We do not envisage that the present technique will be restricted to the determination of sound velocities. It can be used also to study the nature of acoustic vibrations in the vicinity of phase transitions, or to investigate excitations away from the origin of the Brillouin zone. These are the types of problem to be examined in future publications.

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The Use of *MULTAN* to Locate the Positions of Anomalous Scatterers

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Abstract

Observed anomalous scattering differences have been used with the direct-methods program *MULTAN*87 to determine the positions of anomalous scatterers in a variety of metalloproteins and a small molecule. The lack of anomalous differences in the centric data did not prevent the determination of the atom positions and the anomalous scatterers were found in all cases. These results show that the method may be useful to determine the positions of anomalous scatterers in the case of multi-site genetically engineered proteins.

Introduction

Anomalous scattering measurements are increasingly being used in macromolecular crystal structure analysis because of the availability of synchrotron radiation. The variable wavelength makes available multiple-wavelength anomalous-dispersion data from which structures have now been determined by various groups. The technique can be readily applied to the metalloproteins. In addition, it is becoming easier to prepare heavy-atom derivatives and these need not be isomorphous with a native protein if multi-wavelength techniques are used.

The location of the metal atoms is required before phases can be calculated. A Patterson synthesis can be used with anomalous, isomorphous or combined differences, but the interpretation is straightforward when there are only a few sites. For more than about four sites, interpretation becomes less easy. However, with genetic engineering, the possibility of incorporating as many as 20 atoms into a molecule is not unreasonable.

Direct methods have been used to locate metal atoms in proteins on the basis of isomorphous differences [see, for example, Wilson (1978) and Adams, Helliwell & Bugg (1977)]. Given the prospect of a large number of metal sites and the possibility of using multi-wavelength methods, we decided to explore the use of anomalous differences with the direct-methods program *MULTAN*87 (Debaerdemaeker, Germain, Main, Tate & Woolfson, 1987) to locate the metal atoms. We have restricted ourselves to the use of the imaginary component ($\Delta f''$) derived differences, *i.e.* differences measured at one wavelength. These are inherently more accurate than the estimate of $\Delta f'$ from multiple-wavelength experiments because of difficulties with absorption corrections at the different wavelengths. The results are therefore applicable to both conventional and synchrotron X-ray sources.

Method

Anomalous differences can be expressed (Blundell & Johnson, 1976) as

$$\Delta_{\text{ano}} = |F^+| - |F^-| \simeq 2F''_{\text{ano}} \cos(\varphi - \varphi''_{\text{ano}}) = 2F''_{\text{ano}} \cos \Delta\varphi \quad (1)$$

where the symbols are defined in Fig. 1.

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Table 1. *Summary of the data used in the tests*

	Cytochrome c_4	Pea lectin	Hg derivative of α-amylase	Chlorine complex small molecule	
Space group	$P6_122$	$P2_12_12_1$	$C222_1$	$P2_12_12_1$	
Unit-cell dimensions (Å) *a*	62·4	50·8	81·1	5·42	
b	62·4	61·6	98·3	12·03	
c	174·2	137·4	138·0	15·84	
Mol. wt	19 000	49 000	45 000	216	
Wavelength (Å)	1·74	1·86	1·542	1·542	0·7107
Resolution (Å)	3·0	3·0	5·5	1·0	1·0
Mean $\lvert\Delta_{ano}\rvert/\sigma(\Delta_{ano})$	2·0	1·4	10·0	1·5	1·1
Number of reflexions	2940	5239	1313	414	338
Number rejected	48	30	0	0	0
Number in map	400	400	300	125	125
Number of phase sets generated	28	200	100	50	50

Δ_{ano} can be obtained from the experimental anomalous differences, Δ_{obs}, by correcting for the temperature and scale factors B and k respectively:

$$\Delta_{ano} = \Delta_{obs} k^{1/2} \exp[B(\sin^2\theta)/\lambda^2]. \quad (2)$$

It is evident from (1) that the largest $|\Delta_{ano}|$'s will be mostly accompanied by large F''_{ano}'s and large $|\cos\Delta\varphi|$'s. For the largest $|\Delta_{ano}|$'s:

$$|\Delta_{ano}| \simeq 2F''_{ano}. \quad (3)$$

A set of the largest $|\Delta_{ano}|$ may thus be used to determine the positions of the anomalous scatterers. Direct methods are suitable for this purpose since they use mainly the largest E's. However, the observed anomalous differences must be examined carefully before using direct methods. In particular, $|\Delta_{ano}|$ should not exceed the theoretical maximum of $2\sum \Delta f''_j$.

A peculiarity of the use of Δ_{ano}'s alone is that a measurement is only available for acentric data. This is in contrast with isomorphous differences where a measurement is present for all reflexions. A problem which the use of anomalous differences has in common with isomorphous differences is that zero values of F''_{ano}cannot be reliably identified. This removes a source of valuable information for direct phase determination.

