One-Dimensional Disorder in Spinach Ribulose Bisphosphate Carboxylase Crystals

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Abstract

X-ray diffraction photographs of the crystalline ternary complex of spinach ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) with activators Mg^{2+} and CO_2 diffract to high angle while showing diffraction features symptomatic of disorder. Oscillation photographs about the c^* axis taken with a conventional source and collimation show streaks of intensity along c^*. These intensity streaks can be resolved into satellite reflections, with underlying diffuse scattering, by employing synchrotron radiation with slits limiting the horizontal beam convergence from the monochromator and fine collimation. The disorder is discussed in terms of stacking faults between layers of molecules. The space group of a regular stack of layers, repeating every layer, would be $C2$. The observed space group $C222_1$, repeating every alternate layer, is due to the statistical 'back-to-back' arrangement of cells of $C2$ symmetry. Three models are used to predict various features of the diffraction by these crystals. The first model, with every layer randomly shifted by either $a/39$ or $-a/39$ with respect to the previous layer, predicts constant-intensity streaks for the maximally affected reflections. A rectangular modulation of odd layers within a framework of even layers predicts satellite reflections for these reflections. A random distribution of these layers predicts broadening of the affected reflections. The observation of superlattice reflections and broadening is best accounted for by considering an approximate superlattice which produces the satellite reflections with both deviations in period and by additional shifts of $\pm a/39$ leading to the diffuse features.

Introduction

Ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) catalyses the key steps in both photosynthesis and photorespiration in photosynthetic bacteria and plants. The enzyme is activated by forming a ternary complex enzyme-CO_2-Mg^{2+} (Lorimer, Badger & Andrews, 1976). The spinach enzyme, in common with the enzyme from other higher plants, is oligomeric, consisting of eight large and eight small subunits forming an L_8S_8 molecule of total molecular weight about 550 000. The intensity distribution of scattered X-rays and electron microscopy suggests that the molecule has approximate 422 symmetry. The molecular symmetry required in the ternary complex crystals described here will be shown to be 222.

The rubisco crystals

The ternary complex of spinach rubisco with the activators Mg^{2+} and CO_2 was crystallized according to Barcena, Pickersgill, Adams, Phillips & Whatley (1983). These crystals, grown from 7·2% polyethylene glycol at carbon dioxide and magnesium ion concentrations and a pH corresponding to maximum activation, are truncated right-angled prisms. The crystals used in this study were approximately $0.4 \times 0.3 \times 0.1$ mm in dimension. The principal axes were identified by precession photography; the symmetry and the positions of the spots indicate an orthogonal cell of dimensions $a = 158.6$ (5), $b = 158.6$ (5) and $c = 203.4$ (7) Å with $\alpha = \beta = \gamma = 90°$. The absences are: general reflections, $h + k = 2n + 1$; $0kl$, $l = 2n + 1$. Further, the $h0l$ and $hk0$ zones show an additional alternating pattern of absences given in Table 1. The mosaic spread of the $hk0$ zone is of the order of 2° which is higher than that of the other zones whose mosaic spreads are of the order of 0·2°. Even so the crystals diffract remarkably well, to better than 1·8 Å, with the promise of a detailed structural analysis.

The mm symmetry of the precession photographs and the absences are consistent with the space group $C222_1$, with four molecules in the unit cell in special positions of the type $x, 0, 0$. The absence pattern given in Table 1 is a consequence of the special positions. The crystal may be considered as a regular stack of layers along the c axis. All even layers are at the origin in both **a** and **b** but all odd layers are at Δ in **a** and at the origin in **b**. The crystallographic origin of the $C222_1$ cell is then midway in **a** between the origin of the even and odd layers. The phase relationship between adjacent layers for constructive interference is given by

$$2\pi(h\Delta + l/2) = 2\pi n \qquad n = 1, 2, 3, \ldots . \quad (1)$$

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Table 1. *Additional absences*

h0l zone. General absences: $h = 2n + 1$

Range of h	Additional absences
0–4	$l = 2n + 1$
6–14	none
16–24	$l = 2n$
26–32	none
34–42	$l = 2n + 1$

hk0 zone. General absences: $h + k = 2n + 1$

Range of h	Additional absences
0–4	$h = 2n + 1, k = 2n + 1$
5–13	none
15–25	$h = 2n, k = 2n$
26–33	none
34–42	$h = 2n + 1, k = 2n + 1$

The observation is that the l index is odd when the h index is 20 and the l index is even when the h index is 39, hence Δ is either $a/39$ or $-a/39$. This corresponds to rubisco molecules at the special positions $(1/78, 0, 0)$, $(1/2 + 1/78, 1/2, 0)$, $(-1/78, 0, 1/2)$ and $(1/2 - 1/78, 1/2, 1/2)$. These positions are indicated in Fig. 3(c). The cell volume is consistent with four molecules of rubisco per unit cell.

