SHORT COMMUNICATIONS

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On the determination of heavy-atom positions in various elastase derivatives.

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Direct statistical three-dimensional phasing has been applied to difference structure amplitudes at 3·5 Å resolution from three heavy-atom derivatives of the enzyme elastase, using the tangent formula. The metal atoms could be located with good accuracy in two out of three, and with rather less accuracy in the third. Studies on the data set for one derivative at low resolution demonstrate that the methods seem to be reliable to at least 6·0 Å resolution.

Several years ago, Steitz (1968) reported that he was able to determine the heavy-atom positions in the centrosymmetric projections of three derivatives of the enzyme carboxypeptidase with 2 Å resolution data by a direct statistical sign-determining approach with the Sayre (1952) equation. More recently Schevitz *et al.* (1972) have successfully used the same method to locate heavy atoms in a centrosymmetric projection of an osmium derivative of yeast formylmethionine transfer RNA.

This note reports studies of direct phasing techniques applied to three heavy-atom derivatives of the proteolytic enzyme elastase (Watson, Shotton, Cox & Muirhead, 1970), which has a molecular weight of 25000. It is shown here that heavy-atom location by direct methods is not restricted to centrosymmetric projections and that their three-dimensional positions can be determined by phasing with the tangent formula of Karle & Hauptman (1956). Furthermore, the heavy atom sites can be found even for data extending to 6 Å resolution with good accuracy, and to 8 Å with rather less precision.

Method and calculation

Data for this study came from three derivatives of elastase: a *p*-chloromercuribenzene sulphonate (PCMBS-elastase), a uranyl tosyl-elastase and a uranyl PCMBS-elastase. All have been shown to be closely isomorphous with the native protein and to have essentially single-site occupancy (Watson *et al.*, 1970). Data for the tosyl-inhibited native enzyme and the three derivatives were supplied to a resolution of 3·5 Å by Drs Shotton and Watson. The enzyme crystallizes in the space group $P2_12_12_1$, with $a = 51\cdot5$, $b = 58\cdot0$ and $c = 75\cdot5$ Å.

Following Steitz (1968), difference structure amplitudes, $|\Delta F_{\mathrm{H}}|_h$, were calculated for each reflexion where

$$|\Delta F_{\mathrm{H}}|_h = ||F_{\mathrm{HP}}|_h - |F_{\mathrm{P}}|_h|$$

$|F_{\mathrm{HP}}|_h$ and $|F_{\mathrm{P}}|_h$ are the measured derivative and native protein structure amplitudes, respectively. Thus, $|\Delta F_{\mathrm{H}}|_h$ is a measure of the heavy-atom scattering alone. These difference amplitudes were then normalized to give sets of $|\Delta E_{\mathrm{H}}|_h$ values, for the 3·5 Å data by means of least-squares straight-line fits to Wilson plots. The procedure used to obtain ΔE values for the low-resolution ΔF uranyl derivative data will be discussed below.

Phases were determined by tangent formula multi-solu-tion methods (see, for example, Kennard *et al.*, 1971); origin and enantiomorph-determining reflexions, as well as symbolic phases, were chosen by inspection with the standard criteria (Karle & Karle, 1966). The *MULTAN* computer program of Germain, Main & Woolfson (1971) was also used for the 3·5 Å data, after the successful multi-solution phasing attempts.

ΔE maps were calculated for those sets of phases which seemed to be the most internally consistent and which had low values of R (Karle & Karle, 1966), defined as

$$R = \frac{\sum ||\Delta E|_{\mathrm{obs}} - |\Delta E|_{\mathrm{calc}}|}{\sum |\Delta E|_{\mathrm{obs}}}.$$

In all cases the correct solution was checked by comparison of the peak coordinates obtained from the ΔE map, with those of Watson *et al.* (1970) for the heavy atoms.

Results and discussion

For all three derivatives at 3·5 Å resolution, the 'best' sets of phases from the multi-solution phasing were the correct ones, with R ranging from 0·22 to 0·31 [Table 1(*a*)]. These correct solutions were also among the 'best' *MULTAN* ones. Only in the case of the PCMBS derivative was any difficulty encountered in the phasing procedure; this was possibly on account of a markedly abnormal distribution of ΔE values among the parity groups. The temperature factors for all three derivatives from the Wilson plots [Table 2(*a*)] are lower than the ones reported after least-squares refinement.

Table 1. 3·5 Å *and variable resolution results*

(*a*) 3·5 Å resolution results.

