1 and I_2, related to one another by the twin operation, are linear combinations of the intensities J_1 and J_2 which would be observed from an untwinned crystal (Buerger, 1960):

$$I_1 = (1 - x)J_1 + xJ_2,$$

$$I_2 = (1 - x)J_2 + xJ_1,$$

where x is the volume fraction of the smaller crystal in the twin. One can solve this system for J_1 and J_2 to obtain

$$J_1 = \frac{I_1 - x(I_1 + I_2)}{1 - 2x}. \tag{1}$$

Clearly, measured intensities can be corrected for crystal twinning if the volume fraction x can be determined and differs from 0·5.

Having estimated volume fractions for all of the specimens used in the determination of a protein crystal structure, we are able to compare the methods used to estimate them and to discuss the usefulness of the corrections which were made to data obtained from twinned crystals.

Estimation of the volume fraction

We have developed four complementary techniques for assessment of crystal twinning. All four have been used during the solution of the structure of B-phycoerythrin by standard techniques: multiple isomorphous replacement and symmetry averaging (Fisher, Woods, Fuchs & Sweet, 1980). One of them, the 'Britton' technique, allows estimation of the volume fraction for data from a single specimen. The second and third provide an estimate of the approximate difference in twinning between two different twinned crystals. The fourth is optimization of volume fractions during structure-factor least-squares refinement of heavy-atom parameters. Each of these can be described in some detail.

When I_1 is the smaller of two twinning-related intensities, the right-hand side of (1) can become negative for some value of x less than 0·5. This suggests a method by which the volume fraction can be estimated for data from a single twinned specimen. For each specimen used in data collection, twinning-related intensities are compared, and the number of J's which are predicted to be less than zero are tabulated for increasing values of x. Figs. 2(a) and 2(b) illustrate a graphical treatment of such data. We take the x value at the sharp break in the curve shown in Fig. 2(a) as an accurate estimate of the upper bound of x. The broader knee in the curve in Fig. 2(b) does not yield an accurate estimate of x. Since only a few intensities determine the position of the break, the precision of this estimate of x depends strongly upon accurate measurement of the weakest intensities. Britton (1972) used a virtually identical method for the correction of data from twinned crystals of diiodoacetylene (Dunitz, Gehrer & Britton, 1972), and Murray-Rust (1973) used a different interpretation of (1) which we found to be less sensitive.

A consequence of twinning by merohedry is that since observed intensities represent averages of true intensities with uncorrelated values, the frequency of occurrence of very small intensities decreases as the volume fraction of the minor crystal in the twin increases. Rees (1980) has very recently explored the statistics of intensity measurements from crystals twinned by merohedry. He has shown that data from twinned crystals of the protein complex between carboxypeptidase A and the potato carboxypeptidase inhibitor (Rees & Lipscomb, 1980) fit his theoretical model. He has used this model to estimate volume fractions for twinned crystals (Rees, 1980) and found that these values agree well with those determined by the methods of Britton (1972) and Murray-Rust (1973). An important feature of Rees's treatment is that it can be used in the absence of any knowledge of twinning mechanism for the assessment of twinning in a specimen. We did not have the opportunity to apply these ideas to data from B-phycoerythrin crystals.

