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A Comparison of Laue and Monochromatic X-ray Analyses of a Small-Molecule Crystal

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Abstract

As part of the development of the Laue method for quantitative structure analysis we report on a detailed comparison of monochromatic (Mo $K\alpha$ and Cu $K\alpha$) and synchrotron radiation Laue data from the same crystal of a small molecule, (*S*)-2-chloro-2-fluoro-*N*-[(*S*)-1-phenylethyl]ethanamide, $C_{10}H_{11}ClFNO$. The small-molecule crystal was non-centrosymmetric, space group $P2_12_12_1$ ($a = 5\cdot418$, $b = 12\cdot030$, $c = 15\cdot837$ Å), and contained a single Cl atom which is a weak anomalous scatterer as well as 13 other non-H atoms (C, N, O, F). The data sets

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were recorded and processed in the order Mo $K\alpha$, Cu $K\alpha$ and then Laue. Inspection of the data quality, for *MULTAN* and *SHELX* runs, including the molecular geometry, shows that the Laue and monochromatic data compare very favourably. The *SHELX R* factors (on F) were 6·23, 6·45 and 8·19% using 512, 646 and 541 reflections with $I > 2\sigma(I)$ for the Mo $K\alpha$, Cu $K\alpha$ and Laue data sets, respectively. The hand of the molecule was indicated, using the chlorine anomalous signal in each case, Mo $K\alpha$, Cu $K\alpha$ and Laue. The purpose behind the measurement of the two monochromatic data sets was to allow, as a control, intercomparison of separate monochromatic with monochromatic analyses as well as monochromatic with Laue X-ray analyses.

Introduction

The Laue method is best performed using polychromatic, high-intensity synchrotron radiation (SR). Crystallographic data can be collected in a second or less per Laue exposure. Alternatively, very small crystal volumes can be used.

The use of Laue data for quantitative structure analysis is being developed. In this paper we report on a detailed comparison of monochromatic (Mo $K\alpha$ and Cu $K\alpha$) and synchrotron radiation Laue X-ray analyses of a crystal of a small molecule. The small molecule consists of 14 non-H atoms, including C, N, O, F and Cl, and 11 H atoms. The crystal is noncentrosymmetric. The use of the same crystal for each analysis removed sample-to-sample variations as a possible source of errors, an important advantage over a protein crystal as a test system for the development of the method. Also, this sample's absorption correction was modest even at Cu $K\alpha$. The single Cl atom gave a weak anomalous signal.

We have been able to compare the internal consistency of each data set (using *ROTAVATA/AGROVATA*) and the consistency of the observed data against the refined model and the associated calculated F's (using *SHELX*). The accuracy of the F_{calc}'s for a small molecule model is much better than for a macromolecular model, important for pinpointing any errors in the Laue data and processing. We were also able to assess the effectiveness of the Laue data for structure solution (using *MULTAN*) and for the determination of the hand. We have also compared the refined models.

Several earlier papers are relevant to this one. Wood, Thompson & Matthewman (1983) reported on the use of SR Laue photographs in the refinement of a very simple structure, namely Al(PO$_4$). Helliwell, Habash *et al.* (1989) discuss the details of the recording and processing of protein and small-molecule Laue film data, in particular the ways used to derive accurate, wavelength-normalized measurements

without reference to monochromatic data. Hajdu *et al.* (1987) have given details of wavelength normalization based on a ratio method, which relies on monochromatic data, and the subsequent calculation of a difference electron density map of a protein. Harding, Maginn, Campbell, Clifton & Machin (1988) and Clucas, Harding & Maginn (1988) have used Laue data to solve two organometallic crystal structures whereby the metal-atom positions were solved first *via* a Patterson synthesis with the remainder of the structures identified by difference Fourier maps. Farber, Machin, Almo, Petsko & Hajdu (1988) have compared the use of Laue and monochromatic data for the determination of the heavy-atom positions in a protein crystal heavy derivative. Helliwell, Harrop *et al.* (1989) have demonstrated that the Laue data for a mercury heavy-atom derivative of α-amylase is capable of yielding significant reflection phasing from isomorphous and anomalous differences.

The present paper allows, for the first time, direct comparison of complete Laue and monochromatic X-ray analyses (at Mo $K\alpha$ and Cu $K\alpha$) using the same structure and crystal throughout. The comparison of the Mo $K\alpha$ with Cu $K\alpha$ analyses serves as a control to allow more informed assessment of the Laue to Monochromatic comparisons.

