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Application of Known Triplet Phases in the Crystallographic Study of Bovine Pancreatic Trypsin Inhibitor. II: Study at 2.0 Å Resolution

R. H. Mathiesen and F. Mo*

Institutt for fysikk, Norges Teknisk-Naturvitenskapelige Universitet, N-7034 Trondheim, Norway. E-mail: ragmat@phys.ntnu.no

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Abstract

Direct methods strengthened by the application of about 130 triplet phases, assumed known with a mean error of about ±20°, were used to re-determine the structure of BPTI with data at 2.0 Å resolution. The triplet phases served to shift the mean direction and enhance the concentration parameter in the corresponding Cochran distributions. These phases were used in combination with the partial structure extracted from successive density maps to control the gradual expansion and refinement of Fourier coefficients. Single phases were developed iteratively from tangent-formula estimation following the path of the convergence map. The *a priori* triplet-phase information was sufficient to initiate solution of the structure at 2.0 Å.

1. Introduction

In previous studies (Mo *et al.*, 1996; Mathiesen & Mo, 1997) we have shown that a small set of triplet phases could be applied as a starting point and a powerful guide for direct methods in solving macromolecular structures. With this physical information at hand structure solution can also be obtained when the resolution of the data does not meet the present demands of *ab initio* techniques such as *Shake and Bake* (Miller *et al.*, 1993; Weeks *et al.*, 1995) or the combination of direct methods and Patterson methods (Sheldrick *et al.*, 1993). From the investigations on bovine pancreatic trypsin inhibitor, form II (BPTI), (Walter & Huber, 1983; Wlodawer *et al.*, 1984) we found that 90–130 experimentally accessible triplet phases could provide the basis for structure solution both with data at 1.55 and 1.75 Å resolution (Mathiesen & Mo, 1997). Also important in this work was the inclusion of atomic positions assigned from the strongest maxima in the *E* maps. Even in the first map more than 80% of a group of selected maxima were later shown to correspond to true molecular features. The introduction of a new criterion, PHIFOM, based on the assumed known triplet phases was helpful both for the initial selection of the best phase model, and as an aid in discriminating between several possible molecular fragments in the early stages of modelling. However, tests preliminary to the present work made it clear that the techniques that had been successfully applied in the work with BPTI at 1.55 and 1.75 Å resolution were inadequate with data at lower resolution. In particular it became apparent that information on the triplet phases should be used more extensively in the phase refinement and expansion following the *E* map from the initial phase model. This implies that the *a priori* phase information is conserved and combined with the structure information emerging in the first critical steps of expansion.

2. Theoretical considerations

The actual phase refinement was performed using a slightly modified version of the weighted tangent formula (Germain *et al.*, 1971). The estimate, β, for a single structure-factor phase, $\varphi_{\rm H}$, is given as

$$\tan\beta = \frac{\sum_{\rm L} w_{\rm L} w_{\rm H-L}|E_{\rm L}||E_{\rm H-L}|\sin(\varphi_{\rm L}+\varphi_{\rm H-L}+\Phi_3)}{\sum_{\rm L} w_{\rm L} w_{\rm H-L}|E_{\rm L}||E_{\rm H-L}|\cos(\varphi_{\rm L}+\varphi_{\rm H-L}+\Phi_3)} = \frac{T_{\rm H}}{B_{\rm H}}, \qquad (1)$$

where

$$\Phi_3 = \begin{cases} \Phi_3^{\rm est} & \text{for estimated triplet phases} \\ 0 & \text{for all other triplet phases.} \end{cases}$$

$$w_{\rm H} = \tanh[\tau_{\rm H,L}|E_{\rm H}|(T_{\rm H}^2 + B_{\rm H}^2)^{1/2}].$$

$\tau_{\rm H,L}$ are pre-factors associated with the individual triplet-phase relationships (TPR's).

$$\tau_{\rm H,L} = \begin{cases} c_1(|E_{\rm H}E_{\rm L}E_{\rm H-L}|)^{-1} & \\ \times \tanh^{-1}(2P_{\rm H,L}-1) & \text{for a known TPR} \\ \sigma_3\sigma_2^{-3/2} & \text{for all other TPR's.} \end{cases} \qquad (2)$$

$P_{\rm H,L}$ are user-defined probabilities for the individual TPR's, c_1 is an adjustable parameter to enhance the influence of the known TPR's on the phase refinement.