The second set of techniques depends upon the idea that if one crystal is not twinned, or if data from that crystal have been corrected for twinning, these data can be compared to those from other crystals in order to determine the others' volume fractions. One of these techniques involves a search for an x which minimizes $R = \sum_h |J_h - K_h| / \sum_h |J_h|$ where the J's are intensities with no twinning and the K's are from another crystal and are corrected for twinning by (1). The method yields reasonable results, but they are imprecise since the minima in curves of R versus x are broad and shallow. The other technique involves estimation of the volume fraction by comparison of each equivalent pair of twinning-related intensities from two different crystals. If intensities J_1 and J_2 come from an untwinned crystal and equivalent intensities I_1 and I_2
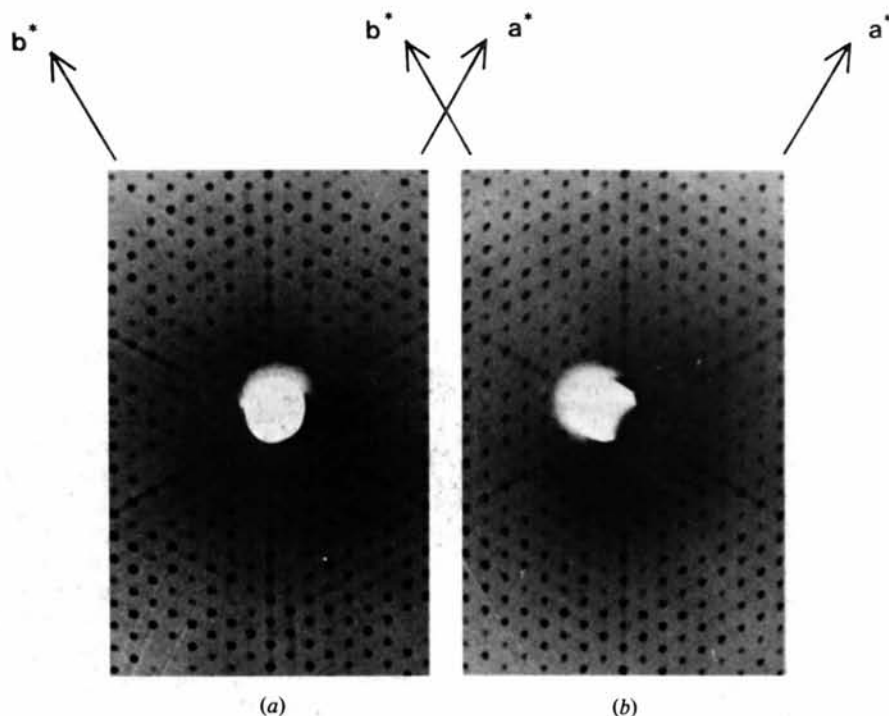

(a) (b)

Fig. 1. Portions of $hk0$-zone precession photographs of (a) slightly twinned ($x = 0.05$) and (b) highly twinned ($x = 0.4$) crystals of B-phycoerythrin.

come from a twin, these intensities should be related by (1). One can then show that each pair of intensities can give an estimate of the volume fraction:

$$x = \frac{sI_1 - J_1}{J_2 - J_1},$$ (2)

where $s = (J_1 + J_2)/(I_1 + I_2)$. In this way the I's are pairwise scaled to the J's in a way which distributes errors equally among the observations. When we compare data from two specimens in this manner, we find that the estimates of x are distributed in an approximately normal distribution, as shown in Fig. 3. These distributions were fit by a least-squares method either to one or to a sum of two Gaussian functions. When the two specimens differ only in volume fraction, the distribution is fit adequately by a single Gaussian function, as shown in Fig. 3(a). When there is a chemical difference between the two specimens used in the comparison, e.g. when one is a native crystal and the other is an isomorphous heavy-atom derivative, a second function is required to give an adequate fit, as shown in Fig. 3(b). This presumably results from large systematic differences in the intensities, owing to heavy-atom substitution. One should note that (2) is formally correct only when the two specimens used in the comparison are chemically identical and when one of the two is not twinned or its data have been corrected for twinning. We found, however, that this method could be used to confirm independent indications of the *difference* in volume fraction, even between crystals with different isomorphous heavy-atom substitution, as long as the volume fractions involved were low.
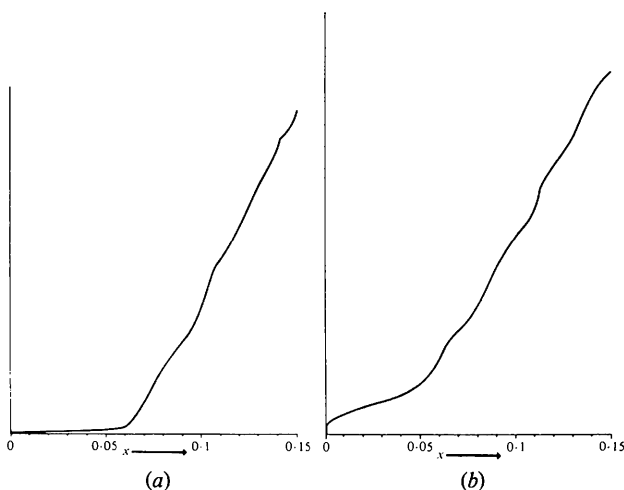
The final technique we have employed for estimation of the volume fraction is its determination during structure-factor least-squares refinement of parameters describing the isomorphous heavy-atom derivatives. We used a refinement procedure similar to that described by Dickerson, Weinzierl & Palmer (1968) to minimize the mean-square lack of closure of the complex structure factors. One can show formally that the data contain information sufficient to determine volume fractions for all the native and heavy-atom-derivative crystals. In practice, however, these parameters are highly correlated and the refinement proceeds only when one or more of them are held fixed at a value determined by an independent method.

