Acta Cryst. (1974). A30, 706

The Application of Direct Methods to the Analysis of Heavy-Atom Derivatives

BY MANUEL A. NAVIA* AND PAUL B. SIGLER

Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, Illinois 60637, *U.S.A.*

(*Received* 28 *February* 1974; *accepted* 16 *May* 1974)

Heavy atoms in isomorphous derivatives of concanavalin-A and formylmethionine transfer RNA have been found by applying the tangent formula in three dimensions to E_Δ's, the normalized moduli of the differences between the scaled amplitudes of the derivative and parent structure factors. A full three-dimensional set of starting phases was computed from trial heavy-atom constellations derived from symbolic-addition analysis of the centrosymmetric axial projections. This strategy compares favorably with the analysis of two- and three-dimensional difference Patterson syntheses usually employed to find heavy atoms, and is flexible enough to incorporate other sources of structural information when available. It is preferable when circumstances, such as high-symmetry space groups, data of poor quality or low resolution, and multiple heavy-atom sites per asymmetric unit make the Patterson syntheses difficult to interpret. Problems peculiar to the application of direct methods to the solution of isomorphous heavy-atom derivatives are discussed.

Introduction

The method of heavy-atom isomorphous replacement generally requires that at least one derivative be interpreted by a Patterson synthesis of the differences in structure-factor amplitudes caused by the introduction of heavy atoms. In this paper we present an alternative initial approach in which a map of the heavy-atom distribution is provided by a Fourier synthesis based on phases derived by a 'direct-methods' analysis of the amplitude differences. This approach grew out of a desire to avoid the difficult task of deconvoluting complicated heavy-atom vector maps encountered when complex biological systems are subjected to X-ray structural analysis.

Direct methods were first used to locate heavy atoms in isomorphous derivatives by Steitz (1968), who applied Sayre's equation (Sayre, 1952) to the centrosymmetric axial projections of carboxypeptidase. Symbolic addition (Hauptman & Karle, 1953; Karle & Karle, 1966) has been successfully used to locate heavy atoms in the centrosymmetric axial projections of hexokinase (Fletterick, Anderson, Hwang & Steitz, 1972), and formylmethionine transfer RNA ($tRNA_f^{Met}$) (Schevitz *et al.*, 1972). Recently, Neidle (1973) has employed multisolution techniques (*e.g.*, Kennard *et al.*, 1971) and the program *MULTAN* (Germain, Main & Woolfson, 1971) to solve three derivatives of porcine elastase in three dimensions.

General procedure

Our approach to the problem of locating the heavy atoms of an isomorphous derivative is summarized in Fig. 1. Parent (P), and isomorphous derivative (PH) data are collected for each of the centrosymmetric axial projections. Two-dimensional differences $\Delta F = (F_{PH} - F_P)$, are normalized to give E_Δ; where $E_\Delta^2 = (\Delta F)^2/(\epsilon^2 \sum_{j=1}^{N} f_j^2)$, f_j are the scattering factors of the N heavy atoms, and ϵ is a symmetry factor (see Karle & Karle, 1966). The term 'ΔE' is avoided, since it would erroneously imply a coefficient $(E_{PH} - E_P)$, analogous to that for ΔF above. The symbolic-addition procedure as programmed by Dewar (1968) is then applied separately to the $|E_\Delta|$'s of each projection to generate sets of signs. A two-dimensional heavy-atom Fourier synthesis is calculated with the set of signs leading to the fewest inconsistent symbol equivalences. The projection maps are examined jointly for three-dimensionally consistent peaks, and the coordinates of the more prominent of these are used to generate a trial heavy-atom constellation. A full three-dimensional set of structure factors is calculated from this potential solution to provide starting phases to be improved *via* the application of the tangent formula (TF) to a complete three-dimensional set of E_Δ's. The decision to proceed or not with the collection of three-dimensional data is made at this point, based on the clarity and internal consistency of the trail constellation and its agreement with auxiliary sources of structural information.

Our use of trial solutions in conjunction with TF parallels the approach of Karle (1968) in that partial structure information is exploited to complete the heavy-atom constellation. Observed $|E_\Delta|$'s and starting phases from a trial solution are supplied to the TF program *TANG* (Coulter & Dewar, 1971) which is allowed to cycle until the average phase change from cycle to cycle is less than a preset value, typically 5°. Once converged, the solution appears stable over many cycles. A three-dimensional Fourier synthesis is calculated with $|\Delta F|$'s, and phases from the final cycle of

* Present Address: Laboratory of Molecular Biology, NIAMDD, NIH, Bethesda, Md. 20014, U.S.A.

TF. The resulting three-dimensional heavy-atom map is examined for: (1) the emergence of sites in addition to those put into the constellation, (2) shifts in the positions of trial sites, and (3) the disappearance of trial sites. Based on these modifications, a new trial constellation is constructed, and used to calculate a new set of starting phases for a further round of TF. This process will be referred to as TF phase development. Finally, when little change in the structure is apparent at the end of a round of TF phase development, heavy-atom coordinates are prepared for least-squares refinement.