Details of the sample

The chlorofluoroacetamide (1) was prepared using the method of Molines & Wakselman (1984), by reaction of chlorotrifluoroethene with (S)-1-phenylethylamine, followed by acidic hydrolysis of the resulting fluoroimine (see below). Crystallization of the crude oily product first gave the (S,S)-isomer (which was further purified by vapour diffusion crystallization before these X-ray diffraction studies). After optimization of the two-step synthesis (overall yield 33%), the diastereoisomers were always found to co-crystallize. Separation could be most readily achieved by flash chromatography (Still, Kahn & Mitra, 1978) using benzene/ethyl acetate (9:1) as eluant, giving (S,S)-(1) (R_F 0·55, m.p. 347–348 K) and (S,R)-(1) (R_F 0·47, m.p. 325–328 K) as white crystalline solids (*cf.* Molines & Wakselman, 1984).
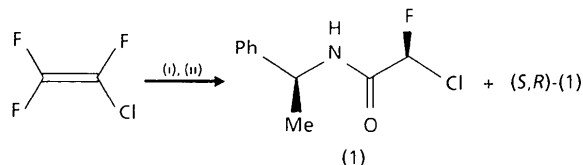
Reagents: (i) (S)-PhCh(Me)NH$_2$/Et$_2$O/sealed tube/20 h
(ii) 10% H$_2$SO$_4$(aq)/reflux/2 h

The chemical formula is C$_{10}$H$_{11}$ClFNO with molecular weight 215·66. The crystal is ortho-

rhombic, space group $P2_12_12_1$ with $a = 5\cdot418$, $b = 12\cdot030$, $c = 15\cdot837$ Å (from Cu $K\alpha$) or $a = 5\cdot414$, $b = 12\cdot001$, $c = 15\cdot801$ Å (from Mo $K\alpha$) and $Z = 4$. The linear absorption coefficient at Cu $K\alpha$ is $3\cdot014$ mm^{-1}, and at Mo $K\alpha$ is $0\cdot302$ mm^{-1}. The density is $1\cdot39$ g cm^{-3}. The sample used measured about $1\cdot0 \times 0\cdot4 \times 0\cdot4$ mm^3 and was cut from a long hexagonal needle crystal and mounted in a glass capillary tube. Note that the cell parameters used in the Laue analysis were those measured using the Cu $K\alpha$ monochromatic beam. The cell ratios can be determined by the Laue method but this was not attempted in this case. The differences in the cell-parameter estimates at Mo $K\alpha$ and Cu $K\alpha$ are due to a somewhat broad rocking width of the sample ($0\cdot7°$ FWHM) and the limited (~ 1 Å) sample resolution limit. The sample was tolerated because it was the only noncentrosymmetric, weak anomalous-scatterer structure that occurred in two years at York.

Data collection and processing

Three data sets were recorded from the same crystal in the sequence Mo $K\alpha$, Cu $K\alpha$ and then Laue.

Monochromatic data sets

The Mo $K\alpha$ wavelength ($0\cdot7107$ Å) data were collected on a specially upgraded Hilger & Watts four-circle diffractometer in the Physics Department of York University. The crystal was fully bathed in the X-ray beam. Cell dimensions were obtained from 30 centred reflections. Intensities of 520 unique reflections, *via* two equivalents and their Friedel mates (*i.e.* four in all for each unique reflection), were measured for $2\theta \leq 42°$ (resolution limit ~ 1 Å) in an $\omega/2\theta$ scan mode with filtered Mo $K\alpha$ radiation. The number of unique reflections with $I \geq 2\sigma(I)$ was 513. An absorption correction was applied according to the method of North, Phillips & Mathews (1968); the relative transmission factors were $1\cdot0$–$1\cdot1$. Lp corrections were applied in the usual way. The measurement of four standard reflections every 200 reflections showed no signs of decay.

The Cu $K\alpha$ wavelength ($1\cdot5418$ Å) data were collected on a Hilger & Watts four-circle diffractometer in the Chemistry Department of York University. The details for the data collection were very similar to the Mo $K\alpha$ except that $2\theta \leq 100°$ (for ~ 1 Å resolution) and that four equivalents and their Friedel mates were measured, *i.e.* eight in all. The number of unique reflections was 684 and 622 had $I \geq 2\sigma(I)$.

