Table 3. *Coefficients of the anisotropic temperature factors* (Å², *standard deviations in parentheses in units of the last digits*) *for* 3,3′-*dimethoxy*-2,2′-*bithiophene*

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C1	0·044 (2)	0·035 (2)	0·041 (2)	−0·001 (2)	0·009 (1)	0·002 (2)
C2	0·066 (3)	0·040 (2)	0·044 (2)	−0·001 (2)	0·012 (2)	0·000 (2)
C3	0·093 (4)	0·052 (3)	0·056 (3)	0·004 (3)	0·019 (3)	−0·007 (3)
C4	0·072 (4)	0·078 (3)	0·068 (3)	0·026 (3)	0·035 (3)	−0·004 (3)
C5	0·094 (3)	0·088 (3)	0·051 (3)	−0·023 (3)	−0·008 (2)	−0·016 (3)
O1	0·064 (2)	0·080 (2)	0·048 (2)	−0·013 (2)	−0·003 (1)	−0·018 (1)
S1	0·050 (1)	0·064 (1)	0·065 (1)	0·006 (1)	0·011 (–)	0·000 (1)

Relative extensions of comparable bond angles determined for the crystal structure are also fairly well reproduced by the calculated parameters. The C–S–C angle is 91·8 (2)° in the crystal structure of DMTDT, and 91·5° in the 4-21G geometry of DHDDT. The differences, (C3–C2–C1) – (C2–C3–C4), and (C2–C1–S1) – (C3–C4–S1) are 1·8 (6) and −4·3 (6)° for crystal DMTDT; and 2·7 and −2·9°, for 4-21G DHDDT, respectively.

It is noteworthy that the bond lengths and angles as predicted by *ab initio* calculations compare well with those in the crystal structure. The origin of the quantitative differences discussed above cannot be determined from the present study. It is nevertheless interesting to find that the order of magnitude of these differences in relative bond distances and angles does not exceed a few hundredths of an ångström and a few degrees.

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Preliminary Study of a Phase Transformation in Insulin Crystals using Synchrotron-Radiation Laue Diffraction

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Abstract

Synchrotron-radiation Laue diffraction photographs have been recorded showing the transformation of single 4Zn insulin crystals [$a = 80{\cdot}7$ (1), $c = 37{\cdot}6$ (1) Å, space group $R3$] to 2Zn insulin [$a = 82{\cdot}5$ (1), $c = 34{\cdot}0$ (1) Å, space group $R3$]. The transformation was brought about by changing the mother liquor in the capillary in which the crystal was mounted. Photographs were taken at 10 min intervals (exposure time 3 s) from 0·5 h after mounting. They showed initially a well ordered 4Zn insulin crystal (d_{min} *ca*

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2·3 Å), then a poorly ordered, sometimes multiple, crystal, and finally a 2Zn insulin crystal, about as well ordered as the initial crystal.

Introduction

The protein insulin may be crystallized in two rhombohedral forms, known as 2Zn insulin and 4Zn insulin (Schlichtkrull, 1958). They have similar cell dimensions, $a = 82{\cdot}5$ (1), $c = 34{\cdot}0$ (1) Å and $a = 80{\cdot}7$ (1), $c = 37{\cdot}6$ (1) Å, for the orthohexagonal cells, with two insulin monomers per asymmetric unit. The three-dimensional structures of both forms have been determined and refined at high resolution (Adams *et al.*, 1969; Dodson, Dodson, Hodgkin & Reynolds, 1979; Bentley, Dodson, Dodson, Hodgkin & Mercola, 1976; Dodson, Dodson, Reynolds & Vallely, 1980). They are generally similar, but there are some important differences in conformation. In particular, the residues B1 to B8 of molecule 2 have an extended conformation in 2Zn insulin, while in 4Zn insulin they are part of a long α-helix (B1–B19), and the residue B1 is displaced by about 25 Å from its position in 2Zn insulin (Fig. 1). Transformation from 2Zn insulin to 4Zn insulin, or its reverse, can take place within the crystal and is controlled by the halide concentration in the mother liquor (Bentley, Dodson & Lewitova, 1978). This transition has also been observed in solution using circular dichroic spectroscopy (Renscheidt, Strassburger, Glatter, Wollmer, Dodson & Mercola, 1984). With conventional X-ray sources it has not been practicable to follow the time course of this transformation.