By correct adjustment of c_1, a convergence map (Germain *et al.*, 1970) can be constructed in which the individual reflections at the bottom of the map are those involved in the known TPR's. All relationships are set up

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in the form

$$Q_{\mathbf{H},\mathbf{L}} = \varphi_{\mathbf{H}} + \varphi_{-\mathbf{L}} + \varphi_{\mathbf{L}-\mathbf{H}} \qquad (3)$$

with an associated weight $\kappa_{\mathbf{H},\mathbf{L}} = \tau_{\mathbf{H},\mathbf{L}}|E_{\mathbf{H}}E_{\mathbf{L}}E_{\mathbf{H}-\mathbf{L}}|$. $Q_{\mathbf{H},\mathbf{L}}$ is initially 0 for all TPR's. For a known TPR, $Q_{\mathbf{H},\mathbf{L}}$ takes on the value of $\Phi_3^{\rm est}$. If structure information is available, all $Q_{\mathbf{H},\mathbf{L}}$ and $\kappa_{\mathbf{H},\mathbf{L}}$, except those TPR's assigned a $\Phi_3^{\rm est}$, are estimated according to Main (1976). Each time new structure information is included, or the convergence map is changed due to introduction of more reflections, c_1 will have to be reoptimized to ensure that all known TPR's remain in the bottom region of the convergence map. This condition will generally not be preserved unless the weights of the known TPR's are increased sufficiently. Modifications of $\kappa_{\mathbf{H},\mathbf{L}}$ due to the structure information will effect relative changes among the $\kappa_{\mathbf{H},\mathbf{L}}$'s, which in turn will usually also change the order in which the reflections appear in the convergence map. Since the phase refinement using (1) based on the convergence map is an iterative process, the known TPR's have their strongest influence if they form the primary basis from which (1) expands and refines new phases.

If the triplet phases to be measured are selected correctly, including reflections suitable for origin definition, the set of Φ_3's provide estimates for single structure-factor phases. Known triplet phases and derived single-phase estimates are information that can be formulated in a special figure of merit defined as PHIFOM (Mathiesen & Mo, 1997). The constant c_1 in (2) can be optimized using PHIFOM as a monitor. A minimal PHIFOM corresponds to known TPR's having an optimal influence on the phase refinement. Other triplet phases can be calculated from the single phase estimates and included in PHIFOM. In this work the latter triplet phases were not input to (1), but were treated as general Φ_3's initially set to 0, and later modified according to the current structure fragment. Thus, PHIFOM will contain information both from the measurements and the structure as long as the expansion is carried out applying (1).

3. Experimental

Intensity data comprising 17 615 unique reflections in the d range 7.2–1.0 Å were kindly provided by Dr M. Schneider of Professor R. Huber's laboratory. Low-resolution data are important for good triplet coupling, so intensities in the shell $7.2 < d < 20$ Å were calculated from published coordinates with an overall isotropic B. Missing data were given a value of $0.5|F_o|_{\rm min}$ within each subshell of thickness 0.025 Å^{-1} in $\sin(\theta)/\lambda$ and then added to complete the data. Three program packages were employed for expansion and refinement of the phases: *MULTAN78* (Main *et al.*, 1978), which was modified locally to accept triplet phases with user-defined weights as input in the *SIGMA2* routine; *MULTAN88E* (Debaerdemaeker, Germain *et al.*, 1988) which employs the *SAYTAN* formalism (Debaerdemaeker *et al.*, 1985; Debaerdemaeker, Tate *et al.*, 1988); and finally the package *Xtal3.2* (Hall *et al.*, 1992) that was used to extend and refine the structure model.