Derivative	Number of ΔE's used	Best R value	Number of symbols used
Uranyl	$150 \geq 1\cdot8$	0·31	2
PCMBS	$147 \geq 1\cdot8$	0·28	3
Uranyl–PCMBS	$191 \geq 1\cdot8$	0·22	3

(*b*) Variable resolution results on uranyl derivative.

Resolution (Å)	Number of ΔE's used	Best R value	Number of symbols used
3·5	$150 \geq 1\cdot8$	0·31	2
6·0	$67 \geq 1\cdot0$	0·34	1
8·0	$59 \geq 0\cdot5$ ($29 \geq 1\cdot0$)	0·38	2

The ΔE Fourier map for each 3·5 Å resolution derivative best set showed peaks in positions corresponding to those given by Watson et al. (1970) [Table 2(a) and Fig. 1(a)]. The maps showed no spurious detail; the heavy-atom peaks were over three times the height of the highest background peaks. The ratio of the heavy-atom peak heights in the double derivative was 0·70. Table 2(a) shows that the positional parameters for the metal atoms, as determined in this study, compare favourably with those from the least-squares refinement reported by Watson et al. (1970). The poorest agreement is for the PCMBS derivative, with a difference of 1·2 Å between the two mercury-atom posi-

tions. This discrepancy could be attributed to relatively poor ΔE determination.

Wilson plots on the 6·0 and 8·0 Å uranyl derivative data gave temperature factors of over 100 Å² in both cases with a wide scatter of points about the least-squares straight lines (in contrast to the 3·5 Å data). Accordingly, the ΔE values for the low-resolution data were calculated with a temperature factor of 18·3 Å² as determined at the higher resolution. Table 1(b) summarizes the phasing results for these data. At 6·0 Å resolution, phasing was straightforward, only one symbolic reflexion being necessary. The 'best' solution, with an R of 0·34, was the correct one, and as

Table 2. Positional parameters and temperature factors

(a) 3·5 Å resolution results.

Derivative	Heavy atom	Positional parameters					
		Present study			Watson et al. (1970)		
		x	y	z	x	y	z
Uranyl	U	0·117	0·585	0·397	0·120	0·585	0·398
PCMBS	Hg	0·913	0·313	0·503	0·910	0·313	0·487
Uranyl- ⎫	U	0·121	0·585	0·397	0·121	0·585	0·398
PCMBS ⎭	Hg	0·912	0·311	0·462	0·915	0·315	0·468

	Temperature factor from Wilson plot, (Å²)	Temperature factor from least-squares refinement (Watson et al., 1970)
Ur	18·3	37
Hg	4·5	22
Ur ⎫	11·3	⎧ 28
Hg ⎭		⎩ 25

(b) Variable resolution results on uranyl derivative.

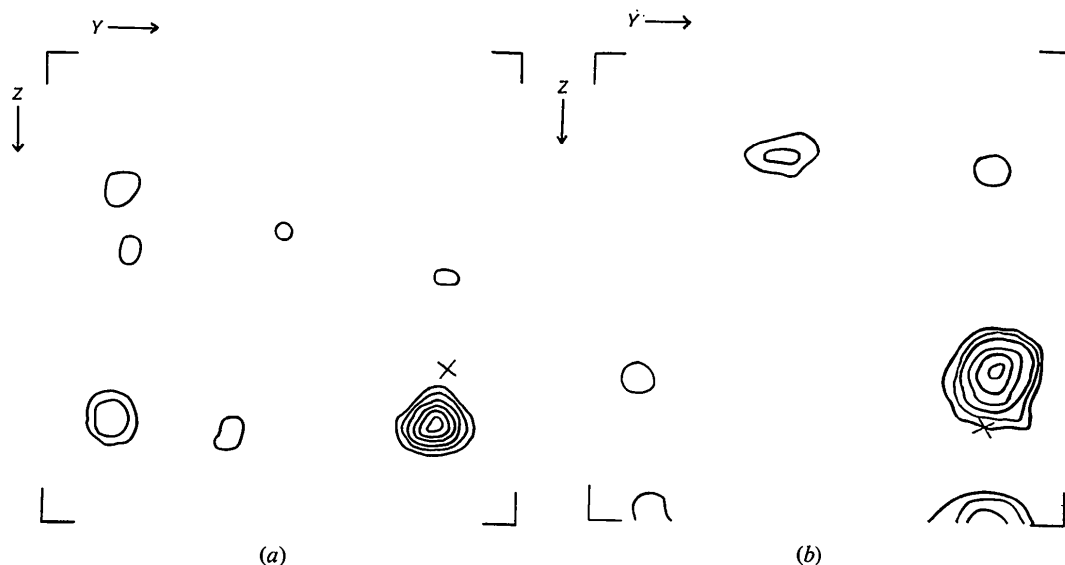| Resolution	Uranium positional parameters						Temperature factor from Wilson plot (Å²)
	Present study			Watson et al. (1970)			
(Å)	x	y	z	x	y	z	
3·5	0·117	0·585	0·397	0·120	0·585	0·397	18·3
6·0	0·120	0·584	0·391	0·120	0·585	0·397	104
8·0	0·126	0·575	0·342	0·120	0·585	0·397	115

Fig. 1. Sections of the ΔE maps for the uranyl derivative (a) at 3·5 Å resolution, (b) at 8·0 Å resolution. The crosses on the maps denote the location of the maximum peak in the 8·0 and 3·5 Å maps, respectively.

