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X-ray Laue diffraction from crystals of xylose isomerase

(glucose isomerase/protein crystallography)

GREGORY K. FARBER[†‡], PELLA MACHIN[§¶], STEVEN C. ALMO[†||], GREGORY A. PETSKO[†], AND JANOS HAJDU[**]

[†]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; [§]Science and Engineering Research Council Daresbury Laboratory, Daresbury, Warrington, Cheshire, WA4 4AD, United Kingdom; [||]Department of Biophysics, Harvard University, Cambridge, MA 02139; and [**]Laboratory of Molecular Biophysics, Oxford OX1 3QU, United Kingdom

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ABSTRACT **The Laue method (stationary crystal, polychromatic x-rays) was used to collect native and heavy-atom-derivative data on crystals of xylose isomerase (EC 5.3.1.5). These data were used to find the heavy-atom positions. The positions found by use of Laue data are the same as those found by use of monochromatic data collected on a diffractometer. These results confirm that Laue diffraction data sets, which can be obtained on a millisecond time scale, can be used to locate small molecules bound to protein active sites. The successful determination of heavy-atom positions also indicates that x-ray crystallographic data collected by the Laue method can be used to solve protein structures.**

The use of the Laue technique (1), which involves a stationary crystal bathed in a beam of polychromatic x-rays, opens up a new realm of possible experiments in protein crystallography (2). Stations 9.6 and 9.7 on the synchrotron radiation source (SRS) at Daresbury, England are well suited for such Laue experiments (3, 4). A number of Laue experiments have been done at the SRS on both small molecules (5) and proteins (6, 7). We report here the successful use of Laue data to characterize a heavy-atom derivative of a protein crystal.

Crystals of xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5) were chosen for Laue experiments for several reasons. The structure of this enzyme has just been solved at 3-Å (1 Å = 100 pm) resolution by the multiple isomorphous replacement method with monochromatic data collected by conventional diffractometry (15). Large crystals that are not especially susceptible to radiation damage are easy to grow. These crystals belong to space group $P22_12_1$ but have strong pseudo body centering. To 3-Å resolution, the systematic absences corresponding to the pseudo space group $I222$ are almost perfectly obeyed. The unit-cell dimensions are a = 98.7 Å, b = 93.9 Å, c = 87.7 Å, and the asymmetric unit of the pseudo space group contains one subunit of molecular weight 43,000. In addition, the reaction catalyzed by xylose isomerase is slow in solution. With glucose as a substrate, the enzyme turns over about 10 times per second (8). Using the Laue method at the SRS, 70% of a complete data set to 2.5-Å resolution can be collected in 1 sec on these crystals (Fig. 1). Thus, if an appropriate trigger for the reaction could be developed, it would be possible to watch catalysis occur in the crystal. Subsequent to the experiments discussed in this communication, we believe that we have found such a trigger (G.K.F., unpublished data).

Before time-resolved protein crystallography of an enzyme–substrate complex can be attempted, it is essential to demonstrate that Laue diffraction data of accuracy sufficient to locate a small molecule bound to the active site of the enzyme can be collected and processed. We chose to try to find the binding sites of europium ions to xylose isomerase. The enzyme, which catalyzes the isomerization of glucose to fructose, requires Mg^{2+} for activity. We decided to use europium as a probe for the divalent cation binding site (9). In addition to being a test of the ability of the Laue diffraction technique to produce interpretable electron-density maps of the binding of small molecules to the active site of a protein, the europium experiment also provides a way to test whether Laue data can be used to locate heavy atoms in a protein crystal.