3.1. *Work at 2.0 Å resolution*

A total of 3716 reflections, of which 3490 had weights $w > 0$, were normalized and sorted in order of descending amplitudes. The 400 largest E's were chosen to set up a total of 7800 TPR's of which 5500 with $\kappa > 0.29$ were used in the convergence map. From the bottom region of this map 130 Φ_3 were selected according to experimental requirements on the relative and total strengths of the amplitudes of the $F_{\mathbf{H}}$, $F_{-\mathbf{L}}$ and $F_{\mathbf{L}-\mathbf{H}}$ structure factors that form the TPR's. The set of Φ_3 was chosen also to set up a sufficient basis for deriving estimates of single phases $\varphi_{\mathbf{H}}$ as described previously (Mathiesen & Mo, 1997). All Φ_3's were assigned values $i \cdot \frac{\pi}{4}$, $i \in \{0, \ldots, 7\}$, closest to the corresponding value calculated from the published structure. Including reflections for the origin definition the selected Φ_3's provided estimates for 133 single $\varphi_{\mathbf{H}}$'s, from which another 537 Φ_3's could be derived yielding a total of $t = 667$ Φ_3's to be used in PHIFOM, *i.e.* 12% of the TPR's used in the convergence map. The permutation of one enantiomer-defining reflection gave two different solutions. The starting set was first refined internally to obtain inner consistency, and then these phases were kept constant while the main body of the phases was refined until convergence. All phases were allowed to vary in two to three final cycles of refinement.

In the studies at higher resolution, the strongest maxima found in an automatic peak search (APS) were included as atoms in the *SAYTAN* algorithm to accomplish further phase expansion and structure modelling (Mathiesen & Mo, 1997). This procedure was also tried initially with the 2.0 Å data. In several separate runs, the 400 single phases from the initial cycle, including information from 20 to 40 APS maxima were recycled in *MULTAN88E*. The corresponding mean triplet-phase errors, $\langle \Delta\Phi_3 \rangle$, from the various runs were all above 83°, even for a small expansion of the phase set from 400 to 500 phases. The values of PHIFOM and $\langle \Delta\Phi_3 \rangle$ indicated that the 400 initial single-phase estimates were of the same quality as had been obtained at the same stage with data at higher resolution. Therefore, a probable explanation for the erroneous phase expansions was an insufficient quality of the structure fragment, defined by the APS maxima, as a basis for structure solution.† As a test, we assigned the 20 top APS maxima to the correct atomic species in their refined positions. Recycling *SAYTAN*, restrained with 400 single phases and the 20 atoms, provided 500 phases with $\langle \Delta\Phi_3 \rangle \simeq 80°$, which may be insufficient for a structure without heavy atoms,

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† The root-mean-square (r.m.s.) deviation from published atomic coordinates for APS maxima was about 0.6 Å at 2.0 Å resolution compared with approximately 0.3 Å with 1.55 Å data.

like BPTI, at this resolution (Mo *et al.*, 1996). This result suggested that the known triplet phases should be retained along with the information on structure and single phases, at least in the first stages of phase expansion and refinement. Starting from a convergence map was preferred over a full *SAYTAN* refinement of all phases simultaneously as the former procedure will iterate new phases from a basis of fixed single phases being internally consistent with the known triplet phases. The program *MULTAN*78 was recoded to incorporate structure information and physically estimated triplet phases simultaneously. The same test, including 400 single phase estimates and 20 correctly located and assigned atoms, was repeated. The resulting 500 phases now had a $\langle\Delta\Phi_3\rangle \simeq 68^\circ$. This is a decisive improvement, demonstrating the importance of triplet-phase information to guide the structure solution at 2.0 Å resolution.

Table 1. *Development of κ_{min}, PHIFOM and the mean triplet-phase error, $\langle\Delta\Phi_3\rangle$, number of E's phased by direct methods, and number of atoms included both from modelling and as APS maxima prior to the refinement.*

$\kappa_{\rm min}$	PHIFOM (°)	$\langle\Delta\Phi_3\rangle$ (°)	No of *E*'s	Mod. + APS input	Algorithm
0.29	15.49	69.12	400	0 + 0	(1)
0.34	21.86	73.54	450	0 + 40	(1)
0.77	17.53	77.38	550	6 + 53	(1)
0.82	17.94	79.71	650	6 + 57	(1)
0.78	18.50	79.68	750	11 + 57	(1)
0.77	18.60	81.86	900	11 + 69	(1)
0.86	18.92	80.04	900	21 + 67	(1)
1.08	19.80	77.93	900	29 + 71	(1)
0.85	42.18	77.81	1100	40 + 67	*SAYTAN*
1.10	39.16	74.90	1300	60 + 68	*SAYTAN*
1.55	39.01	69.35	1300	84 + 67	*SAYTAN*