Special problems

(1) *The 'hand' of individual atoms*

In constructing a non-centrosymmetric three-dimensional constellation of heavy atoms from mutually perpendicular centrosymmetric views one is faced with the difficulty of individually assigning the correct 'hand' to each of the heavy atoms; that is, deciding whether the ith atom is to be located at (x_i, y_i, z_i), or $(x_i, y_i, -z_i)$. It is impossible from an analysis of such projections alone to correlate correctly the relative positions of the heavy atoms within a constellation. Our strategy has been to make an arbitrary choice of 'hand' for only one heavy-atom site, allowing the other sites to emerge during TF phase development, thus completing the 'partial structure' in a manner consistent with the initial choice. Alternative approaches to this problem are compared in the *Discussion*.

(2) *Accuracy required of trial coordinates*

The positions of peaks on the various heavy-atom Fourier maps in Figs. 2 and 5 do not necessarily correspond to 'correct' least-squares refined coordinates; implying that the three-dimensional coordinates assigned on the basis of these peaks would not be accurate. Studies with 6 Å model data indicated that a trial heavy-atom site which was not located within 3 Å of a correct site would fail to initiate a productive TF phase development. These same studies showed, however, that depending on other factors, approximately ten incorrect sites could be removed from the final map if one correct site were present. Therefore, to allow for uncertainty in the positioning of trial atoms, we routinely initiated the first round of TF phase development by constructing a cluster of six-atoms symmetrically arranged 3 Å away from a seventh central atom located at the site considered most likely to be correct from the projections. All atoms were initially of equal occupancy and were assigned an overall isotropic temperature and form factor equal to that derived experimentally from the difference amplitudes. For subsequent rounds of TF phase development, greater relative occupancy was assigned to the central atom of clusters constituting new trail solutions in proportion to one's confidence in a site.

(3) *Criteria of correctness*

The interpretation and evaluation of heavy-atom derivatives cannot draw on known chemical constraints, as is the case with covalently bonded molecular structures (Karle, 1968). Two confidence criteria have been applied to the results of TF phase development: (1) the capacity of any atom in the trial solution to regenerate the rest of the solution, and (2) the capacity of the TF phase development to remove from a trial solution an incorrect or 'bogus' site purposely included with the occupancy of the largest trial site.

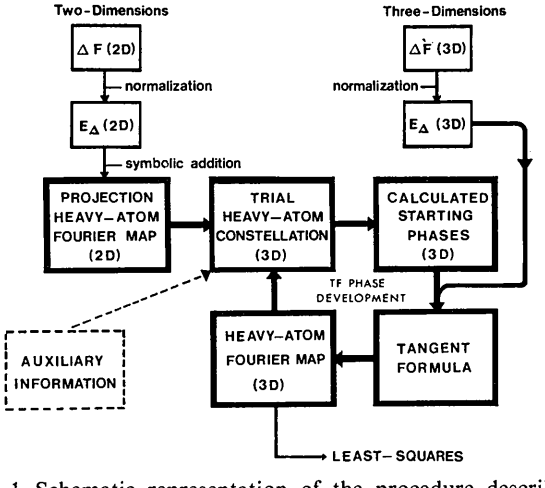


Fig. 1. Schematic representation of the procedure described in the text for the interpretation of heavy-atom derivatives by direct methods. The term 'round' as used in the text, indicates the completion of passage through the four elements of TF phase development. Each round includes a specified number of cycles (usually 6 to 12) of the tangent formula.

Experimental results

(1) *Concanavalin-A*

Concanavalin-A (Con-A) data to 2·4 Å resolution was kindly provided by Dr Karl Hardman, Argonne National Laboratory. Con-A crystallizes in the space group $I222$ (eight general positions, one protomer per asymmetric unit) with unit-cell dimensions $a=63{\cdot}15$, $b=86{\cdot}91$ and $c=89{\cdot}25$ Å. Our analysis was limited to 3 Å in projection, and 5 Å in three dimensions. It was carried out with no knowledge of heavy-atom sites or occupancies in order to create a sufficiently difficult and realistic test of the method. The mercuric acetate [$Hg(AcO)_2$] derivative of Con-A, with six sites per asymmetric unit is described in Table 1. This derivative, which had among the poorest refinement parameters of all the derivatives used in the structure determination, was solved by the difference Fourier method using parent phases obtained from previously solved derivatives (Hardman & Ainsworth, 1972). Details are given here for the direct methods solution of this $Hg(AcO)_2$ derivative. A simpler two-site $PtCl_4^{2-}$ derivative was also considered and solved without difficulty.