Laue diffraction photographs have been used recently at the SERC Daresbury Laboratory and at other synchrotron-radiation sources to record diffraction data on protein and other crystals with very short exposure

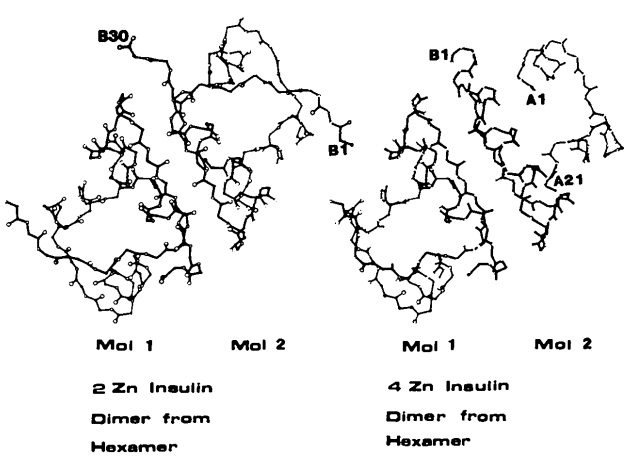


Fig. 1. The conformation of the insulin dimer in crystalline 2Zn insulin and 4Zn insulin; the most significant difference, in the N-terminal part of the B chain (residues B1 to B8) is seen in the upper right part of this view. The A chains are drawn with thinner lines, the B chains with thicker lines.

times (1 s or less) (Helliwell, 1985). It is thus possible to take a series of exposures at short intervals in time to study structural changes in crystals. This has been exploited by Hajdu *et al.* (1987) to study the interaction of phosphorylase-b with a substrate analogue; a sequence of exposures were made in minutes, each of which could, in principle, be processed to give an electron-density map or, better, a difference map showing the changes in electron density from one time to the next. We propose to perform a similar study of the transformation of 4Zn insulin to 2Zn insulin and make a preliminary report here.

Experimental

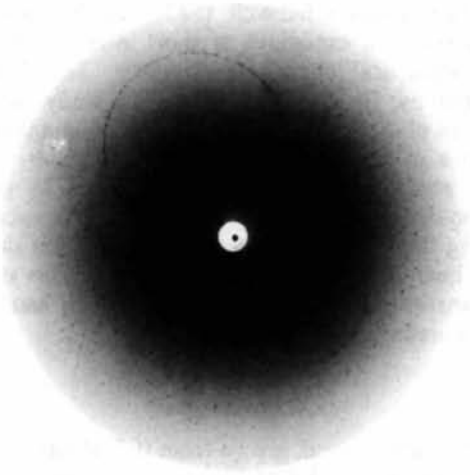
A 4Zn insulin crystal, grown from 0·2 *M* citrate buffer, pH 6·3, containing 0·6 *M* NaCl, was mounted in a glass capillary. The 4Zn insulin mother liquor was removed and replaced with 2Zn insulin mother liquor (*i.e.* chloride-free 0·2 *M* citrate buffer, pH 6·3) and the capillary sealed. The 2Zn insulin mother liquor was not allowed to bathe the crystal, but only to affect the crystal through its vapour pressure – 'distillation' from the new mother liquor will dilute the chloride ion in the film of liquid around the crystal. The capillary was quickly transferred to the X-ray camera on the SRS workstation 9·7 (Helliwell *et al.*, 1988). The first Laue diffraction pattern could be recorded 34 min after the buffer-solution change; subsequent photographs were taken at intervals of 10 min over a period of 2–3 h. The crystal–film distance was 81 mm, collimator 0·2 mm diameter, exposure time 3 s with the SRS running at 2 GeV and *ca* 200 mA, 40 s with the SRS in single-bunch mode with beam current of *ca* 10 mA. Seven crystals were examined; in four cases, including that illustrated below, the crystal was translated between successive exposures to allow the examination of a fresh volume of crystal, undamaged by radiation.