Comparing these two conventional source data sets it is clear that the better one is the set at Cu $K\alpha$ in the sense that the number of observable reflections is greater than for the set at Mo $K\alpha$.

Table 1. *Comparison of merging R's and resolution with numbers of reflections*

(a) Comparison of the merging R's and numbers of reflections as calculated by *ROTAVATA/AGROVATA* (Evans, unpublished)

	Mo $K\alpha$	Cu $K\alpha$	Laue
R	3·3	7·3	8·7
N_I	520	684	541
N_o	2216	5149	4587
d_{min}	1·0	1·0	0·90

Notes: $R = \sum_{hkl}\sum_i(I_i - \bar{I}_{hkl})/\sum_{hkl}n\bar{I}_{hkl}$, where i is a count over n equivalents in a given hkl symmetry set. N_I is the number of independent reflections, N_o is the number of measurements, d_{min} is the resolution limit.

(b) Number of independent reflections *versus* resolution

d_{min}(Å)	Mo $K\alpha$	Cu $K\alpha$	Laue
2·85	35	39	2
2·01	60	64	4
1·64	67	73	16
1·42	77	83	18
1·27	80	93	64
1·16	75	104	81
1·08	58	95	72
1·01	64	121	96
0·95	4	12	95
0·90	–	–	93
	520	684	541

Notes: the resolution limit for the monochromatic data sets is $\sim 1\cdot0$ Å in each case as mentioned in the text, hence the very few reflections in the 0·95–1·01 Å band. The resolution intervals chosen are dictated by the Laue-data resolution limit since we wished to use identical resolution bands for each data set for ease of comparison.

Laue data

The Daresbury Synchrotron Radiation Source station 9.7 was used to record Laue data on photographic film. The crystal was aligned with the a axis parallel to the rotation axis. At $\varphi = 0°$ the c axis was parallel to the incident X-ray beam. A $0\cdot2$ mm diameter collimator was used. A total of 13 exposures were recorded at intervals of $8°$ from $\varphi = -6$ to $90°$. The exposure time per filmpack was $0\cdot5$ s with the SRS operating at 2 GeV, 179 mA (on average, start current 181 mA and end current 177 mA) and the wiggler at 5 T. Each film pack contained six films and one Al foil (between the 5th and 6th films).

The films were scanned at 50 μm raster on a Scandig-3 microdensitometer at York. The data were processed using software developed at Daresbury. The crystal misorientation angles as well as $\lambda_{min} = 0\cdot2$, $\lambda_{max} = 2\cdot6$ Å and $d_{min} = 0\cdot90$ Å were estimated using the program *LGEN* (Elder & Machin, unpublished) on a PERQ computer. The resolution limit figure was determined directly from the Laue photographs by comparison with predicted patterns at various resolutions between $1\cdot0$ and $0\cdot8$ Å. The density of spots and the nearest-neighbour spots to nodal spots are particularly informative on this parameter. The high-angle parts of the pattern gave information on λ_{max} and the low-angle parts of the

Table 2. *The largest E's used by MULTAN for the data sets*

Mo $K\alpha$				Cu $K\alpha$				Laue			
h	k	l	E	h	k	l	E	h	k	l	E
5	1	1	2·570	0	2	6	2·502	2	4	15	2·787
0	2	6	2·549	2	5	8	2·444	0	13	1	2·783
0	3	9	2·389	1	0	10	2·384	2	5	8	2·684
2	5	8	2·332	0	3	9	2·360	3	5	7	2·391
3	6	8	2·330	3	6	8	2·342	3	6	8	2·371
0	1	7	2·282	5	1	1	2·315	5	3	5	2·209
3	5	7	2·206	3	5	7	2·307	2	6	9	2·201
1	0	10	2·191	0	9	5	2·208	0	9	5	2·146
0	9	5	2·177	1	0	15	2·185	2	5	10	2·093
1	5	0	2·163	2	6	9	2·171	3	3	2	2·080
0	2	3	2·151	0	1	7	2·155	3	3	13	2·036
3	4	0	2·138	0	2	3	2·131	3	5	11	1·988