The results of the application of these techniques to data from twinned crystals of B-phycoerythrin are shown in Table 1. Five different specimens were used in the analysis, one of which was the native protein; two were the same heavy-atom derivative. We found that when the measured data were of high quality, 'Britton' plots like the one shown in Fig. 2(a) yielded seemingly
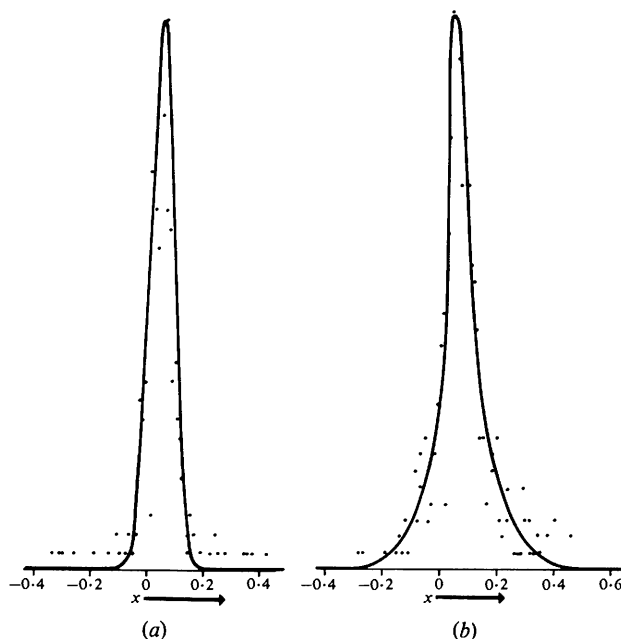

(a)   (b)

Fig. 3. Frequency distribution of x determined by relation (2). The smooth curves are fits of the data either to one or to a sum of two functions of the form $f(x) = (A/\sigma\sqrt{2\pi}) \exp[-(x - x_o)^2/2\sigma^2]$. (a) Three-dimensional, 5·0 Å-resolution data from crystals of B-phycoerythrin measured by rotation photography. The data compared are from the same isomorphous heavy-atom derivative (crystals 2 and 3 in Table 1). The volume fraction for the less-twinned crystal was estimated by other methods as 0·9%. The value of a σ for the single function in the fit is $\sigma = 4\cdot3\%$. (b) The same sort of data, coming from a native and a heavy-atom derivative crystal (crystals 1 and 2 in Table 1). The less-twinned crystal is the same as that used in (a). The relative integrals and values of σ for the two functions in the fit are $I_1 = 0\cdot41$, $\sigma_1 = 3\cdot2$, $I_2 = 0\cdot59$ and $\sigma_2 = 11\cdot3$. Values of x_o were calculated to be the same within experimental error for the two functions in the sum.