Preliminary interpretation of photographs

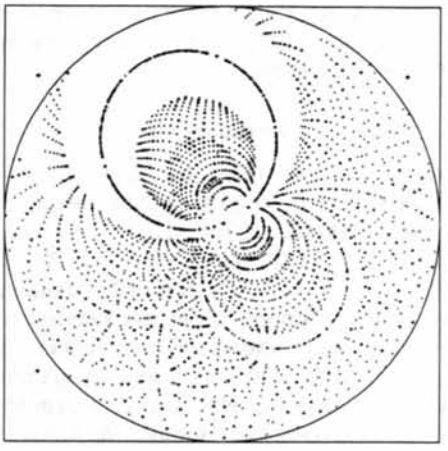
For most crystals three stages could be distinguished; they are illustrated in Figs. 2(*a*), 2(*b*) and 2(*c*), selected from thirteen photographs taken of one crystal.

Stage 1: a photograph taken 54 min after buffer change (Fig. 2*a*) showed sharp reflections, characteristic of a good-quality crystal; detailed analysis, in one case, confirmed that the crystal was 4Zn insulin (see below).

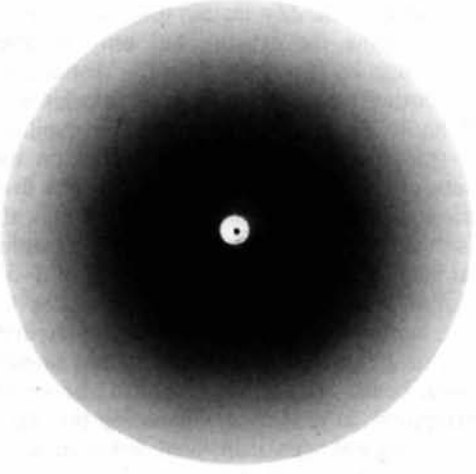
Stage 2: a photograph 64 min after buffer change (Fig. 2*b*) showed weaker more-diffuse reflections and fewer of them; this sparser distribution of spots indicates that some disordering of the crystal occurs relative to its state in stage 1. In some crystals two or three rings appeared, indicating two or three distinct crystals with slightly different cell dimensions and/or orientations.



(*a*)

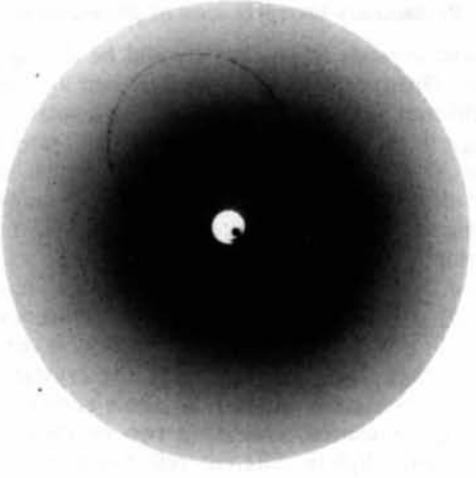


(*d*)

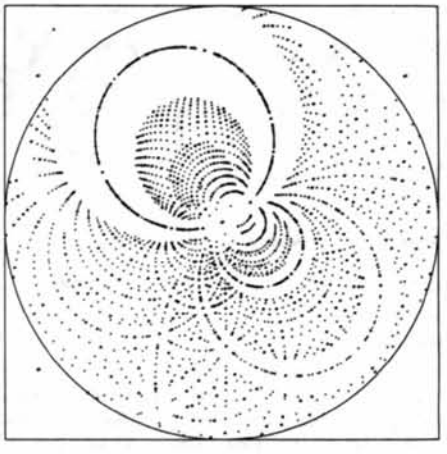


(*b*)