Oscillation photographs, with the c^* axis coincident with the oscillation axis, show unusual diffraction features. Some of the reciprocal-lattice lines contain sharp spots while others contain streaks drawn out along c^* (Fig. 1). The sharp and diffuse spots vary as a function of h and the sharp spots exhibit the alternating absence pattern discussed above. The spots drawn out along c^* occur in the regions for which there are no additional absences. Sharp reflections are to be found around h equal to 0, 20 and 39 and diffuse reflections drawn out along c^* around h equal to 10 and 30. The intensity of the broadened reflections shows some fine structure which can be resolved by using the Synchrotron Radiation Source at Daresbury with a finely collimated X-ray beam. This was achieved by limiting the convergence of the X-ray beam from the monochromator in the horizontal plane to 0·6 mrad and employing a 0·2 mm collimator. Satellite-type reflections with diffuse background can then be seen in the region of h equal to 10 and 30 (Fig. 2). These strange diffraction effects suggest that the crystals are disordered.

The total amplitude of X-rays diffracted along c^* by a regular stack of layers along the c axis may be written as

$$A(w) = F \sum_{z=0}^{N-1} \exp(2\pi i w z), \qquad (2)$$

where F is the structure factor of a layer and the disorder to be considered is in the stacking of layers; z and w are fractional positions in the real lattice along c and in the reciprocal lattice along c^* respectively; and N is the number of unit cells along the c axis. The summation over z is the Laue function $[G(w)]$ which is equal to N, provided N is large,

when w is equal to l (where l is an integer) but zero otherwise. This corresponds to sharp reflections along c^*. A stacking fault perturbs the periodicity along c so that the Laue condition is no longer valid; diffuse intensity then occurs. The $0kl$ zone which is free of diffuse scattering and satellite reflections and the evenly spaced $00l$ reflections imply this disorder is limited to displacements parallel to a.

The absence pattern, diffuse reflections and disorder

Suppose there is a random distribution of layers with each successive layer displaced by $\pm\Delta$ parallel to the a axis with respect to the previous layer. Then every alternate layer can have its origin coincident with that of the original layer. The unit cell of a single repeat, as shown in Fig. 3, is $C2$ with two rubisco molecules per unit cell at special positions of the type $(0, y, 0)$. The rubisco molecules are required to have a molecular twofold axis coincident with the crystallographic twofold axis along the b axis. The statistical repeat of this random distribution is two layers with a statistical twofold axis parallel to b and a statistical 2_1 axis parallel to c. The space group of the averaged
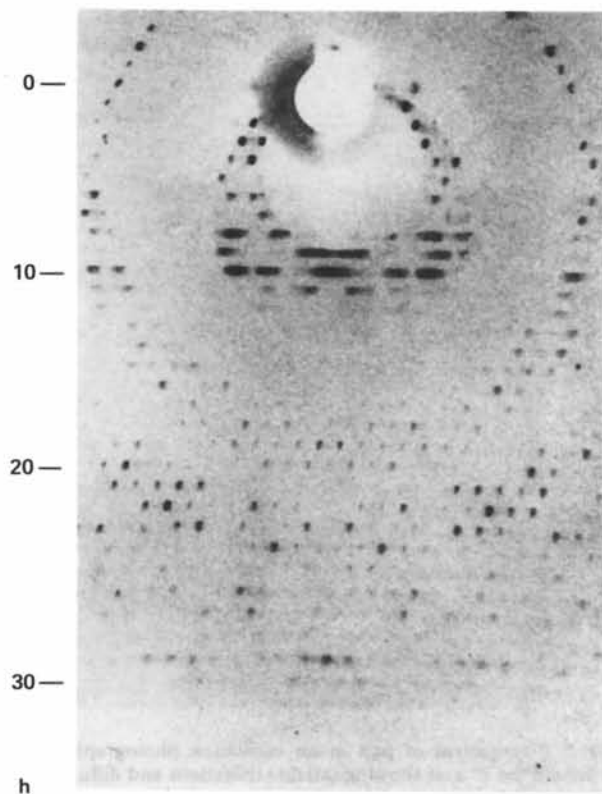