(a)   (b)

Fig. 2. The number of intensities predicted to be negative by relation (1) as a function of the twinning fraction x. We choose to call this a 'Britton' plot. (a) Three-dimensional, 5·0 Å-resolution data from crystals of B-phycoerythrin measured by rotation photography (crystal 1 in Table 1). (b) The same sort of data from an isomorphous heavy-atom derivative of B-phycoerythrin (crystal 3 in Table 1).

well defined volume fractions. Otherwise, pairwise estimation of twinning differences was used to provide an initial estimate of the volume fraction for preliminary studies, such as calculation of difference-Patterson functions. Structure-factor least squares was used to provide volume fractions for the final structure solution. One should note that least-squares-refined volume fractions lie within the range of values suggested by the 'Britton' plots and the values determined by R minimization lie close to these. Values obtained by pairwise estimation fit less well. We have taken steps to ensure that these differences are not a consequence of the effective weighting of observations in each technique. In the 'Britton' method, the volume fraction is defined by the few intensity pairs with the smallest values of $I_1/(I_1 + I_2)$. In least-squares refinement and, by analogy, in R minimization, the contribution of each pair of measurements depends upon $d(J_1)/dx$ which is proportional to $(I_2 - I_1)$. Thus, in these three cases the contribution is greatest when the difference between twinning-related intensities is large. In order to mimic these weighting schemes we have used only those intensity pairs for which $I_1/(I_1 + I_2)$ is less than 0·25, where I_1 is the smaller intensity, in preparation of Figs. 3(a) and 3(b). When this limit is not imposed, the curves become broader but the estimates of the volume fraction remain the same. We have not been able to deduce the reason for the large deviation of pairwise-estimated volume fractions from those estimated in other ways.

Accuracy of twinning-corrected data

To assess the efficacy of the corrections we have made on diffraction data from B-phycoerythrin crystals, we shall analyze the propagation of errors into the corrected intensities, discuss briefly the results of our low-resolution structure solution, and analyze the errors which would be introduced into an electron-density map if no twinning correction was made.

A conventional expression for estimation of error propagation is that given by Hamilton (1964): for $f(x_1, x_2, \ldots)$,

$$\sigma_f^2 = \sum_i \left(\frac{\partial f}{\partial x_i}\right)^2 \sigma_{x_i}^2.$$

One can apply this relation to (1) to obtain

$$\sigma_{J_1} = \left[(1-x)^2 \sigma_{I_1}^2 + x^2 \sigma_{I_2}^2 + \left(\frac{I_1 - I_2}{1 - 2x}\right)^2 \sigma_x^2\right]^{1/2} \bigg/ (1 - 2x). \qquad (3)$$

A few simple manipulations will reveal the behavior of this function. In the best case, σ_x is small. If $\sigma_{I_1} \simeq \sigma_{I_2}$, (3) reduces to

$$\sigma_{J_1} = \frac{(1 - 2x + 2x^2)^{1/2}}{1 - 2x} \sigma_I$$

which becomes $1 \cdot 1 \sigma_I$ for $x = 0 \cdot 1$, $1 \cdot 6 \sigma_I$ for $x = 0 \cdot 25$ and $2 \cdot 2 \sigma_I$ for $x = 0 \cdot 33$. If the error in x is substantial and the difference between I_1 and I_2 is large, the situation will be much worse than this. To assure oneself that the correction is indeed greater than the error introduced, one can compare (1) to its expected standard deviation. Expression (1) can be rearranged to get

$$J_1 = I_1 + \frac{x}{1 - 2x}(I_1 - I_2).$$

If σ_I is small, (3) becomes

$$\sigma_{J_1} = \frac{\sigma_x}{(1 - 2x)^2} |I_1 - I_2|.$$

Therefore, so as long as $\sigma_x/(1 - 2x)^2$ is less than $x/(1 - 2x)$, or $\sigma_x < x(1 - 2x)$, the correction is significant.

Table 1. *Twinning fractions (%) estimated for diffraction data from crystals of B-phycoerythrin*

The 3200 data in the analysis were measured by rotation photography to 5·0 Å resolution. Those twinning fractions marked * were judged to be accurately estimated from 'Britton' plots and were fixed at the values shown for least-squares heavy-atom refinement. In columns labeled a and b, the data from the crystal marked † were corrected for twinning according to the fraction shown and these corrected data were used as the basis for estimation of the other twinning fractions in that column. In column c the estimates were prepared by use of uncorrected data from crystal 2 as a basis for comparison. A constant value was then added to all estimates to bring the entire column to the approximate levels of columns a and b for that method of estimation.