Fig. 2. (*a*), (*b*), (*c*) Laue diffraction patterns for (*a*) 4Zn insulin crystal, 54 min after mounting in the presence of halide-free buffer; (*b*) the same crystal (but a different portion) 64 min after mounting; (*c*) the same crystal (a third portion) 134 min after mounting. (*d*), (*e*) Predictions of Laue diffraction patterns for (*d*) 4Zn insulin and (*e*) 2Zn insulin giving the closest match with (*a*) and (*c*).



(*c*)



(*e*)

Stage 3: a photograph 134 min after buffer change (Fig. 2*c*) showed good sharp reflections similar in quality and in resolution to stage 1, indicating that the crystal has 'reordered'. A detailed analysis of reflection positions, below, showed that the pattern now corresponded to 2Zn insulin.

The other crystals examined showed the same stages, although the times varied somewhat from crystal to crystal, presumably depending on the amounts of old and new buffer sealed in the capillary. If the crystal was allowed to come into contact with the new buffer the transformation started before we could take the first Laue photograph.

Identification of crystal forms from Laue diffraction patterns

A program *LGEN* (Elder, 1987; Helliwell *et al.*, 1988) was used to confirm that the Laue diffraction at stage 1 can be well accounted for by the 4Zn insulin unit cell and not so satisfactorily by 2Zn insulin; conversely, the stage 3 pattern was shown to fit 2Zn insulin better than 4Zn insulin.

The program *LGEN* on an ICL PERQ computer allows input of nodal spot positions with the puck and tablet, indentification of the crystal orientation (defined by three angles) when the unit-cell edges and crystal–film distance and lattice type have been specified. Refinement is performed by adjusting the orientation angles to minimize the r.m.s. displacement of observed and calculated spot positions. The results are shown in Table 1.

Clearly the stage 1 photograph matches 4Zn insulin and the stage 3 photograph matches 2Zn insulin. The same conclusion had been reached, qualitatively, by comparing the full-pattern simulations; the best-fitting simulations are shown in Figs. 2(*d*) and 2(*e*); they also show that d_{min} is *ca* 2·2 Å for the initial 4Zn insulin crystal and also for the final 2Zn insulin crystal.

Concluding remarks

We have shown, by a sequence of Laue diffraction photographs, that, when a 4Zn insulin crystal is brought into contact with the vapour from the crystallization buffer of 2Zn insulin, the 4Zn insulin crystal is transformed to a reasonably well ordered crystal of 2Zn insulin within about 2 h, and that while the transformation is taking place the crystal is less well ordered.

A similar disordering followed by reordering was observed by Hajdu *et al.* (1987) when phosphate buffer was introduced to their phosphorylase-b crystal. We intend to use a flow cell at 277 K to take photographs suitable for intensity measurements at various stages of this transformation and its reverse, process them and calculate electron-density maps to reveal the course of the reaction.

Table 1. *Refinement of orientation angles to account for nine nodal spots on Laue diffraction photographs*

The angles φ_x, φ_y, φ_z represent rotation about z, then y, then x, from an initial orientation with c parallel to z, the camera spindle, and a^* parallel to x, the X-ray beam.

Photo	Unit cell	φ_x(°)	φ_y(°)	φ_z(°)	R.m.s. error (mm)
Stage 1	2Zn insulin	173·9	−0·6	106·1	6·43
	4Zn insulin	−104·3	−3·8	84·9	**0·49**
Stage 3	2Zn insulin	−104·5	−6·7	85·2	**0·39**
	4Zn insulin	170·8	−7·9	129·6	1·31

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