Fig. 1. Enlargement of part of an oscillation photograph taken around the c^* axis showing broad diffuse reflections in the region of h equal to 10. This photograph was taken using a conventional rotating-anode source ($\lambda = 1·5418$ Å, 40 kV, 40 mA). The crystal-to-film distance was 150 mm corresponding to a resolution of 4·14 Å at the edge of the film. A 0·6 mm collimator, an oscillation range of 3·0° and exposure time of 250 min were used.

structure is $C222_1$ with four molecules in the unit cell, as observed. The rubisco molecule apparently possesses 222 symmetry since the arrangement in $C2$ demands a molecular twofold axis parallel to the b axis and the statistical space group $C222_1$ requires a molecular twofold axis parallel to the a axis. The absences are not consistent with the space group $C222$. A statistical space group was encountered in imidazole methaemoglobin (Bragg & Howells, 1954; Cochran & Howells, 1954) and in catalase (Glauser & Rossmann, 1966). From the statistical argument of Cochran & Howells (1954) as employed by Glauser & Rossman (1966) the Laue function may be shown to be

$$G(w) = \frac{[1 - K^N \exp(\pi i N w)]}{[1 - K \exp(\pi i w)]}, \qquad (3)$$

where K is $\cos(2\pi h/39)$. When h is 0 or 39 then K is $+1$ and $|G(w)|$ is equal to N for w equal to even values of l and when h is 20 then K is -1 and $|G(w)|$ is equal to N for w equal to odd values of l. For h equal to 10 or 30 K is 0 and $|G(w)|$ is equal to 1 for all values of w. The alternating diffuse scattering
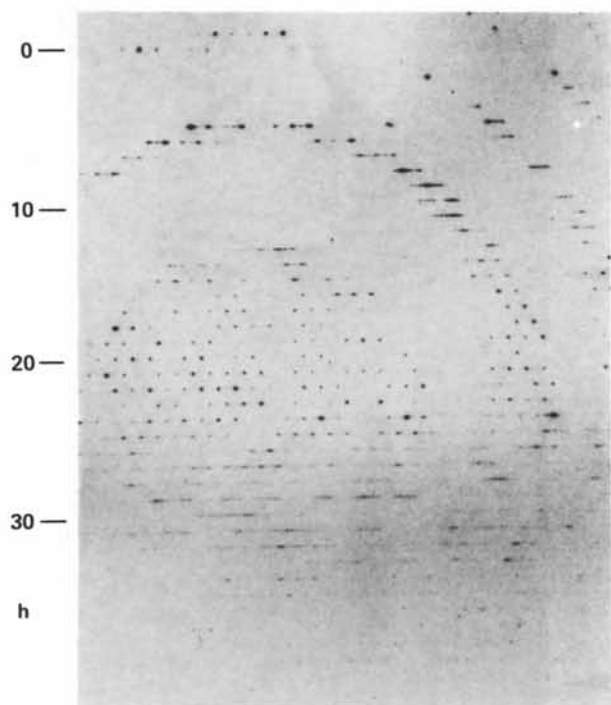

0 —

10 —

20 —

30 —

h

Fig. 2. Enlargement of part of an oscillation photograph taken around the c^* axis showing satellite reflections and diffuse scattering in the regions of h equal to 10 and 30. This photograph was taken using the Daresbury Synchrotron Radiation Source ($\lambda = 0.88$ Å, 1.8 GeV, 235 mA). In order to resolve the satellite reflections a 0.2 mm collimator was used and the horizontal beam convergence was limited to 0.6 mrad. The crystal-to-film distance was 144 mm corresponding to a resolution at the edge of the film of 2.28 Å. An oscillation range of 1.5° and exposure time of 75 min were used.

pattern predicted by this model is clearly in agreement with the observed pattern of absences in l as a function of h. This is to be expected because the statistical space group is $C222_1$ with the rubisco molecules at special positions. The variation in the breadth of the reflections along c^* depending on the value of h is also in agreement with observation. The most diffuse reflections are for h equal to 10 and 30 for which the prediction is a constant streak along c^* and sharp reflections are predicted for h equal to 0, 20 and 39. While the variation of the disorder with h is correctly predicted the observation for h equal to 10 and 30 is not constant intensity along c^* but under conditions of fine collimation a series of satellite reflections along c^*.