Table 1. *Refinement parameters for the* $Hg(AcO)_2$ *derivative of Con-A*

Refinement parameters for the $Hg(AcO)_2$ derivative taken from the joint refinement at 2·4 Å resolution of all available Con-A derivatives for final parent phases (Hardman & Ainsworth, 1972). Coordinates are expressed in fractional units corresponding to the grid used in computing the Fourier syntheses (Fig. 4).

Derivative	Site	$X/48$	$Y/64$	$Z/64$	A^a	B^b
$Hg(AcO)_2$	*A*	3·4	−0·4	1·1	35·9	4·2
	B	2·2	0·5	3·7	46·8	4·3
	C	1·2	0·2	9·3	38·7	22·5
	D	15·7	15·0	25·3	67·8	3·6
	E	5·6	23·8	16·5	35·7	3·5
	F	13·5	16·9	21·7	31·0	1·7

R^c	RMSE^d	$\langle f_H \rangle^e$	Ratio^f
0·623	102·1	108·7	1·06

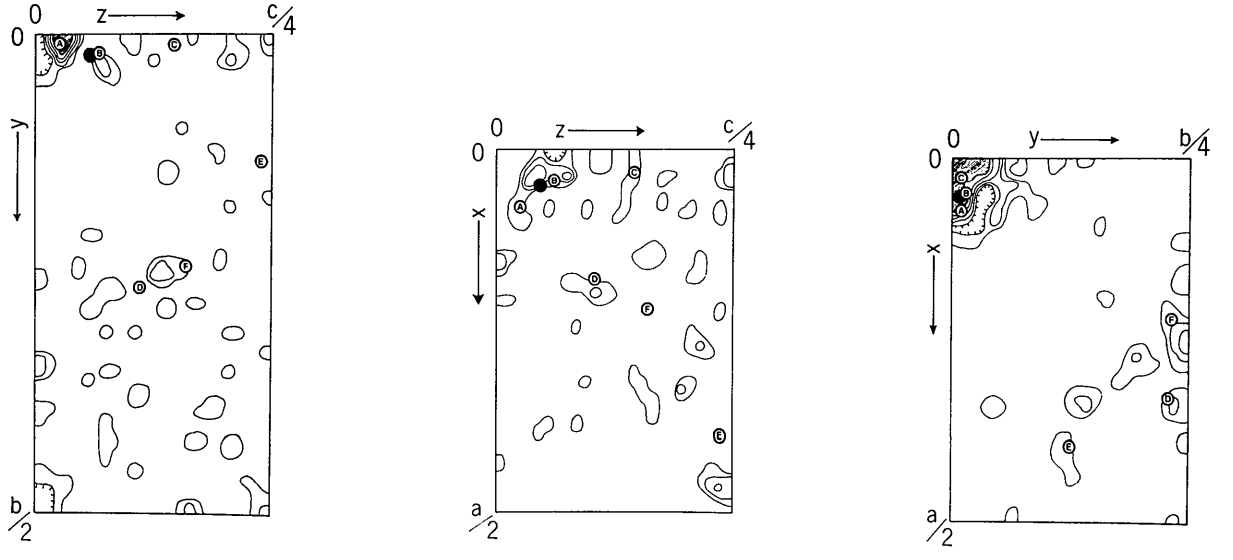
[a] Occupancy in units of electrons.
[b] Temperature factor in Å^2.
[c] Residual, $R = \sum |F_{PH(obs)} - F_{PH(calc)}| / \sum |F_{PH(obs)} - F_{P(obs)}|$
[d] Root-mean-square lack of closure in units of electrons.
[e] Root-mean-square calculated heavy-atom scattering factor in units of electrons.
[f] $\langle f_H \rangle$/RMSE.

Fig. 2 represents two-dimensional heavy-atom maps phased *via* symbolic addition for the (0*kl*), (*h*0*l*), and (*hk*0) centrosymmetric axial projections of the $Hg(AcO)_2$ derivative. Fig. 3 is also the Harker section $(u,v,0)$ given for comparison. As described in *Methods*, starting phases for the first round of TF phase development were calculated from a cluster of equal-occupancy heavy atoms constructed about a compromise position considered the most likely site from the projection studies. In spite of the fact that this compromise site was initially 4 Å from a 'correct' site, the process of TF phase development was successful in locating five of the six atoms found by Hardman & Ainsworth (1972) with only one spurious peak observed above background. Fig. 4 documents the evolution of the $Hg(AcO)_2$ heavy-atom constellation over three rounds of TF phase development, each round involving approximately six cycles of TF. For the final round, a 'bogus' site was inserted at the location marked ⊗ in Fig. 4(*c*). Its disappearance is taken as an indication of success.

(2) *Transfer RNA*

Yeast $tRNA_f^{Met}$ crystallizes in the space group $P6_422$ (twelve general positions, one molecule per asymmetric unit) in a unit cell of dimensions $a = 115·3$, $c = 136·9$ Å (Johnson, Adolph, Rosa, Hall & Sigler, 1970). Crystals are 82·5% solvent by volume, and moderately radiation sensitive with a large temperature factor of 155 Å^2 for the parent intensities. All data were collected by precession photography to 6 Å resolution using monochromated Cu $K\alpha$ radiation. Table 2 describes the 3-pyridyl-mercuric acetate derivative ($PyrHg^+$) of $tRNA_f^{Met}$ considered below.