Data Collection

All data were collected at station 9.7 at Daresbury on CEA Reflex 25 film (12.5 × 12.5 cm). Each film pack had 6 or 10 films (A–F or A–J); no metal foils were interleaved between the films. Crystals were mounted with c^* along the capillary tube and perpendicular to the x-ray beam. The approximate locations of a^* and b^* were determined by crystal morphology. Three sets of photographs with crystal-to-film distances of 110–120 mm were taken on each crystal. The x-ray beam was aligned along a^* or b^* or was 45° between the two. The crystal was translated between each photograph. Exposure times were 1 or 2 sec, using polychromatic x-rays with an effective wavelength range of 0.2–2.5 Å. The A–F films of all three orientations for both crystals were scanned at Daresbury on a Scandig 3 rotating-drum microdensitometer with a 50-μm raster size.

Data Reduction

The data were reduced using a suite of programs written at Daresbury. The programs have been described in detail elsewhere (6, 10), so only an outline is presented here. Data reduction has four phases: finding and refining the crystal orientation, indexing spots and measuring their intensity, scaling the intensities of spots measured on films A–F, and normalizing these intensities based on the wavelength causing diffraction of a particular reflection.

Rough orientation parameters for the crystal were determined by comparing observed and calculated diffraction patterns on a ICL PERQ graphics terminal. These rough parameters were refined using program GENLAUE. The orientation parameters were the same as those used in the oscillation photography processing program IDXREF (11). GENLAUE produces a list of reflections that should be observed on the film. The intensities of these reflections were determined by integration using INTLAUE. INTLAUE is a modification (12) of the MOSFLM program used for oscillation photographs (11). AFSCALE was used to scale the data from the different films in a film pack together by using the equation $I_A = a\, I_B \exp(-b\lambda^3)$. This program also applies the obliquity correction (11) and the Lorentz-polarization correction. Finally, LAUENORM was used for wavelength normalization. This program normalizes reflections based on

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Abbreviation: SRS, synchrotron radiation source.
[‡]To whom reprint requests should be addressed.
[¶]Deceased March 7, 1987.