The superlattice approximation

Satellite reflections are the result of a periodic modulation of the layers within the crystal. Consider an extension of the argument used to arrive at (1). All even layers are at the origin in both $\mathbf{a}$ and $\mathbf{b}$, as before, but instead of all odd layers being at either $a/39$ or $-a/39$ they are modulated periodically such that $M/2$ odd layers are at $a/39$ followed by $M/2$ odd layers at $-a/39$. The modulation is assumed to be symmetric on average because there is no reason to assume that either $a/39$ shifts or $-a/39$ shifts are preferred in the crystal. The superlattice period is M odd layers and the modulation may be expressed as

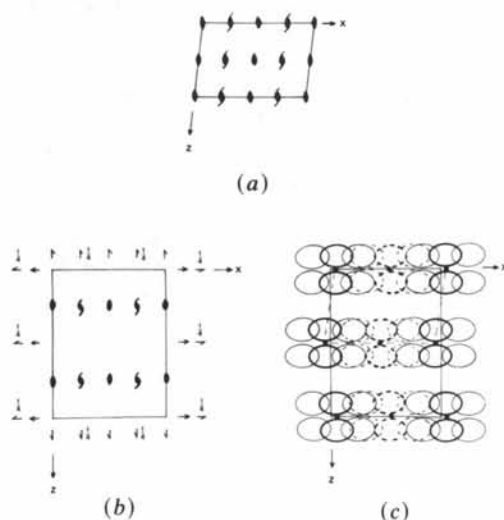$$\mathbf{x} = \mathbf{a}x(z). \qquad (4)$$

(a)

(b)   (c)

Fig. 3. (a) The $C2$ cell of a regular stack of layers repeating every layer. (b) The statistical $C222_1$ cell arising from the average 'back-to-back' arrangement of cells of $C2$ symmetry. (c) The packing of the rubisco molecules in the $C2$ and $C222_1$ cells. One large plus one small subunit (LS) of the rubisco oligomer is represented by an ellipse, molecules centred at $y = 0$ are solid and those centred at $y = \frac{1}{2}$ are dashed. Bold lines indicate a superposition of two LS units in projection.

The total amplitude diffracted from a periodic modulation of layers as a function of w may be written as

$$A(w) = F \sum_{z=0}^{N-1} \exp 2\pi i[wz + hx(z)], \qquad (5)$$

where F is the structure factor of a layer and where there are N odd layers in the crystal. The function $x(z)$ is periodic such that

$$x(z) = x(qM + s) = x(s) \qquad (6)$$

where $q = 0, 1, 2, \ldots, (N/M)$ and $s = 0, 1, 2, \ldots, (M-1)$. This allows the amplitude to be split into two sums, one over whole periods (q) and one within periods (s). If one assumes that N is an integral multiple of M then

$$A(w) = F \sum_{q=0}^{(N/M)-1} \exp (2\pi i q M w)$$

$$\times \sum_{s=0}^{M-1} \exp 2\pi i[ws + hx(s)]. \qquad (7)$$

Use of the Laue condition, as applied to (2), and substitution of $x(s)$ as $+1/39$ for $0 \le s < M/2$ and $-1/39$ for $M/2 \le s < M$ gives

$$A(w) = FN/M \left[\sum_{s=0}^{(M/2)-1} \exp 2\pi i(ws + h/39) \right.$$

$$\left. \times \sum_{s=M/2}^{M-1} \exp 2\pi i(ws - h/39) \right]. \qquad (8)$$

This can be simplified by the use of trigonometric identities to yield the amplitudes of the main and satellite reflections (Böhm, 1975):

$$A(P/M) = NF|\cos 2\pi h/39| \quad \text{main reflections}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad (9)$$
$$A(P/M) = NF\frac{|\sin 2\pi h/39|}{|\sin (n\pi/M)|} \quad \text{satellite reflections,}$$

where $P = Mr + n$, r is an integer and $-M/2 \le n \le M/2$, where n refers to the order of the satellite between two main reflections.

The contribution of the modulated layers to the sharp reflections varies as $\cos (2\pi h/39)$. This is in agreement with the conclusion drawn from (3). The satellite reflections vary as $\sin (2\pi h/39)$. The region for which (3) predicted maximum disorder now corresponds to the region for which the satellites are maximum. The observed satellites cannot easily be indexed but the average modulation period is of the order of 2000 Å. The alternating pattern of absences is accounted for in the same way as before since the average space group is $C222_1$ with four molecules at special positions in the unit cell.