Table 3. *Comparison of SHELX refinements*

	Mo $K\alpha$		Cu $K\alpha$		Laue	
	Correct hand	Incorrect hand	Correct hand	Incorrect hand	Correct hand	Incorrect hand
R (%)	6·23	6·30	6·45	7·53	8·19	8·41
wR (%)	5·66	5·74	7·12	8·66	8·11	8·48
Weighting-scheme parameters* a	13·3247	13·4031	21·1606	17·1821	10·3413	10·2809
b	0·000052	0·000051	0·000113	0·000209	0·000233	0·000275
f''	0·16 e		0·7 e		0·04 to 0·25 e	
f'	0·12 e		0·35 e		−0·02 to 0·19 e	
					$0\cdot 35 < \lambda < 0\cdot 9$ Å	
No. of independent reflections						
with $I > 2\sigma(I)$	512		646		541	
with Friedel equivalent reflections kept separate	848		1059		937	

* The weighting scheme used was of the form $a/[\sigma^2(F) + bF^2]$.

pattern information on λ_{min}. The program *GEN-LAUE* (Zurek, unpublished) was used to refine the crystal misorientation angles with the coordinates of a set of nodal reflections. Data were integrated using the program *INTLAUE* (Greenhough, unpublished). The singlet reflection spots were used in the subsequent analyses and there were no spatially overlapped spots. Films were scaled together using the program *AFSCALE* (Clifton, unpublished). Wavelength normalization was achieved using the symmetry equivalents recorded at different wavelengths (Campbell, Habash, Helliwell & Moffat, 1986) and the program *LAUENORM* (Campbell, unpublished). Spots stimulated by wavelengths between 0·35 and 0·9 Å were used. Outside this wavelength range the data statistics were unacceptable. This is due to the weakness of the very short wavelength data. Also, the lack of a suitable Laue absorption correction program at present meant that longer wavelength spots were significantly in error. Details of the recording and analyses of protein and small-molecule Laue data, and the Daresbury processing software, can be found in Helliwell, Habash *et al.* (1989).

Table 1(*a*) compares the merging *R*'s and number of reflections in the three datasets. Table 1(*b*) shows the number of independent reflections *versus* resolution for the Laue data, illustrating that there are very few low-resolution data measured as single-wavelength-component Laue spots.

Structure solution

The Mo $K\alpha$ data input into *MULTAN* (Debaerdemaeker, Tate & Woolfson, 1985) with all the defaults yielded a best phase set with values for ABSFOM, PSIZERO and RESID of 1·084, 1·132 and 19·90. This showed all the non-H atoms. The Cu $K\alpha$ data input to *MULTAN*87, using the *SAYTAN* option (Debaerdemaeker *et al.*, 1985) yielded a best phase set with values for ABSFOM, PSIZERO and RESID of 1·098, 1·033 and 16·57. This showed all the non-H atoms of the structure.

The wavelength-normalized Laue data were input to *MULTAN*86 (Debaerdemaeker *et al.*, 1985) running under defaults. The bulk of the low-resolution shell of data, *i.e.* to 1·9 Å, was missing since only single-wavelength-component spots were used [for a discussion of this see Cruickshank, Helliwell & Moffat (1987)]. A phase set with the values for the figures of merit ABSFOM, PSIZERO and RESID of 1·101, 0·0 and 26·58, respectively, gave 12 out of 14 of the non-H atoms.

Table 2 shows the reflections with the largest *E*'s for each data set.

Structure refinement

SHELX (Sheldrick, 1976) was used to solve the structure by anisotropic least-squares refinement of the non-H atoms with the different data sets Mo $K\alpha$, Cu $K\alpha$ and Laue. Table 3 compares the *R* factors and numbers of reflections used. Table 4 compares the atomic parameters.* These are very similar. The F atom is disordered to some extent probably due to it being attached to a terminal C atom. Fig. 1 shows a view of the structure. The H atoms were placed in their stereochemically calculated positions and refined with isotropic temperature factors.