40 APS maxima with coordinates from the first *E* map were labelled C atoms and included as a partial structure in the subsequent refinement and expansion by (1). The primary strategy for this process was to use (1) with the following additional information. (*a*) 130 known TPR's; (*b*) 133 single-phase estimates from the TPR's above, and 267 phases with values obtained from the initial refinement cycle; (*c*) *m* APS maxima input as C atoms, and *n* modelled atoms for recalculation of general $Q_{\rm H.L}$ and $\kappa_{\rm H.L}$ (Main, 1976).

The weighting scheme for the TPR's made it possible to recycle and expand the phase set using information both from reciprocal (known TPR's) and real space (structure fragment) simultaneously. By varying the value of c_1 in (2) the known TPR's were weighted appropriately to remain at the bottom of the convergence map despite the relative changes among the weights of general TPR's as the amount of structure information increased.

Of the 40 APS maxima in the first *E* map there were three pairs with an interdistance 2.75 Å or less. As a test to identify possible S—S bridges among these maxima PHIFOM was calculated for four models resulting from recycling the following partial structure alternatives: 40 C atoms and three different combinations of 38 C + 2 S atoms. Only one of the latter combinations gave a PHIFOM lower than that from the model with 40 C atoms. In order to maximize the influence of the structure fragment in this test, c_1 was kept at a constant low value. Structure modelling was started based on this tentative identification of an S—S bridge. It was possible to localize the Cβ atoms in a map calculated from the 2 S + 38 APS maxima recycled once more by (1) in order to reoptimize c_1.

New APS maxima were included from the following map, and the Cα and N atom in one of the Cys residues were localized. The N-atom position corresponded to one of the 40 APS maxima selected from the first map. As in our previous work at higher resolution, the structure modelling was initially carried out in *E* maps by carefully adding atom by atom to the fragment using rather conservative restraints on bond distances and angles. Modelling in the *E* maps was made using the Alberta/Caltech *TOM/FRODO* program (Israel *et al.*, 1994). In the top section of Table 1 the refinement and expansion of phases using (1) can be studied in detail. Expansion was carried out in a conservative manner, aiming to increase the average number of relations for each reflection slowly to ensure that the weaker *E*'s had sufficient statistics for phase estimation. Only TPR's with $\kappa > \kappa_{\rm min}$ were used in the convergence map. New APS maxima were included from each new map, and old APS maxima decreasing in density below a selected cutoff value were filtered out. During the first cycles of expansion, c_1 was kept at very high values (55–60) to allow the known triplet phases dominate the refinement. When about 80 atoms (Model + APS maxima) had been included, c_1 was reduced to enhance the weight of the accumulated structure information.

When the number of atoms exceeded 100, expansion and refinement was continued using the *SAYTAN* algorithm coded in *MULTAN*88*E*. Known triplet phases were then no longer retained as input to the program. All estimates of $Q_{\rm H.L}$ and $\kappa_{\rm H.L}$ were calculated from the current structure fragment alone (Main, 1976). Results from the refinement with *SAYTAN* are shown in Table 1. The increase in PHIFOM is due to the exclusion of the known triplet phases as restraints to the refinement algorithm, when *SAYTAN* is used instead of (1). After constructing a molecular model consisting of 84 connected atoms, and in addition 67 unconnected APS maxima, it became difficult to expand the structure fragment based on *E* maps. Further expansion and refinement was attempted, but phasing more than 1300 reflections by direct methods became difficult, as a modest increase in the number of *E*'s resulted in a dramatic increase in the number of TPR's needed. The lowering of $\kappa_{\rm min}$, necessary to process TPR's involving new unphased reflections, also results in processing a large number of TPR's which are redundant with respect to the phases refined earlier.