Random distribution of odd layers shifted by either $a/39$ or $-a/39$

Instead of a modulation of the odd layers consider a random distribution of these layers. The ordered arrangement of even layers and random distribution of odd layers provide a situation intermediate between the models already discussed. The even layers are defined as A layers and the odd layers are either B layers (shifted by $a/39$ with respect to A layers) or C layers (shifted by $-a/39$ with respect to A layers). The variation of the intensity along c^* as a function of h and w may be calculated for this model using the approach of Wilson (1949). If the probability of a fault is α and is the same for either B faulting to C or vice versa then, assuming that the first B or C layer is a B layer, the probability of any odd layer being a B layer is given by

$$B_n = \tfrac{1}{2}[1 + (1 - 2\alpha)^{(n-1)}]. \qquad (10)$$

The contribution of the random distribution of odd layers to the total intensity may now be calculated using the approximations of Wilson (1949, ch. V):

$$I(w) = NF_A^2[\cos^2 (2\pi h/39) + \tfrac{1}{2} \sin^2 (4\pi h/39)]$$

$$+ NF_A^2\{\alpha/[\alpha^2 + (2\pi w)^2 \sin^2 (2\pi h/39)]$$

$$- \pi w/[\alpha^2 + (2\pi w)^2 \sin (4\pi h/39)]\}. \qquad (11)$$

The same result is obtained if a C layer is chosen as the first odd layer.

The amplitude of the sharp reflections varies as $\cos (2\pi h/39)$. The intensity of the reflections for h indices of 10 and 30 are broadened along c^* by an amount proportional to α:

$$I(w) = NF_A^2\alpha/[\alpha^2 + (2\pi w)^2]. \qquad (12)$$

These results are in good agreement with the predictions from the previous approaches. Instead of constant intensity or satellites, broadening of the affected reflections is predicted. This model also predicts a displacement of the intensity from the origin for reflections for which h is other than 0, 10, 20, 30 or 39. This last term in (11) is not periodic in w but falls off with increasing w. Further, the contribution of this term is maximum when there are also contributions from all the other terms. It is therefore not surprising that this effect has not been experimentally separated from the broadening and satellite effects.

Concluding remarks

The models discussed above predict various features of the diffraction by rubisco crystals:

(i) The models have an average space group of $C222_1$ with four molecules at special positions which account for the alternating pattern of absences.

(ii) The model with each successive layer displaced by $\pm a/39$ with respect to the previous layer correctly

indicates within which part of reciprocal space reflections are nearly sharp or diffuse. The observed diffuseness is less than the predicted constant-intensity streak which implies the disorder in the crystal is less than in this model.

(iii) A regular rectangular modulation of odd layers, within a rigid framework of even layers, gives rise to satellite reflections which vary with h in the same way as the diffuse reflections of (ii). The situation in the crystal is clearly somewhere between this and the previous model.

(iv) The random distribution of odd layers predicts broadening of the reflections for which the other models suggested constant-intensity streaks and satellite reflections respectively.

The situation in the crystal may be described as a combination of these effects. There is an approximate superlattice which gives rise to the satellite reflections and variation from this superlattice, in period and also by shifts of $\pm a/39$, which gives rise to the diffuse features observed on the photographs. There is clearly a tendency for alternate layers to be the same and it is the crude modelling of this by the last approach that leads to the prediction of reflection broadening rather than constant-intensity streaks.

The implication of this result is that an electron density map calculated from these intensities would correspond to a superposition of enzyme molecules separated by about 4 Å. It is therefore not possible to determine the structure of the enzyme from these crystals. The most probable cause of this disorder is heterogeneity in the amino termini of the large subunit. This is being investigated with the aim of growing ordered crystals.

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Röntgenkleinwinkelbrechung an Metalldrähten, Glasfäden und hartelastischem Polypropylen

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Abstract

Very thin equatorial small-angle scattering of Cu $K\alpha$ radiation is observed from metallic wires, glass fibres and hard elastic polypropylene which can be explained quantitatively as a refraction phenomenon if the excess refractive indices $n = 1 - \varepsilon$ of the samples are smaller than unity. For Cu $K\alpha$ radiation and SiO_2 glass ε has a value of $7 \cdot 2 \times 10^{-6}$. The intensity I_s of reflection within a total reflection angle $\delta = 3 \cdot 8 \times 10^{-3} = 13'$ is negligibly small compared with the intensity of refraction I_B. In addition to refraction, diffraction by gaps and holes in strained polypropylene was observed. The scattering of 60 μm particles of Li–Ba silicate glass powders [Nau & Brückner (1971). *Kolloid Z. Z. Polym.* 244, 223-233] can be explained by transforming the observed scattering of spheres to cylinders yielding the refraction I_B as calculated.

I. Einleitung

An den untersuchten dünnen metallischen Drähten, Glasfäden oder hartelastischen Polymerfasern tritt stets eine bisher wenig beachtete Röntgenkleinwinkelstreuung auf, die sich als schmaler Streifen längs des Äquators hinzieht. Fig. 1 zeigt z.B. die mit Cu $K\alpha$-Strahlung erzeugte Kleinwinkelstreuung einer 40 μm