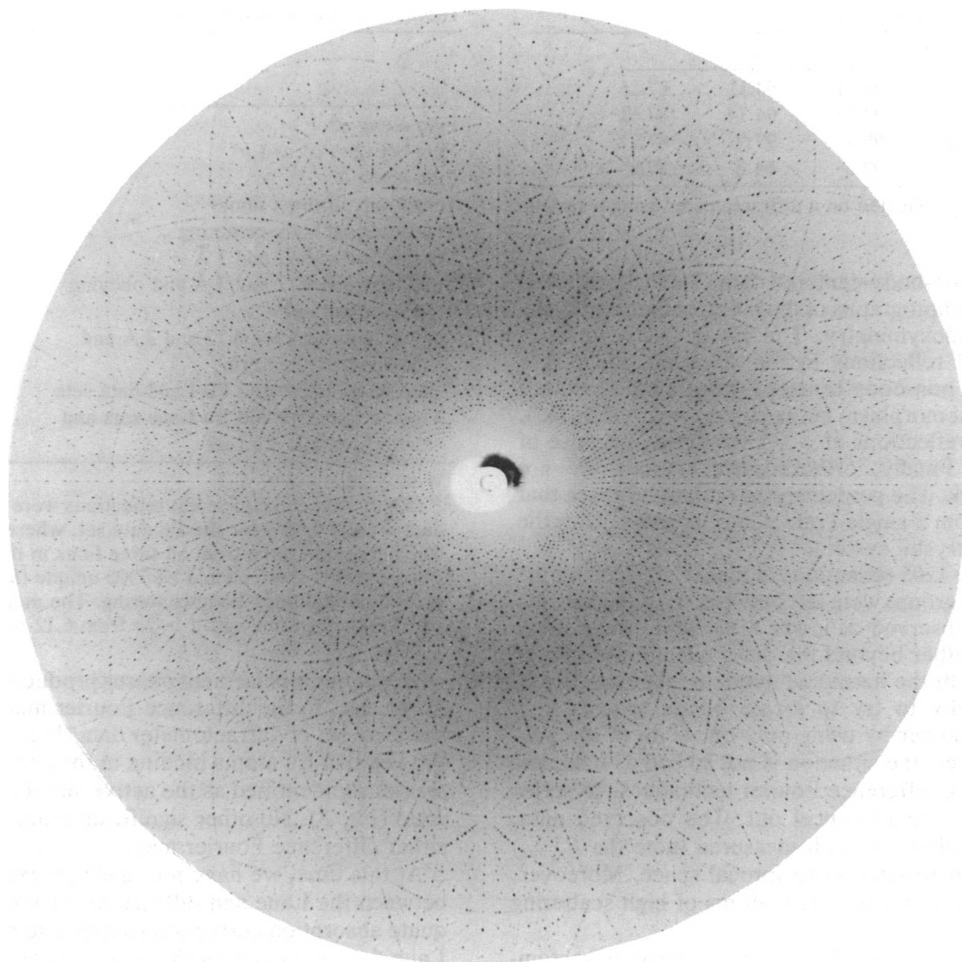

FIG. 1. The A film from the Eu-derivative crystal aligned along a^*.

the intensities of Friedel equivalent reflections observed at different wavelengths. This method of normalizing the intensity distribution differs from that used by Hajdu *et al.* (7, 13) in their Laue diffraction study of the binding of an oligosaccharide to crystals of glycogen phosphorylase. Those data were normalized by using the corresponding native structure amplitude from a reference data set obtained with monochromatic radiation. The method employed here does not require any reference data to scale intensities and depends solely upon the Laue diffraction intensities themselves.

During data reduction, we were careful to discard any questionable spots. Note that in Laue work, a distinction must be made between spots and reflections. A spot on a film may be composed of more than one Bragg reflection. Spatial overlaps, where two reflections are too close to be resolved, were discarded in GENLAUE. These were not a serious problem for our unit-cell dimensions at the crystal-to-film distance employed. Harmonic overlaps, where reflections superimpose exactly [e.g., the (1,1,1) and the (2,2,2)], were also discarded. Cruickshank *et al.* (14) have shown that contrary to earlier expectation, the number of harmonic overlaps is a small percentage of the total number of recorded reflections. Although methods are being tested to deconvolute the intensities of harmonic overlaps, it is comforting to know that they can be discarded with impunity. The results of data reduction are shown in Tables 1–3.

Discussion and Results

To judge the usefulness of the Laue data, we first calculated Patterson maps in an attempt to find heavy-atom positions. We ran into two problems during these calculations. The Eu-derivative crystals are not as strongly pseudo-body-centered as the native crystals. Because of this, we were

Table 1. Crystal orientation

	Orientation along a^*	Orientation 45° from a^* and b^*	Orientation along b^*
	Native crystal		
ϕ_x	−0.50°	−0.16°	0.16°
ϕ_y	1.60°	1.93°	1.94°
ϕ_z	−0.56°	−0.53°	−0.47°
Crystal-to-film,† mm	117.57	118.48	117.59
rms deviation‡	0.027°	0.020°	0.023°
No. of spots used for refinement	114	363	171
	Eu-derivative crystal		
ϕ_x	3.10°	2.76°	2.43°
ϕ_y	0.77°	1.06°	1.08°
ϕ_z	−0.34°	−0.31°	−0.32°
Crystal-to-film, mm	109.17	109.19	109.18
rms deviation‡	0.032°	0.029°	0.027°
No. of spots used for refinement	490	485	493

ϕ_x, ϕ_y, and ϕ_z are the crystal mis-setting angles (11).
†The film packs along a^* and b^* for the native crystal had 10 films. All other film packs had six films. This accounts for the different crystal-to-film distances.
‡The root-mean-square deviation in degrees between the observed and calculated diffraction vectors.