Fig. 5 shows two-dimensional heavy-atom maps of the (0*kl*) and (*hk*0) centrosymmetrical axial projections of the $PyrHg^+$ of $tRNA_f^{Met}$ phased by symbolic addition. When viewed down the *a* axis, the general positions of this three-dimensional space group are seen as



(*a*) (*b*) (*c*)

Fig. 2. Two-dimensional heavy-atom Fourier synthesis for the (0*kl*), (*h*0*l*), and (*hk*0) centrosymmetric axial projections of the Con-A $Hg(AcO)_2$ derivative; coefficients are $|E_d| \cos \varphi$, where E_d is as defined in the text, and φ is 0 or π as determined by symbolic addition. The projections are calculated to 3 Å resolution in the plane group *cmm* using all 198, 192, and 181 reflections respectively. Temperature factors exhibited by the $|\Delta F|$'s were 19·9, 12·4, and 3·8 Å^2. Letters indicate 'correct' sites as listed in Table 1. Trial phases for TF phase development are calculated from coordinates of the compromise site indicated by the symbol ●. Contours in all figures are drawn at equal and arbitrary values above zero. Cross-hatched areas represent very high density.

triplets of related points within the asymmetric unit of the plane group, *pmm*. More specifically, for a general point (x,y,z) in the space group $P6_422$, one expects the triplet within the planar asymmetric unit to have coordinates (x,z), $(x,\frac{1}{3}-z)$, and $(x-y,\frac{1}{3}+z)$. Since this relationship is not imposed by the form of the calculation of the $(0kl)$ projection map, we have routinely used the relative positions of peaks in projection to distinguish plausible heavy-atom sites from background noise (Schevitz *et al.*, 1972). Fig. 6 shows the $(u,v,0)$, $(u,v,\frac{1}{3})$, and $(0,v,w)$ Harker sections of the three-dimensional difference Patterson synthesis given for comparison.

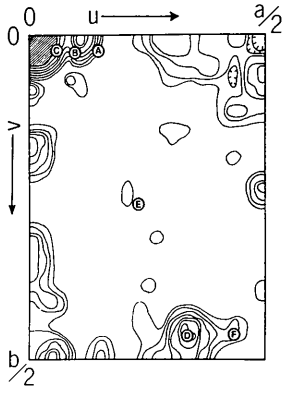


Fig. 3. Harker section $(u,v,0)$ of the three-dimensional difference Patterson synthesis of the Con-A $Hg(AcO)_2$ derivative calculated to 5 Å resolution. Coefficients used are $(\Delta F)^2$ where ΔF is as defined in the text. Letters indicate the expected Harker-section vectors for the sites listed in Table 1.

Table 2. *Refinement parameters for the* $PyrHg^+$ *derivative of* $tRNA_f^{Met}$

Refinement parameters for the $PyrHg^+$ derivative taken from the final joint refinement at 6 Å resolution for parent phases of all currently available derivatives of $tRNA_f^{Met}$ (Schevitz *et al.*, 1974). Coordinates are expressed in fractional units corresponding to the grid used in computing the Fourier synthesis (Fig. 7).

Derivative	Site	*X*/64	*Y*/64	*Z*/72	*A*[a]	*B*[b]
$PyrHg^+$	*A*	2·4	33·4	8·3	101·9	234·0
	B	9·4	24·4	12·0	57·8	1·2
	C	14·2	25·6	52·3	146·5	186·6
	D	9·1	10·0	11·1	27·0	150[c]
	E	19·0	21·8	36·9	36·8	150[c]
	F	6·2	9·5	28·2	45·6	150[c]
	G	14·4	22·0	3·2	34·8	150[c]
	H	10·0	21·2	8·5	25·3	150[c]
	I	9·6	26·9	10·4	20·0	150[c]
	J	18·2	27·2	53·4	35·4	150[c]

R[d]	RMSE[e]	$\langle f_H \rangle$[f]	Ratio[g]
0·57	218·4	428·2	1·96

[a] Occupancy in arbitrary units.
[b] Combined temperature-form factor in Å².
[c] Temperature–form factor held constant
[d] Residual as defined in Table 1.
[e] Root-mean-square lack of closure in the same units as occupancy.
[f] Root-mean-square heavy-atom scattering in the same units as occupancy.
[g] $\langle f_H \rangle$/RMSE.