The hand of the molecule was determined in the following way. The *hkl* and *$\overline{hkl}$* reflections were kept separate and the structure refined firstly as a set of *xyz*'s and then *$\overline{xyz}$*'s. The correct hand is that indicated by the lower *R* factor. It can be seen from Table 3 that all three data sets are consistent in the preference for one enantiomer. The Cu $K\alpha$ run is the most significant as is expected from the larger value of *f''*. As an approximation it is assumed that the *f''* for the Laue data took an average value of 0·15 e⁻,

Table 4. *Comparison of atomic parameters*

(a) Coordinates and temperature factors for non-H atoms with e.s.d.'s in parentheses

		Mo $K\alpha$	Cu $K\alpha$	Laue
C1	x	−0·9284 (19)	−0·9312 (13)	−0·9316 (17)
	y	−0·4168 (6)	−0·4168 (5)	−0·4123 (6)
	z	−0·2894 (6)	−0·2874 (4)	−0·2860 (4)
	U	0·078 (7)	0·078 (4)	0·060 (4)
C2	x	−0·9843 (20)	−0·9894 (14)	−0·9905 (13)
	y	−0·4709 (6)	−0·4697 (4)	−0·4727 (5)
	z	−0·2033 (5)	−0·2034 (3)	−0·2032 (4)
	U	0·054 (6)	0·060 (4)	0·042 (3)
N3	x	−0·7961 (13)	−0·7930 (9)	−0·7942 (10)
	y	−0·5117 (5)	−0·5107 (4)	−0·5102 (4)
	z	−0·1617 (4)	−0·1618 (3)	−0·1619 (3)
	U	0·057 (5)	0·060 (3)	0·042 (3)
C4	x	−0·8178 (12)	−0·8170 (10)	−0·8156 (10)
	y	−0·5652 (6)	−0·5625 (5)	−0·5626 (5)
	z	−0·0790 (5)	−0·0793 (3)	−0·0781 (3)
	U	0·057 (6)	0·061 (4)	0·037 (3)
C5	x	−0·6179 (15)	−0·6176 (12)	−0·6188 (14)
	y	−0·6502 (6)	−0·6490 (5)	−0·6492 (6)
	z	−0·0667 (5)	−0·0668 (4)	−0·0669 (5)
	U	0·071 (6)	0·081 (4)	0·058 (4)
Cl6	x	−0·7963 (5)	−0·7983 (4)	−0·7994 (6)
	y	−0·2874 (2)	−0·2866 (2)	−0·2857 (2)
	z	−0·2723 (2)	−0·2725 (1)	−0·2720 (2)
	U	0·111 (2)	0·116 (2)	0·098 (2)
F7	x	−1·1458 (17)	−1·1396 (15)	−1·1449 (23)
	y	−0·3943 (6)	−0·3954 (6)	−0·3906 (9)
	z	−0·3273 (4)	−0·3287 (4)	−0·3283 (6)
	U	0·179 (7)	0·186 (6)	0·164 (8)
O8	x	−1·2028 (12)	−1·2045 (9)	−1·2051 (10)
	y	−0·4803 (5)	−0·4813 (4)	−0·4811 (5)
	z	−0·1803 (3)	−0·1804 (3)	−0·1804 (3)
	U	0·082 (4)	0·084 (3)	0·063 (3)
C9	x	−0·8156 (12)	−0·8170 (9)	−0·8146 (11)
	y	−0·4761 (6)	−0·4763 (4)	−0·4767 (5)
	z	−0·0084 (4)	−0·0089 (3)	−0·0091 (3)
	U	0·048 (5)	0·049 (3)	0·030 (2)
C10	x	−1·0060 (13)	−1·0019 (10)	−1·0004 (10)
	y	−0·4740 (6)	−0·4759 (4)	−0·4743 (5)
	z	0·0517 (5)	0·0512 (4)	0·0516 (4)
	U	0·055 (5)	0·058 (3)	0·042 (3)
C11	x	−0·9989 (15)	−1·0035 (12)	−1·0030 (12)
	y	−0·3971 (7)	−0·3981 (5)	−0·3990 (6)
	z	0·1154 (5)	0·1155 (4)	0·1152 (4)
	U	0·064 (6)	0·066 (4)	0·049 (3)
C12	x	−0·8109 (16)	−0·8143 (11)	−0·8144 (12)
	y	−0·3204 (7)	−0·3205 (5)	−0·3197 (5)
	z	0·1213 (5)	0·1214 (4)	0·1214 (4)
	U	0·066 (6)	0·064 (4)	0·047 (3)
C13	x	−0·6255 (13)	−0·6268 (10)	0·6275 (10)
	y	−0·3210 (6)	−0·3205 (4)	−0·3218 (6)
	z	0·0603 (6)	0·0616 (4)	0·0618 (4)
	U	0·062 (6)	0·064 (4)	0·046 (3)
C14	x	−0·6275 (13)	−0·6273 (10)	−0·6271 (9)
	y	−0·3984 (6)	−0·3984 (5)	−0·3975 (5)
	z	−0·0030 (5)	−0·0032 (4)	−0·0017 (4)
	U	0·055 (5)	0·061 (3)	0·041 (3)