Table 2. Unit-cell dimensions (Å)

Crystal	*a*	*b*	*c*
Laue native	99.21	94.11	87.25
Laue Eu	99.25	94.14	87.61
Mono native	98.7	93.9	87.7
Mono Eu	99.5	94.2	87.8

Mono refers to data collected on a diffractometer using a sealed-tube x-ray source.

forced to discard non-body-centered data (Table 3). Fifteen-degree precession photographs of the *h0l* zone confirmed this difference in pseudosymmetry. The Eu derivative has 24 non-body-centered reflections in the *h0l* zone; the native crystal has only 11 non-body-centered reflections. The other problem was nonisomorphism between the two crystals (15). We discarded all reflections at >3-Å resolution because of nonisomorphism. Finally, orthorhombic crystals are not ideal for Laue work. The percentage of reciprocal space that can be covered from a single Laue photo increases with the symmetry of the crystal system.

As a result, only 1205 reflections of a possible 7300 (in the *I*222 pseudo space group) were left between 6.12 and 3 Å. No reflections were observed at lower resolution, since these reflections were either beyond the outer edge of the film or were discarded with the harmonic overlaps. At first glance, it would seem folly to try to locate bound species in a 43,000-dalton monomer by using only one-sixth of the possible data. However, the situation is not so bad as it seems. We intended to use difference Fourier techniques, so series termination errors would cancel out. The observed data, though few in number, are well measured (note the R_{symm}) and are broadly distributed in reciprocal space. Moreover, we are only trying to locate a few atoms of high scattering power.

Patterson maps calculated from both Laue and diffractometer data with only this small set of reflections were uninterpretable. However, a difference Fourier map between Laue native and Laue Eu data with monochromatic multiple isomorphous replacement phases produced a map identical in all respects to the difference Fourier map calculated with a complete set of diffractometer data (Fig. 2). The map clearly showed two Eu atoms binding in the central cavity that was previously identified as the active site (G.K.F., unpublished data) (Fig. 3). No other significant features were present in either difference Fourier map.

At this time, we have not made an extensive comparison between the Laue and diffractometer intensities, since adequate absorption correction routines do not yet exist for the Laue data and many of the other aspects of data processing are still under development. We believe that as long as native and substituted crystal data are collected in the same way, systematic errors tend to cancel out.

Table 3. Data-reduction statistics

	No. of reflections†	
	Native	Eu
Film along a^*	3,933	4,798
Film between a^* and b^*	5,145	8,334
Film along b^*	3,457	6,394
Total from all three films‡	11,499	16,970
Total obeying body-centering	6,286	7,653
Total between 6.12 and 3 Å	3,592	5,115
Total between 6.12 and 3 Å and obeying body-centering§	1,868	2,303
Unique total between 6.12 and 3 Å and obeying body centering	1,348	1,680
Common to native and Eu Laue data sets	1,205	
Common to native and Eu Laue sets and monochromatic data set	1,021	

†With intensity > 0.

‡*R* factors between equivalent reflections were 0.0577 for the native data set and 0.0652 for the Eu data set, where $R = (\Sigma I_i - \bar{I})/(\Sigma\bar{I})$. The intensities come from all three films in the data set.

§Theoretically, there should be 7300 unique (*hkl*) from 6.12- to 3-Å resolution that obey body-centering. The monochromatic data set had 5597 reflections with $I > 2\sigma$ from 6.12 to 3 Å.