From this point on, all 3490 reflections, with weights $w > 0$, were phased from least-squares refinement. At first, only the scale and an overall isotropic atomic displacement factor were refined. From ΔF maps, $C\beta$ positions along the modelled chain could be localized as well as new atoms at the terminals of the partial backbone. At this stage, and with the 2.0 Å data, it was advantageous to continue with a combination of ΔF and $2F_o - F_c$ maps. Details from this part of the work are given in Table 2. When the backbone between the two cysteines Cys30 and Cys51 was nearly complete, it was apparent that the two separate parts were shifted relative to one another; r.m.s. deviations with respect to the published coordinates were 0.423 and 0.476 Å for the connected molecule and for the molecule + APS maxima (related to their closest counterpart in the published structure), respectively. Least-squares refinement of the atomic coordinates, using restraints for idealized bond lengths and angles, was now performed regularly towards the end. No restraints or constraints were applied on torsion angles or planarity of groups of atoms. After convergence of the first refinement, the r.m.s. value of the connected molecular fragment was reduced to 0.355 Å. The work was terminated when 451 atoms had been fitted to the model; 131 of the atomic positions originated from APS maxima. The 451 atoms completed most of the protein structure with the exception of a few atoms in the front and rear-end residues and some incomplete lysine residues. At the stage of termination, disordering of residues 7 and 53 that was observed with data at 1.0 Å resolution (Wlodawer *et al.*, 1984) was not evident in the maps. The 18 largest unconnected APS maxima had densities above 1.25 e Å^{-3} in the $2F_o - F_c$ maps and were tentatively included as water O atoms. A check against the published structure proved all these assignments to correspond to water structure within 0.6 Å. Of the remaining 19 APS maxima, ten were close to solvent structure positions, but they could not be distinguished from the other nine by their densities alone. The r.m.s. discrepancy between our final 451-atom model and the corresponding fragment from the crystallographic refinement with 1 Å data was 0.239 Å.

Fig. 1 shows four maps in identical cuts through the cell. The actual layer is defined by a least-squares plane through the disulfide bridge S30G—S51G and backbone atoms in surrounding residues. The E map in Fig. 1(*a*) and the F_o map in Fig. 1(*b*) have been calculated from the 400 Fourier coefficients developed in the first refinement cycle. In the E map sharp features appear close to several of the crosses symbolizing final atomic positions. The most dense features represent some of the APS maxima that were selected initially. In comparison, the F_o map does not possess the same amount of sharply defined electron density, but it shows more connectivity that might be used to make initial hypotheses on secondary structure. In Figs. 1(*c*) and 1(*d*) the same layer is presented, 1(*c*) with identical amplitudes as in Fig. 1(*b*), but with phases calculated from the refined structure. Fig. 1(*d*) is based on all 3490 F_o coefficients from the final stage and labels are given for all atoms closer than 0.45 Å to this layer. These atoms are marked with crosses in the other maps.

Table 2. *History of the crystallographic refinement of BPTI at 2.0 Å.*

PHIFOM (°)	$\langle\Delta\Phi_3\rangle$ (°)	Mod. + APS input	Variables	R.m.s.† (Å)
39.17	65.01	112 + 81	Scale, $U_{\rm overall}$	—
37.93	62.01	143 + 79	Scale, $U_{\rm overall}$	—
36.35	60.31	155 + 90	Scale, $U_{\rm overall}$	0.423
35.88	56.44	180 + 90	Scale, $U_{\rm overall}$, xyz	0.335
26.90	44.51	254 + 73	Scale, $U_{\rm overall}$, xyz	0.293
19.31	19.51	451 + 19	Scale, $U_{\rm overall}$, xyz	0.239

† R.m.s. gives the root-mean-square deviation in atomic positions between the modelled structure fragment and the corresponding fragment from the crystallographic refinement with 1 Å data.