(b) Bond lengths (Å)

	Mo $K\alpha$	Cu $K\alpha$	Laue
C1—C2	1·538 (10)	1·507 (9)	1·532 (10)
C2—N3	1·307 (9)	1·346 (8)	1·328 (10)
N3—C4	1·460 (8)	1·453 (7)	1·474 (8)
C4—C5	1·500 (9)	1·513 (8)	1·501 (10)
C1—Cl6	1·730 (9)	1·740 (7)	1·698 (9)
C1—F7	1·348 (11)	1·331 (9)	1·361 (14)
C2—O8	1·242 (9)	1·229 (8)	1·222 (9)
C4—C9	1·546 (9)	1·523 (8)	1·505 (8)
C9—C10	1·402 (9)	1·382 (7)	1·392 (8)
C10—C11	1·366 (9)	1·382 (8)	1·355 (10)
C11—C12	1·376 (10)	1·390 (8)	1·401 (10)
C12—C13	1·392 (10)	1·389 (8)	1·385 (10)
C9—C14	1·383 (9)	1·394 (7)	1·397 (9)
C13—C14	1·365 (9)	1·388 (8)	1·357 (10)

(c) Bond angles (°)

	Mo $K\alpha$	Cu $K\alpha$	Laue
Cl6—C1—C2	108·8 (6)	110·3 (5)	113·7 (5)
F7—C1—C2	107·9 (6)	109·7 (6)	109·6 (8)
F7—C1—Cl6	104·5 (6)	104·1 (4)	104·5 (6)
N3—C2—C1	116·7 (8)	114·9 (6)	114·6 (6)
O8—C2—C1	118·9 (8)	120·6 (6)	119·4 (6)
O8—C2—N3	124·1 (7)	124·2 (5)	126·1 (6)
C4—N3—C2	123·5 (6)	121·8 (5)	121·8 (5)
C5—C4—N3	110·9 (6)	110·4 (5)	110·3 (5)
C9—C4—N3	109·9 (6)	111·5 (4)	111·1 (5)

Table 4 *(cont.)*

	Mo $K\alpha$	Cu $K\alpha$	Laue
C9—C4—C5	111·8 (6)	111·9 (4)	112·9 (5)
C10—C9—C4	119·7 (7)	120·4 (5)	120·9 (5)
C14—C9—C4	121·1 (6)	120·4 (4)	122·1 (5)
C14—C9—C10	119·2 (7)	119·2 (5)	117·0 (5)
C11—C10—C9	119·4 (7)	120·9 (5)	122·3 (5)
C12—C11—C10	121·6 (8)	120·0 (5)	120·0 (5)
C13—C12—C11	118·9 (7)	119·5 (5)	118·2 (5)
C14—C13—C12	120·3 (7)	120·2 (5)	121·2 (5)
C13—C14—C9	120·7 (7)	120·2 (5)	121·2 (5)

i.e. 0·04 e⁻ − 0·25 e⁻. The stereochemistry of the C4 atom is known from the previous chemistry and agrees with these results.

It is important that if *SHELX* is used with Laue data that the anomalous dispersion should be negligible. In the presence of a strong anomalous scatterer *SHELX* would need to use the Laue data unmerged and with the stimulating wavelength for each reflection carried through on the file, so that appropriate values of f' and f'' could be given. Also, the *LAUENORM* procedure relies on there being negligible anomalous dispersion.
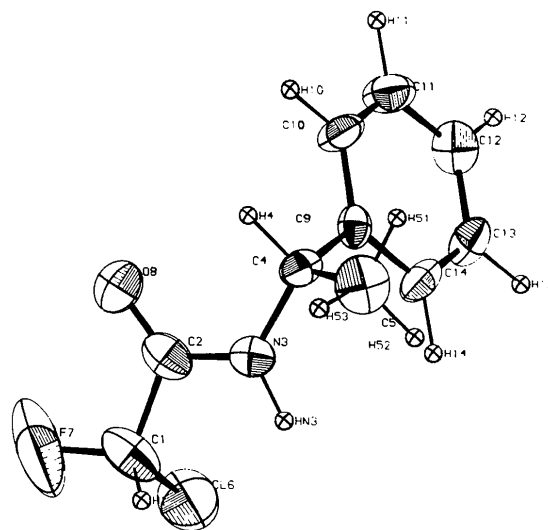
(a)
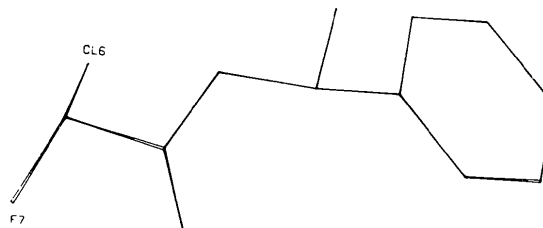
(b)