			Method of estimation							
		Heavy-atom								
		least	R minimization				Pairwise estimation			
Crystal	'Britton' plot	squares	a	b	c	mean	a	b	c	mean
1. Native	5·9–6·2	6·0*	6·0†	5·0	5·7	5·6	6·0†	8·0	7·0	7·0
2. Derivative 1	0·85–0·95	0·9*	−0·45	0·9†	1·5	0·65	−0·75	0·9†	0·4	0·2
3. Derivative 1'	1·9–4·5	2·9	4·5	2·3	3·0	3·3	4·6	4·8	4·2	4·5
4. Derivative 2	1·9–4·3	2·2	3·7	1·4	2·1	2·4	1·8	2·7	3·3	2·6
5. Derivative 3	7·2–8·9	7·5	8·7	6·3	7·0	7·3	9·5	8·8	8·3	8·9

This means that for small x σ_x must be less than x. The largest upper limit for σ_x is 0·125 at $x = 0·25$.

Results of the structure determination of B-phycoerythrin at 5·0 Å resolution will be described in detail elsewhere (Fisher, Woods, Fuchs & Sweet, 1980). Some of the conventional indicators of the quality of the multiple isomorphous replacement phasing are shown in Table 2. The evidence that the structure solution, derived from twinning-corrected data, is substantially correct is as follows. (1) The indicators in Table 2 are consistent with a correctly determined structure. (2) The electron-density map clearly reveals a molecular symmetry element which we had not confirmed in any other way. (3) The structure consists largely of extended regions of connected density. (4) The molecule contains a region of uniform low density which is presumably occupied by a single disordered polypeptide chain (Sweet, Fuchs, Fisher & Glazer, 1977; Fisher, Woods, Fuchs & Sweet, 1980). From these results we conclude that the corrections were at least sufficient to provide a solution to the structure. Whether they were necessary is examined through an additional analysis of the data.

To assess quantitatively the effect of the twinning correction on the final electron-density map, an artificial heavy-atom refinement was performed. Data which had not been corrected for twinning, coming from both the native and the heavy-atom-derivative crystals, were used for the refinement; the starting parameters for the heavy atoms were those which were used in the structure solution (Fisher, Woods, Fuchs & Sweet, 1980). At the conclusion of the refinement the least-squares residuals and other indicators were virtually the same as those shown in Table 2. The lack of improvement in phasing statistics after correction for twinning may result from the slight increase in random error, described earlier, which accompanies correction of the systematic twinning error. The new and incorrectly phased transform values $(mF)_{uncorr}$ were clearly different from the correctly phased, twinning-corrected ones, $(mF)_{corr}$. Values of $\Delta(mF) = (mF)_{uncorr} - (mF)_{corr}$, and phase differences, $\Delta\varphi$, between F_{corr} and F_{uncorr} were evaluated for each reflection, where m is the figure of merit of the phase (Dickerson, Weinzierl &

Table 2. *Statistics of phasing and refinement*

$F_c = \langle f_H^2 \rangle^{1/2}$; $E = \langle \varepsilon^2 \rangle^{1/2}$; $R_K = \langle \varepsilon \rangle / \langle F_{PH} \rangle$; $\varepsilon = |F_{PH}| - |F_P + f_H|$. F_P, F_{PH} and f_H are structure factors for native protein, protein derivative and the heavy atom, respectively. Means are evaluated for all reflections. Structure factors are on an arbitrary scale.

Derivative	F_c	E	R_K (%)
1	0·75	0·43	6·7
1'	0·72	0·39	6·3
2	0·85	0·46	7·2
3	0·41	0·26	4·0

Palmer, 1968). The most significant trends in values of $|\Delta\varphi|$ and $|\Delta(mF)|$ were seen when average values were tabulated as a function of the ratio $Z = J_1/(J_1 + J_2)$. Although the mean value of $|\Delta(mF)|$ varies over less than a factor of two throughout the range of Z, it is largest at the extremes. As one might expect, $|\Delta\varphi|$, which as a mean of 22° for all data, is largest at small values of Z, decreasing smoothly as Z increases to 1·0.