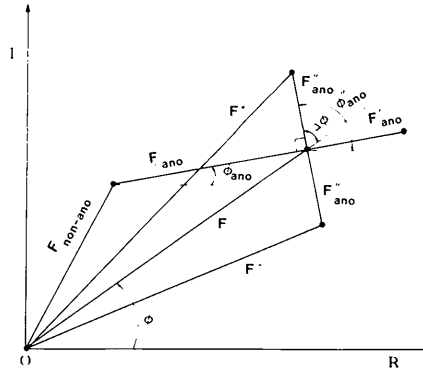

Fig. 1. Vector diagram for the structure factors for the direct ($\mathbf{F}^+$) and inverse ($\mathbf{F}^-$) reflections. The structure factor of the inverse reflection is shown reflected across the real axis.

Results

The crystal structures used in the tests are set out in Table 1. The table also includes an indication of the size of the anomalous signal as the ratio of mean $|\Delta_{ano}|$ to $\sigma(\Delta_{ano})$. For each test, any anomalous measurement arising from $F_{obs} < 4\sigma(F_{obs})$ or $|\Delta_{ano}| > 5$ times the root mean square of Δ_{ano} was excluded from the calculation. The numbers of reflexions so excluded are also given in Table 1. These rejection criteria are similar to those used by other workers using anomalous differences for structure determination (Hendrickson & Teeter, 1981).

(i) *Cytochrome c_4*

Cytochrome c_4 is a dihaem protein containing two iron atoms in the asymmetric unit. The structure determination is in progress (Sawyer and co-workers, to be published). The observed anomalous scattering data, collected at $\lambda = 1{\cdot}739$ Å ($f''_{Fe} = 3{\cdot}9$ electrons, Fe K absorption edge at 1·743 Å), were used in our analysis (Helliwell, 1984).

The heights of the iron peaks in the Harker sections of the anomalous-difference Patterson map with $|\Delta_{ano}|^2$ as coefficients ranged between 15 and 30 compared with an origin peak of 1000. The highest peak in a general position not associated with an Fe–Fe vector was of height 90. The Patterson map was therefore difficult to interpret.

The figures of merit suggested the phase sets were of considerably lower quality than those expected for small-molecule studies, but similar to those of isomorphous derivative analysis with *MULTAN* (Wilson, 1978). An E map calculated from the phase set with the highest ABSFOM (0·341), lowest PSIZERO (3·039) and lowest RESID (58·51) revealed the two Fe sites (Table 2).

(ii) *Pea lectin*

The anomalous scatterer structure of pea lectin was analysed by Einspahr, Suguna, Suddath, Ellis, Helliwell & Papiz (1985) at 3 Å resolution. The asymmetric unit contains a dimer with each monomer

Table 2. *Comparison of results from the best E map with the known positions of the anomalous scatterers*

Peak height		x	y	z	Atom	x	y	z
Cytochrome c_4								
	1318	0·454	−0·003	0·316	Fe1	0·457	0·003	0·318
	1141	0·653	0·102	0·082	Fe2	0·656	0·102	0·082
	307	0·826	0·096	0·851				
Pea lectin								
	868	0·899	0·102	0·101	Mn1	0·89	0·14	0·13
	814	0·600	0·147	0·259	Mn2	0·66	0·10	0·29
	694	0·865	0·066	0·806				
α-amylase (Hg derivative)								
	3090	0·109	0·230	0·529	Hg	0·140	0·229	0·528
	741	0·282	0·172	0·619				
Small molecule (Cu $K\alpha$ data)								
	2828	0·807	0·288	0·770	Cl	0·798	0·287	0·773
	837	0·191	0·137	0·543				
Small molecule (Mo $K\alpha$ data)								
	2855	0·857	0·299	0·808	Cl	0·798	0·287	0·773
	788	0·799	0·166	0·754				
Small molecule (calculated differences for Cu $K\alpha$)								
	3021	0·807	0·283	0·770	Cl	0·798	0·287	0·773
	780	0·977	0·181	0·753				

binding one Mn^{2+} ion and one Ca^{2+} ion. The data were collected at $\lambda = 1{\cdot}86$ Å ($f''_{Mn} = 3{\cdot}8$ electrons), near the Mn K absorption edge using synchrotron radiation at the Daresbury Synchrotron Radiation Source. Despite this, the size of the anomalous signal is very small as seen in Table 1.

The Harker sections of the anomalous-difference Patterson map were almost featureless. The known Mn–Mn vectors lay at peak hieghts of between 0 and 50 compared with the highest unexplained peak height in the Harker sections of 120 - clearly a difficult map to interpret.

In the E map computed from the best phase set as given by the combined figure of merit (ABSFOM 0·312, PSIZERO 2·848, RESID 55·33), the two highest peaks were about 5 Å away from the known Mn positions as shown in Table 2. It should be noted that the maximum ABSFOM had a value of 0·320, the minimum PSIZERO was 2·777 and the minimum RESID was 54·66.