Fig. 2 displays three maps with Fourier coefficients as in Figs. 1(*a*), 1(*b*) and 1(*d*), respectively. This layer shows a different spatial region close to the front and rear-end α-helices of BPTI. In all the maps we clearly see a solvent region of the cell. Notice that none of the maps evince significant electron density in the solvent region. Fig. 2(*c*) also shows two of the APS maxima not bonding to the protein, but with electron densities above 1.25 Å, and therefore accepted as water O atoms (OW). By comparison of (*a*), (*b*) and (*c*) it is evident that the 400 initial phases are of adequate quality to distinguish between protein and solvent. This implies that the quality of the maps can be improved by solvent flattening. In fact, we have verified such improvements later by applying the map-modification techniques of Wang (1985*a*,*b*). In retrospect, a joint use of E maps and F_o maps might be advantageous in the early stages of structure modelling and phase expansion. As shown in this work, E maps provide structure information in the form of APS maxima distributed over most of the protein region. Furthermore, the enhanced atomicity of the E maps will facilitate structure modelling in localized regions. F_o maps are more continuous by nature, and will therefore reveal better the connectivity which is required for the application of programs like *Bones* (Israel *et al.*, 1994). In the early stages of the phase expansion, the number of Fourier coefficients available is limited. Therefore, F_o maps will not necessarily reflect the actual resolution available in the data. The actual resolution of the maps is improved using E's.

This study has shown that the single phases derived from direct methods restrained with the known triplet phases, are of sufficient quality to initiate solution of macromolecular structures at 2.0 Å resolution. More work has to be carried out to optimize the real-space interpretations, and also to improve the strategies for phase expansion.

3.2. *Using a priori phase information in direct-methods procedures*

The prominent direct methods for solution of the phase problem are based on a probability distribution of the triplet phase invariant due to Cochran (1955). It predicts the triplet phase to be 0, with a variance dependent on the magnitudes of the structure factors involved and on the complexity of the structure through κ. Given the complexity of macromolecules the variance increases to the extent that the distribution becomes nearly uniform in the range $[-\pi, \pi]$, hence nearly non-informative. Introducing *a priori* phase information will improve this situation. Triplet phases, derived for instance from physical measurements, are one such source of information. They can be employed to shift the mean direction of the triplets towards their correct values, and the variances can be reduced by empirical enhancement of the κ's. The effect of this in the first crucial steps of structure solution can be decisive.

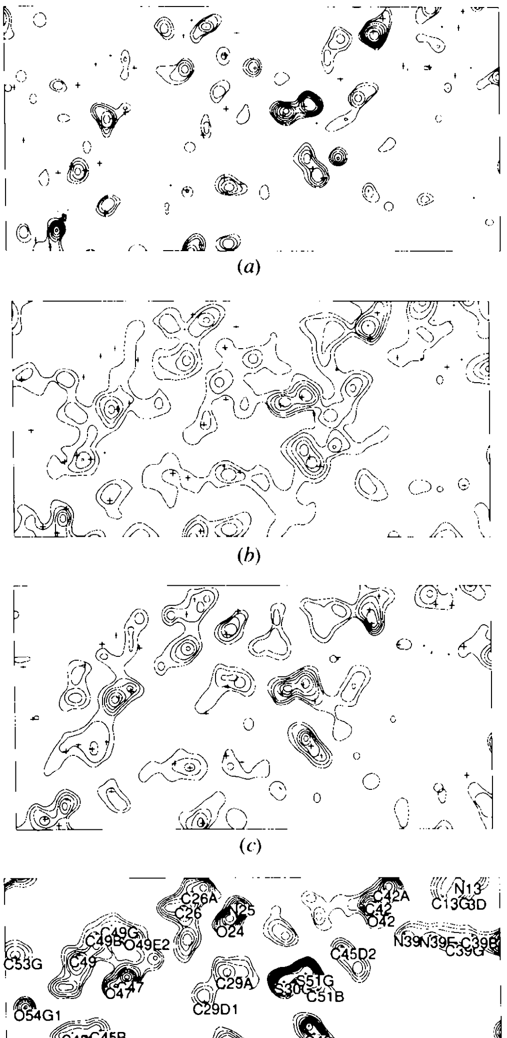

Fig. 1. Four identical sections calculated from a least-squares plane through the atoms C29A C29 N30 C30A C30 S30G N31 C31A S51G. Refined atomic positions closer than 0.45 Å to this plane are marked with crosses in all the maps, map (*d*) also shows the atom labels; (*a*) 400 *E*'s from the initial refinement cycle, (*b*) 400 F_o's from the initial refinement cycle, (*c*) 400 F_o's with amplitudes identical to (*b*), but phases calculated from the final structure model, (*d*) 3490 F_o's from the final model. Lowest contour level and increment in density are: 0.27, 0.07 (*a*), 0.38, 0.07 (*b*), 0.45, 0.07 (*c*), and 0.7, 0.25 e Å^{-3} (*d*), respectively.