More to the point of structure determination, one can analyze, in terms of the $\Delta(mF)$, the errors which would be introduced into an electron-density map by failure to correct for crystal twinning. One can show that the r.m.s. magnitude of an inverse Fourier transform is proportional to the r.m.s. modulus of the transform. Thus we can calculate the 'relative error' in an electron-density map as

$$\frac{\langle \Delta\rho^2 \rangle^{1/2}}{\langle \rho^2 \rangle^{1/2}} = \frac{\langle \Delta(mF)^2 \rangle^{1/2}}{\langle (mF)^2 \rangle^{1/2}}.$$

When F_{000} is included in the mean, the relative error is 0·05. This means that an electron-density map calculated from uncorrected data would contain errors, the r.m.s. value of which is equivalent to one twentieth of the total matter in the structure. On the other hand, one rarely calculates electron-density maps which include F_{000}. When this term is excluded the relative error becomes 0·3. Maps calculated from $(mF)_{corr}$, $(mF)_{uncorr}$, and $\Delta(mF)$ confirm that this theoretical treatment of error is quantitatively correct. The maps also show, however, that a mean phase difference of 22° is not particularly significant: although there are differences in detail, the general features of the electron density are quite similar in the maps calculated from corrected and uncorrected data. Thus it seems likely that the twinning corrections we have made are important to our having determined the correct structure, although features of the correct structure may have been apparent without them. Further studies will show if the significance of the twinning corrections increases for a higher-resolution structure and if the corrections can be made at all.

The methods we have developed are able to correct errors in diffraction data which result from twinning by merohedry. Our analysis of the twinning correction of B-phycoerythrin data shows that because the crystals used in data collection were twinned very little, the overall improvement of the final structure was small. However, the analysis does suggest that problems in macromolecular crystallography with substantially larger degrees of twinning can be tackled with confidence of success.

A number of features of the twinning-correction problem lead to suggestions in experimental design which may be of use to others. The first is that if diffraction intensities are to be measured one at a time, e.g. on a difractometer, extra effort could be put into measuring the data which show the largest differences

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The Acoustic Gyrotropic Tensor in Crystals

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Abstract

The acoustic gyrotropic tensor is a fifth-rank tensor characterized by $d_{ij,l} = -d_{ji,l}$, with $i, j = 11, 22, 33$, $(23, 32), (31, 13), (12, 21)$, $l = 1, 2, 3$, and controls the acoustical activity in crystals. With the employment of group theoretical methods, the number of independent coefficients of this tensor and the character for this tensor under proper and improper rotation are worked out. A classification of the acoustically active classes is given.

Introduction

The phenomenon of acoustical activity refers to the rotation of the plane of polarization of a transverse acoustic wave propagating along the acoustic axis. According to Portigal & Burstein (1968), who predicted this effect, acoustical activity arises due to first-order spatial dispersion contributions to elastic constants, just as optical activity is the result of first-order spatial dispersion contributions to the dielectric constant. Consequently, the velocity degeneracy of the linearly polarized transverse acoustic phonons at $k = 0$ is lifted at finite k where k is the phonon wave vector. The two split modes are left and right circularly polarized along the acoustic axis and they propagate with different phase velocities. This phase-velocity difference leads to the rotation of the plane of polarization.

Direct observation of acoustical activity in α-quartz by Brillouin scattering techniques has been reported by Pine (1970). The splitting of the degenerate optical phonons due to first-order spatial dispersion has also been observed by Pine & Dresselhaus (1969) in the low-temperature Raman spectrum of α-quartz.

Portigal & Burstein (1968) have identified the non-vanishing coefficients of the acoustic gyrotropic tensor for point groups T, T_d and O from symmetry considerations. We have derived the number of non-vanishing coefficients of this tensor and identified