Table 2(b) shows, the uranium-atom position corresponded remarkably well to the correct one. Reeke & Lipscomb (1969) have also reported that the tangent formula works at 6·0 Å.

The 8·0 Å data, however, did not give as good a result. Various attempts at multi-solution phasing with ΔE greater than 1·0 and 0·5 produced a number of phase sets, only two of which had R less than 0·40. The less consistent of the two had the lower R of 0·38, by nearly 0·02, and was the better solution, as judged by the ΔE map. Fig. 1(b) [Table 2(b)] shows that the uranium atom, determined at 8·0 Å resolution was over 4 Å away from its true position. The ΔE map did not show the good peak discrimination encountered earlier; the strongest spurious peak had half the strength of the correct one. It is possible that significant errors in the ΔE values at this low resolution were to some extent responsible for these results.

It thus seems that direct three-dimensional phasing of ΔF data sets can reliably locate heavy atoms in biological macromolecules, even when only 5 or 6 Å resolution data are available, provided that the individual heavy atoms are well resolved. Not too much reliance can be placed on lower-resolution results. As Steitz (1968) has pointed out, these methods could be of considerable use in the structure analysis of molecules with multiple heavy-atom binding sites. Where high-symmetry space groups are involved, Patterson map interpretation becomes especially difficult and statistical phasing to locate heavy atoms is particularly advantageous.

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La₂O₂S structure refinement and crystal field.*

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Parameters on the structure of La₂O₂S have been refined by the least-squares method using 396 Mo $K\alpha$ intensity data. In space group $P\bar{3}m1$, the La³⁺ ion occupies 3(m) sites surrounded below by three S²⁻ ions (3·037 Å) and above by three O²⁻ ions (2·424 Å) and one axial O²⁻ ion (2·423 Å). A new crystal-field analysis has been performed using this data.

Introduction

The rare-earth oxysulfides activated with trivalent rare-earth ions form an important technological class of materials with high luminous efficiency (Struck & Fonger, 1971; Dobrov & Buchanan, 1972). In particular, neodymium-activated lanthanum oxysulfide, La₂O₂S:Nd, has received some attention as a promising new high-gain laser material and, hence, has stimulated studies on the growth of the required single crystals (Alves, Buchanan, Wickersheim & Yates, 1971; Baughman, 1973). The simplicity of the crystal structure as well as the many various isomorphous members and/or dopants makes the material ideal for analysis of crystal-field parameters (Sovers & Yoshioka, 1969; Newman & Stedman, 1971). Unfortunately, only approximate atomic parameters obtained by the powder method were available (Wyckoff, 1960) leading Newman & Stedman to obtain values for superposition model parameters (in their crystal-field analysis) which were difficult to interpret. Crystal structure parameters determined on 1 wt.% Nd doped

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La₂O₂S material kindly supplied by Baughman are reported in this note.

Experimental details and results

A single-crystal specimen was ground to a radius of 0·0121 cm. The specimen was examined by long-exposure photographic methods to insure that $P\bar{3}m1$ is the correct space group. Lattice constants were determined to be $a_0 = 4·049$ (1) and $c_0 = 6·939$ (2) Å by measurements made on a Picker diffractometer. The θ–2θ scan technique and a scintillation detector employing pulse-height discrimination were used to measure a complete hemisphere (to 95° 2θ) of Mo $K\alpha$ intensity data. A unique data set of 396 intensities was obtained by averaging the symmetry-related values provided the differences were less than $\sigma_{ave}/\sqrt{n}$, where σ_{ave} is the average σ for n measurements with the usual definition of $\sigma = (N_{SC} + K^2 N_B)^{1/2}$, where N_{SC}, N_B and K are the total scan count, background counts and the time ratio of the scan to background respectively. In the few instances where an intensity did not agree to within this criterion, it was discarded and averaging was performed on the remaining intensities. All reflections were considered observed. Spherical absorption corrections (μ[Mo $K\alpha$] = 190 cm⁻¹) were ap-