(iii) Hg *derivative of α-amylase*

The structure of this protein is being analysed by Z. Derewenda (to be published). The Hg derivative data which we used were collected on a Xentronics area detector at York using Cu $K\alpha$ radiation ($f''_{Hg} =$ 7·7 electrons). The anomalous signal was quite strong as shown in Table 1.

The Harker sections of the anomalous-difference Patterson map showed Hg–Hg peaks as the highest non-origin peaks. An E map from the phase set corresponding to the highest ABSFOM (0·854), lowest PSIZERO (5·084) and lowest RESID (15·18) revealed the Hg site, though it is displaced by about 2·5 Å in the x direction compared with that obtained in the MIR study (Table 2).

(iv) *A small molecule*

Anomalous-dispersion data were available for the known structure of $C_{10}H_{11}ClFNO$ (Gomez de Anderez *et al.*, 1989) in which chlorine is the anomalous scatterer. This gave the opportunity to perform calculations on a crystal where all the atomic coordinates were known. The anomalous differences from both Cu $K\alpha$ and Mo $K\alpha$ data, measured on the same crystal, were used in the analyses. With Cu $K\alpha$ data, the best two phase sets both showed the Cl position as the highest peak in the E map. The best set had an ABSFOM of 0·690, PSIZERO 2·743 and RESID 28·16. For the Mo $K\alpha$ data, the phase set characterized by the highest ABSFOM (0·717) and lowest RESID (29·68) also showed the Cl position, but displaced by about 0·65 Å from the true position. This was thought to be due to the weak anomalous signal from Cl for Mo radiation ($f'' = 0{\cdot}16$ electrons as opposed to 0·7 electrons for Cu $K\alpha$).

Knowing the complete structure, we were able to repeat the calculation using calculated anomalous differences instead of those observed. For the calculated Cu $K\alpha$ differences, the best figures of merit were ABSFOM 0·956, PSIZERO 1·579, RESID 14·04, and the E map gave a single large peak at the Cl atom position. This illustrates nicely that anomalous differences can, in principle, be used with *MULTAN* to locate the anomalous scatterers.

Concluding remarks

We have demonstrated that it is feasible to locate metal atoms from the use of anomalous differences using direct methods of phase determination. An E map for the phase set with the highest value of combined figure of merit led to a correct structure in all

the cases studied. Both the ABSFOM and RESID figures of merit were found to be good indicators of the best phase sets. PSIZERO was also found to be acceptably good despite the fact that reflexions with zero Δ_{ano} cannot be reliably identified. The missing centric data did not prevent success in these trials. Further work is needed to see if the technique will handle many anomalous scatterer sites as might occur with genetically engineered proteins.

We are grateful to a number of people for supplying data sets. Dr M. Helliwell measured the small-molecule data and Mrs E. J. Dodson calculated the anomalous differences. Dr Z. Derewenda provided the mercury derivative α-amylase data. Other data sets have already been referenced. Professors M. M. Woolfson and Fan Hai-fu are thanked for valuable discussions. We are grateful to the Science and Engineering Research Council for grant support.

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Combined Use of Monochromatic and Laue Diffraction Techniques for Macromolecular Structure Determination

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Abstract

A novel strategy of macromolecular structure analysis is described which combines the use of monochromatic scanning Laue (SCL) and white-beam Laue (WBL) diffraction techniques. It provides, when applied with an area detector with on-line capabilities, a means of interactively determining and optimizing experimental parameters; it further makes rapid data evaluation feasible, also with off-line detector systems. These new procedures have been applied to a protein structure, β-trypsin, using a FAST area detector (Enraf-Nonius) and image plates (Fuji) on a double-focusing synchrotron beamline at DORIS. Structure factors, which were derived from FAST Laue data, were empirically scaled by comparing equivalent reflections in different wavelength bins. A $2F_o - F_c$ difference Fourier map, which was calculated at 1·8 Å resolution using these structure-factor moduli together with phases from the known structural model, showed well defined electron density distribution ($R = 22\%$). Image-plate exposures showed diffraction to 1·2 Å resolution. The effect of crystal mosaicity on the maximum wavelength bandwidth for Laue exposures has been investigated. SCL techniques, which involve rapid scanning (with a crystal or multilayer monochromator or a tunable undulator) through a defined wavelength range, extend the applicability of Laue techniques to crystals with broadened mosaic spread.

1. Introduction

Laue diffraction techniques provide a means of measuring integrated reflection intensities for crystal structure analysis by exposure of a stationary crystal to an incident (neutron or X-ray) beam of broad spectral distribution (Lowde, 1956; Buras, Mikke, Lebech & Leciejewicz, 1965). They were first employed in the neutron structure determination of small biological structures (Hohlwein, 1977; Klar, Hingerty & Saenger, 1980) using a modified Laue technique (Maier-Leibnitz, 1967). The same technique was used in the first (neutron) Laue diffraction study of a protein structure (Hohlwein & Mason, 1981). It was recently demonstrated that Laue techniques could be applied in protein diffraction studies with syn-

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