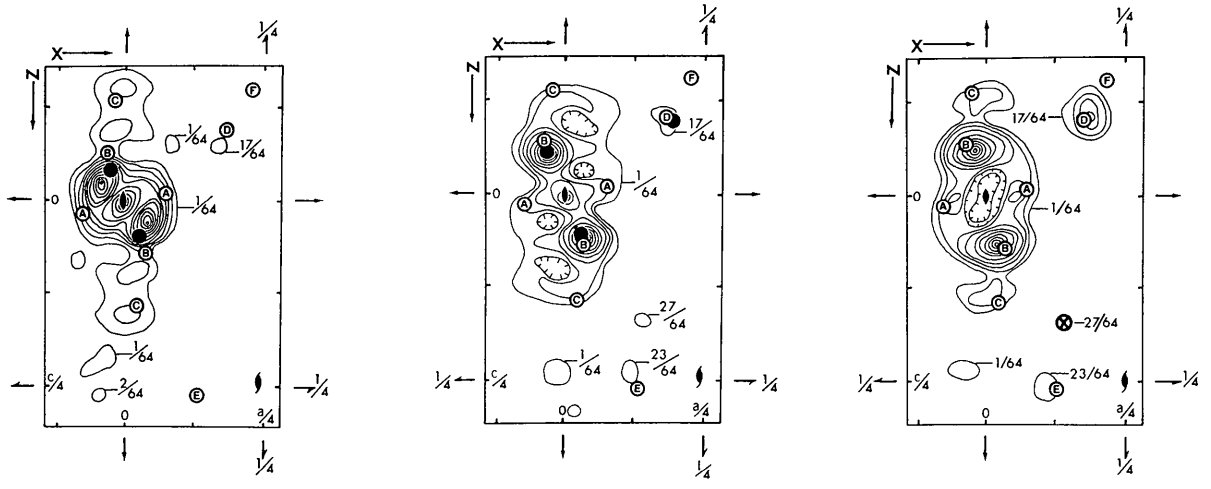


Fig. 4. Three-dimensional heavy-atom Fourier synthesis for the Con-A $Hg(AcO)_2$ derivative, documenting the evolution of TF phase development. Coefficients used were $|\Delta F| \exp(i\varphi_i)$, where ΔF is as defined in the text, and φ_i are the phases obtained after the first (*a*), second (*b*), and third (*c*) rounds of TF phase development. The $|\Delta F|$ data to 5 Å resolution exhibit a temperature factor of 59·7 Å². The maps are composite electron-density sections parallel to (010) (chosen to display an unobstructed view of every major peak), each calculated with the 376 unique reflections whose corresponding $|E_\Delta|$'s were greater than 0·9. The fractions adjacent to the contours indicate their depth in the unit cell along the y direction. Letters locate the sites listed in Table 1. As expected, the highest peak in (*a*) locates the compromise site used to calculate starting phases for the first round of TF phase development. Sites used to initiate subsequent rounds of TF phase development are indicated in (*a*) and (*b*) by the symbol ●. A 'bogus' site, as described in the text, was inserted in the final round at the site marked ⊗ in (*c*).

Unlike the case of Con-A above, the compromise most likely site from which starting phases were calculated for TF phase development was practically superimposable on the 'correct' site *A* (Table 2).

The first round of TF phase development produced a peak on site *A*, a second large peak near site *B*, and a smaller peak near site *C*. To challenge the validity of the interpretation at this stage, a round of TF phase development was initiated with only site *B*. This too produced a map with peaks corresponding to sites *A*, *B*, and *C*.

For the final round of TF phase development, starting phases were calculated from the coordinates of four sites; two near *A* and *B*, a third near *C*, and a 'bogus' site as described above. The results of this final round are shown in Fig. 7. The three highest occupancy sites (*A*, *B*, and *C*) correspond to the dominant peaks on the map. Site *F*, the fourth highest in occupancy corresponds to a peak three contours above background. Near-background peaks are found near sites *E* and *J*, while the other refined sites in Table 2 remain below background. The presence of site *I* may be reflected in the distortion of the large peak assigned to *B*. Two peaks, three contours in height, appear to be spurious. The 'bogus' site indicated by ⊗ is completely eliminated.

Discussion

We have carried out the analysis of two complicated derivatives to the point where their interpretation represents an adequate start for least-squares refinement and further analysis by the difference Fourier technique. The procedure set forth here offers a distinct advantage over the conventional difference Patterson approach in cases where the number of heavy atoms in the unit cell is unusually large, such as in oligomeric enzymes, protein–protein and protein–nucleic acid complexes, whole viruses, and virus components. This advantage follows from two facts. First, the number of vectors in a Patterson synthesis increases roughly as