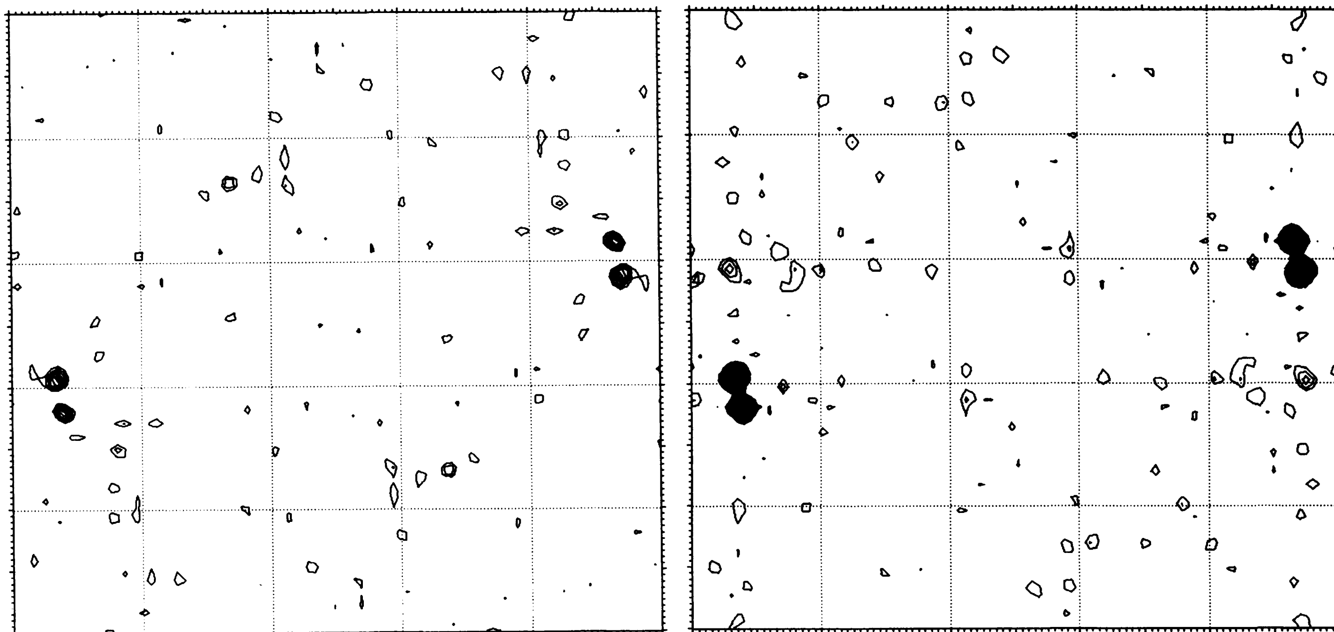

FIG. 2. (*Left*) The $z = 0.16$ section of the difference Fourier map between native xylose isomerase and the Eu derivative of xylose isomerase. This difference Fourier map was computed from Laue intensities and monochromatic phases. The lowest contour level is 2σ; subsequent contour levels are 1σ apart. The *x* axis is horizontal in this map, and the *y* axis is vertical. Each Eu gives rise to one peak. The other pair of peaks is due to crystallographic symmetry. (*Right*) The difference Fourier map computed from a complete data set of diffractometer measured monochromatic intensities and monochromatic phases. The σ levels and map parameters are the same as those in *Left*.

FIG. 3. Stereo view of the α-carbon backbone of xylose isomerase, with the entire difference map between Laue native and Laue Eu superimposed. The Eu density in the active site is the only significant feature of the map. The lowest contour level is 4σ.

Conclusions

Using the intensities from Laue data sets and phases from monochromatic data, we have been able to locate heavy-atom positions in a protein. The crystals used in these experiments provided a difficult test case for the Laue method. There were problems with pseudosymmetry and with nonisomorphism. In addition, the low symmetry of the crystals made it impossible to collect a complete Laue data set on a single photograph. Despite these problems, the data from the Laue experiments were good enough to find the heavy atoms. The Laue difference Fourier map was, in fact, as clean and interpretable as the corresponding monochromatic difference Fourier map.

Data collected by the Laue method have also been used to calculate interpretable difference maps of the binding of an organic molecule to one of the substrate binding sites of glycogen phosphorylase (7). The experiments that we report here confirm the utility of Laue diffraction for the examination of ligand binding to the active sites of enzymes and indicate that Laue diffraction, which can give a complete data set in less than a second in favorable cases, should be good enough to solve unknown structures.

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