Fig. 1. (a) The structure (based on the Laue data) and numbering scheme. The shapes of the ellipsoids correspond to 50% probability contours of atomic displacement. (b) The three overlapped structures.

In the present case, to illustrate the strength of the anomalous signal of the Cl atom, we show sections of difference Fourier maps from the Mo $K\alpha$, Cu $K\alpha$ and Laue data. Firstly, we show sections of the anomalous-difference Fouriers based on the observed anomalous difference, $F(hkl) - F(\overline{hkl})$ for each case using calculated phases from the rest of the structure (excluding Cl) advanced by 90° – see Figs. 2(a) (Mo $K\alpha$), 2(b) (Cu $K\alpha$) and 2(e) (Laue); Fig. 2(c) shows the equivalent calculated map for the Mo $K\alpha$ case. Secondly, a difference Fourier based on $F_{Cu\ K\alpha} - F_{Mo\ K\alpha}$, and so proportional to f', with calculated phases (without the Cl) was computed – see Fig. 2(d). It is noteworthy that in Fig. 2(b) the highest peak is at the Cl position and clearly very significant; this was used to determine the hand of the molecule, but the peak in Fig. 2(a) was only just the highest peak in the map. In Fig. 2(d) the peak, although the highest, was at the noise level. The Laue data used was primarily stimulated by λ's < 1·0 Å for which we can say that the change in f' over that wavelength range and the size of f'' within that range are not large. The anomalous-difference f'' Fourier for the Laue data showed a peak at the Cl position but is a noisy map (Fig. 2e).

The separate refined structures were overlapped one with another by a least-squares determination of a rotation matrix and translation vector. The root-mean-square deviation in Å and maximum single-atomic displacement (given in brackets) for the Mo $K\alpha$:Cu $K\alpha$, Mo $K\alpha$:Laue and Cu $K\alpha$:Laue molecular models are 0·023 (0·044), 0·029 (0·050) and 0·025 (0·052).

Concluding remarks

The main findings of the paper are as follows. Firstly, the merging R factors of the data sets are similar although clearly the monochromatic data were better than the Laue data. Secondly, the structure solution using the Laue data and *MULTAN* was successful despite the fact that the bulk of the low-resolution data were missing. Thirdly, the refinements in *SHELX* show very similar quality crystallographic R factors and all agree in their indication of the hand of the molecule. Fourthly, the refined molecular models agree within 0·03 Å, on average. Finally, the temperature factors are somewhat less in the Laue case.