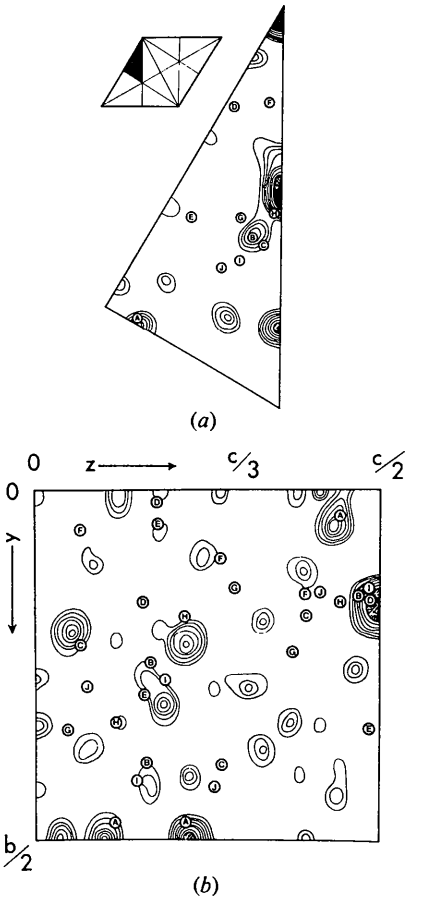


Fig. 5. Heavy-atom Fourier syntheses for the (*hk*0) and (0*kl*) centrosymmetric axial projections of the $tRNA_f^{Met}$ $PyrHg^+$ derivative. Coefficients are as defined in Fig. 2. The projections are calculated to 6 Å resolution in the plane groups *pmm* and *p6mm* respectively, using all 174 and 175 unique reflections. Temperature factors exhibited by the $|\Delta F|$'s are 300·0 and 164·1 Å^2. Letters indicate 'correct' sites as listed in Table 2. Starting phases for the first round of TF phase development are calculated from a heavy-atom constellation with coordinates nearly coincident with those of site *A* on the projection maps.

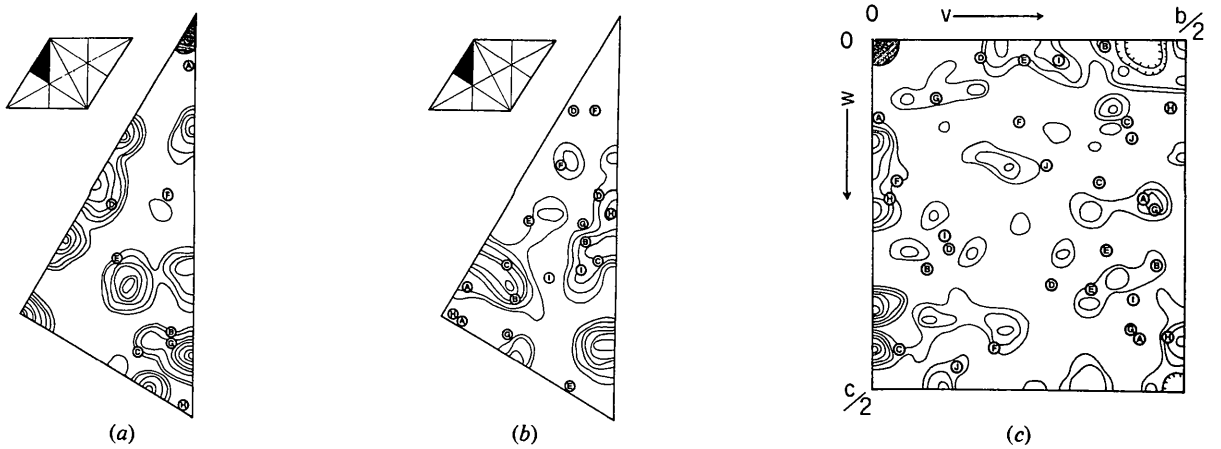


Fig. 6. Harker sections $(u,v,0)$, $(u,v,\frac{1}{2})$, and $(0,v,w)$ of the three-dimensional difference Patterson synthesis of the $tRNA_f^{Met}$ $PyrHg^+$ derivative calculated to 6 Å resolution. Coefficients used are $(\Delta F)^2$, where ΔF is as defined in the text. Letters locate the expected Harker-section vectors for the sites listed in Table 2.

the square of the number of heavy atoms in the unit cell. Given N heavy-atom sites per asymmetric unit in a space group with S general positions, one needs to resolve (and deconvolute) N^2S-N interatomic vectors per asymmetric unit. On the other hand, the number of peaks expected in the Fourier synthesis produced by TF phase development increases only linearly with the number of sites per asymmetric unit and is independent of the symmetry of the cell. Second, assuming that the peaks are readily resolved, the interatomic vectors must be deconvoluted before an interpretation is achieved. In the Fourier map, however, heavy atoms are represented directly, with a common origin and a consistent 'hand'.

In this regard, TF phase development may have potential in extending the usefulness of the difference Patterson synthesis to heavy-atom derivatives of intermediate complexity. The problem of the 'hand' of individual atoms, as discussed above under *Special problems*, arises when two-dimensional interpretations of difference Patterson projections or Harker sections are expanded to three dimensions. With a molecule of known covalent structure, there exists a body of prior knowledge about bond lengths and angles, and the geometry of substructures which allows one to correlate the positions of the individual atoms. Unfortunately, the positions of heavy atoms in an isomorphous derivative are not so constrained. In the absence of reliable phase information, the correlation of the 'hand' of the individual atoms is dealt with by an analysis of the cross vectors of the difference Patterson synthesis, or by a trial-and-error procedure in which least-squares parameters are compared for the various choices. These procedures will tend to break down in complicated space groups with multiple sites per asymmetric unit; in the first instance, because of the reduced interpretability of Patterson maps, and in the second, because of the rapid increase in the number of choices with the number of sites. Where this represents a limitation to Patterson methods, the tangent formula as used above may prove particularly useful. In any case, it is highly unlikely that derivatives of the type presented in this work could have been solved as 'first' heavy-atom derivatives by a difference Patterson synthesis.

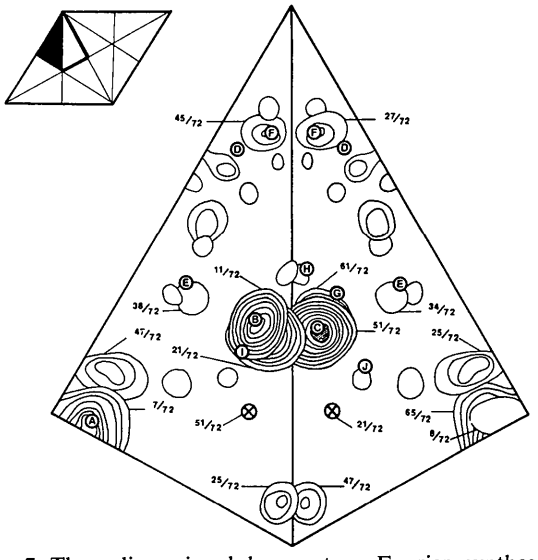


Fig. 7. Three-dimensional heavy-atom Fourier synthesis for the $tRNA_f^{Met}$ $PyrHg^+$ derivative. Coefficients used are $|\Delta F|\exp(i\varphi)$, where ΔF is as defined in the text, and φ is the phase obtained in the last of three rounds of TF phase development. The $|\Delta F|$ data to 6 Å resolution exhibit a temperature factor of 176·8 Å^2. The map is a composite of electron-density sections parallel to (001) calculated with 389 unique reflections whose corresponding $|E_\Delta|$'s were greater than 0·9. Two asymmetric units are shown so that there is an unobstructed view of every major peak. They are related by a twofold axis at $z=\frac{1}{2}$, and are displayed in the context of the whole unit cell in the inset. The fractions adjacent to the sections indicate their depth in the unit cell along the z direction. Letters locate the sites as listed in Table 2. A 'bogus' site, as described in the text, was inserted in the final round at the site marked ⊗.

The method also provides a built-in operational criterion with which efficiently to screen potential heavy-atom derivatives, namely, the ability to construct a three-dimensionally consistent provisional heavy-atom constellation from an interpretation of heavy-atom Fourier maps of centrosymmetric axial projections. This feature is especially attractive at the early stages of an analysis, since collecting projection data to high resolution represents a minimal commitment of experimental resources.

This approach to direct methods allows (indeed requires) the investigator to actively intervene in the process of phase development through the construction of a trial constellation based on an interpretation of heavy-atom projections of three-dimensional maps resulting from the preceding round of TF phase development (see Fig. 1). Such an interpretation involves visual discrimination between 'true' peaks and noise in constructing the trial constellation. In effect, this type of intervention, commonly used in the structure determination of small molecules (Karle, 1968), allows real-space constraints to be imposed on phase development in reciprocal space, and provides a convenient way of incorporating into the phase development supplementary structural information which independently might be considered marginal. For example, the weight of a prospective heavy-atom site can be adjusted to the extent that it agrees with a difference Patterson map, or obeys constraints imposed by molecular dimensions, chemical stoichiometry, or local non-crystallographic symmetry.

Finally, TF phase development can be very useful even when approximate parent phases are available from a solved or partially solved derivative. In the difference Fourier and 'heavy-atom' methods, starting phase information tends to remain dominant; hence, if substantial error exists in the provisional phases, it is passed on directly to the Fourier maps used in those analyses. In TF, however, the provisional phases are used only to prime an amplitude-dominated procedure.

With each cycle, the structural information contained in the amplitudes improves the phases, thereby purging erroneous structural information carried over from previously solved derivatives. We have routinely used TF phase development in just this way to confirm interpretations of difference Fourier syntheses phased with poor parent phases.