Clearly, the structure solution with Laue data and *MULTAN* is feasible, provided that the unit-cell parameters are known already from monochromatic exposures. Also, refined structural details based on Laue data can be trusted. In the Laue case the total exposure time was 6·5 s and in the monochromatic cases in excess of 24 h for this crystal and equipment.
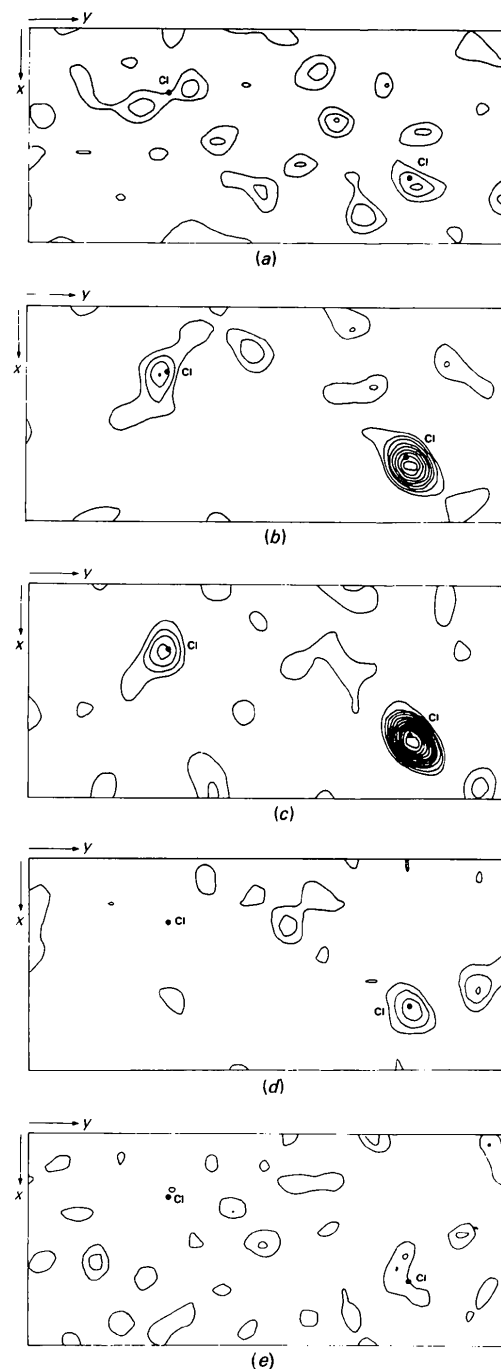

Fig. 2. Anomalous-difference Fourier maps based on f'' for (a) observed Mo $K\alpha$ difference, (b) observed Cu $K\alpha$ difference and (c) calculated Mo $K\alpha$ difference. In (d) the observed (Cu $K\alpha$ – Mo $K\alpha$) term is proportional to the change in f'. In (e) the f'' map is shown for the Laue case and should be compared with (a) which is of approximately the same wavelength as the mean wavelength of the Laue data. Each map is contoured at one standard deviation calculated for each map. 60 sections were calculated in Z and the section shown is number 16. The Cl atom marked at bottom right occurs at section 16·2, the weaker one at top left actually occurs at section 13·8 and so is tailing off in electron density.

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Crystallographic Refinement by Incorporation of Molecular Dynamics: Thermostable Serine Protease Thermitase Complexed with Eglin c

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Abstract

In order to investigate the principles of protein thermostability, the crystal structure of thermitase from Thermoactinomyces vulgaris, a thermostable member of the subtilisin family of serine proteases, has been determined in a complex with eglin c. Eglin c is a serine protease inhibitor from the leech Hirudo medicinalis. After data collection with a television area-detector diffractometer and initial structure solution by molecular-replacement methods, crystallographic refinement proceeded with incorporation of molecular-dynamics techniques. It appeared that this refinement procedure has a large convergence radius with movements of more than 5 Å for many atoms. Two procedures for the crystallographic molecular-dynamics refinement have been tested. They differed mainly in time span and weight on the X-ray 'energy'. The best results were obtained with a procedure which allowed the molecular-dynamics technique to search a large area in conformational space by having less weight on the X-ray restraints and allowing more time. The use of molecular-dynamics refinement considerably simplified the laborious and difficult task of fitting the model in its electron density during the refinement process. The final crystallographic R factor is 17·9% at 2·2 Å resolution.

Introduction

Thermitase is a heat-stable serine protease from Thermoactinomyces vulgaris containing 279 amino-acid residues (Meloun, Baudys, Kostka, Hausdorf, Frömmel & Höhne, 1985; Frömmel & Höhne, 1981; Teplyakov, Strokopytov, Kuranova, Popov, Harutyunyan, Vainshtein, Frömel & Khene, 1986; Dauter, Betzel, Höhne, Ingelman & Wilson, 1988). It is homologous to the subtilisins Carlsberg, BPN' and proteinase K, for which high-resolution X-ray structures have been reported (Bode, Papamokos & Musil, 1987; McPhalen, Schnebli & James, 1985; Katz & Kossiakoff, 1986; McPhalen, Svendsen, Jonassen & James, 1985; Betzel, Pal & Saenger, 1988). The amino-acid sequences of these enzymes have been compared by Meloun et al. (1985). The observed sequence identity between subtilisins Carlsberg and BPN' is 69%, whereas the sequence identity of either of the subtilisins with thermitase is only 47%. A difference of 25 K in optimum tempera-