A commonly raised objection to the application of direct methods in this context is the question of insufficient resolution. Unlike the refinement and extension of parent phases (Coulter, 1965; Weinzierl, Eisenberg & Dickerson, 1969; Reeke & Lipscomb, 1969; Coulter & Dewar, 1971; Barrett & Zwick, 1971; Hendrickson, Love & Karle, 1973; Hendrickson & Karle, 1973), interpreting heavy-atom derivatives by direct methods involves locating a relatively small number of isolated atoms in a large asymmetric unit. Even at low resolution, the number of observations is large relative to the number of parameters to be fixed, and the chance of density overlap is remote. It is not surprising, therefore, that both Neidle's (1973) study and this work have succeeded in locating heavy atoms at 8 Å and 6 Å resolution respectively. Indeed, we submit that difficulties arise more from the fact that we are analysing a difference density than from questions of resolution. For example, it is likely that because of a lack of isomorphism a certain degree of negative difference density will exist, undermining to some extent the non-negativity assumption on which direct methods are based (Sayre, 1952; Sikka, 1969). Moreover, there exist inherent errors in difference structure amplitudes, as discussed by Henderson & Moffat (1971). Chief among these is the fact that for the non-centrosymmetric reflection $|\Delta F|$ can be a poor estimate of the true heavy-atom structure amplitude f_H. Studies are currently under way to evaluate a better estimate of f_H provided by differences in anomalous scattering (Matthews, 1966).

We thank Dr Richard Schevitz of this Department for his general advice and for providing the tRNA data; Dr Karl Hardman of the Argonne National Laboratory for supplying the Con-A data; Dr R. Fletterick of Yale University for advice on the use of certain programs; Dr Martin Zwick and Mr John Hughes of this Department for stimulating discussions, and Ms Lynn Kubin and Mr Gerald Grofman for preparation of figures.

This work was supported by grants from the National Science Foundation (GB 27645X) and the U.S. Public Health Service (GM 15225). M.A.N. was supported by the Danforth Foundation and U.S. P.H.S. Training Grant GM 780 from the National Institue of General Medical Sciences. P.B.S. was the recipient of a Research Career Development Award from NIGMS (GM 22801).

References

BARRETT, A. N. & ZWICK, M. (1971). *Acta Cryst.* A**27**, 6–11.

COULTER, C. L. (1965). *J. Mol. Biol.* **12**, 292–295.

COULTER, C. L. & DEWAR, R. B. K. (1971). *Acta Cryst.* B**27**, 1730–1740.

DEWAR, R. B. K. (1968). Ph. D. Thesis, University of Chicago.

FLETTERICK, R., ANDERSON, W., HWANG, K. & STEITZ, T. A. (1972). Presented at 1st Northeast Protein Crystallography Workshop; Oct. 29–Nov. 1.

GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.

HARDMAN, K. D. & AINSWORTH, C. F. (1972). *Biochemistry*, **11**, 4910–4919.

HAUPTMAN, H. & KARLE, J. (1953). *Solution of the Phase Problem. I. The Centrosymmetric Crystal.* A.C.A. Monograph No. 3. Pittsburgh: Polycrystal Book Service.

HENDERSON, R. & MOFFAT, J. K. (1971). *Acta Cryst.* B**27**, 1414–1420.

HENDRICKSON, W. A. & KARLE, J. (1973). *J. Biol. Chem.* **248**, 3327–3334.

HENDRICKSON, W. A., LOVE, W. E. & KARLE, J. (1973). *J. Mol. Biol.* **74**, 331–361.

JOHNSON, C. D., ADOLPH, K., ROSA, J. J., HALL, M. D. & SIGLER, P. B. (1970). *Nature, Lond.* **226**, 1246–1247.

KARLE, J. (1968). *Acta Cryst.* B**24**, 182–186.

KARLE, J. & KARLE, I. L. (1966). *Acta Cryst.* **21**, 849–859.

KENNARD, O., ISSACS, N. W., MOTHERWELL, W. D. S., COPPOLA, J. C., WAMPLER, D. L., LARSON, A. C. & WATSON, D. G. (1971). *Proc. Roy. Soc.* A**325**, 401–436.

MATTHEWS, B. W. (1966). *Acta Cryst.* **20**, 230–239.

NEIDLE, S. (1973). *Acta Cryst.* B**29**, 2645–2647.

REEKE, G. N. & LIPSCOMB, W. N. (1969). *Acta Cryst.* B**25**, 2614–2623.

SAYRE, D. (1952). *Acta Cryst.* **5**, 60–65.

SCHEVITZ, R. W., NAVIA, M. A., BANTZ, D. A., CORNICK, G., ROSA, J. J., ROSA, M. D. H. & SIGLER, P. B. (1972). *Science*, **177**, 429–431.

SCHEVITZ, R. W., NAVIA, M. A., CORNICK, G. PASEK, M., ROSA, J., HUGHES, J., KRISHNAMACHARI, N., HEWITT, W. & SIGLER, P. B. (1974). Work in progress.

SIKKA, S. K. (1969). *Acta Cryst.* A**25**, 539–543.

STEITZ, T. A. (1968). *Acta Cryst.* B**24**, 504–507.

WEINZIERL, J. E., EISENBERG, D. & DICKERSON, R. E. (1969). *Acta Cryst.* B**25**, 380–387.