A well known alternative source for phase information are single–wavelength anomalous scattering (SAS) experiments. SAS phases can be combined with direct

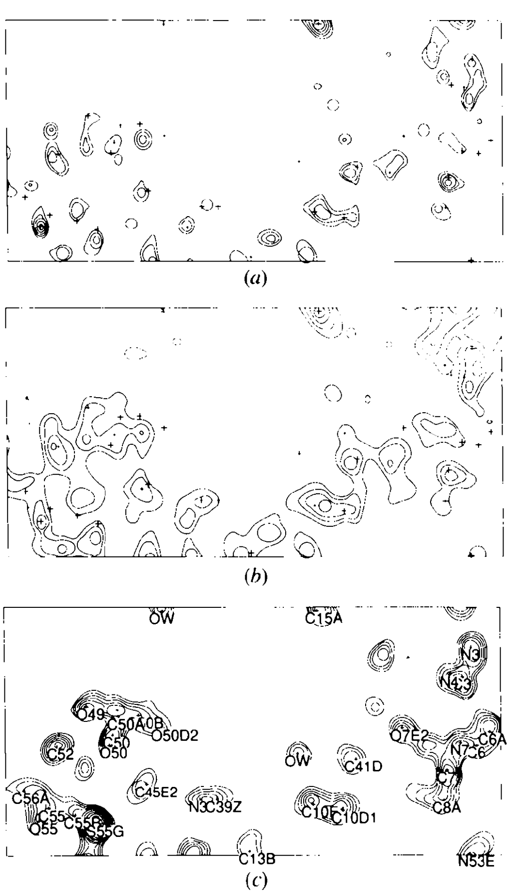

Fig. 2. Three identical sections calculated from a least-squares plane through the atoms of the backbone . Refined atomic positions closer than 0.6 Å to the plane are marked with crosses in all the maps. In map (*c*) also the atom labels are printed; (*a*) 400 *E*'s from the initial refinement cycle, (*b*) 400 F_o's from the initial refinement cycle, (*c*) 3490 F_o's from the final model. Lowest contour level and increment in density are as for the corresponding Figs. 1(*a*), (*b*) and (*d*), respectively.

methods (Hauptman, 1982; Giacovazzo, 1983; Fan & Gu, 1985) and their potential for structure solution has been demonstrated in several test cases (Mukherjee *et al.*, 1989; Sha *et al.*, 1995; Guo *et al.*, 1997).

In the present and previous work (Mo *et al.*, 1996; Mathiesen & Mo, 1997) we have been concerned with optimizing the application of the tangent formula (Karle & Hauptman, 1956; Germain *et al.*, 1971; Main, 1976) restrained with known triplet phases. Similar or even better results could well arise if this kind of information was fed into *SAYTAN* procedures (Debaerdemaeker *et al.*, 1985; Debaerdemaeker, Tate *et al.*, 1988), maximum-entropy methods (Bricogne, 1984; Gilmore, 1996) or the *Shake & Bake* method (Miller *et al.*, 1993; Hauptman, 1995). Inherent in *ab initio* multisolution methods for macromolecular work is the need to develop and examine very large numbers of models. If data are available at atomic resolution, about 1.2 Å, correct models can be identified after numerous trials of phase refinement and structure-fragment expansion in a fully automated procedure like *Shake & Bake*. With data at lower resolution even more models are required. A further complication then is the lack of a unique FOM that converges fast enough to identify good models after the initial refinement. This seems to limit the applicability of present *ab initio* techniques with data at less than atomic resolution to studies of structures containing heavy atoms. *A priori* triplet phase information will reduce or may eliminate the need for exploring a large number of trial models. The information forms the basis for a unique FOM, and the resolution in the data can be lowered.

The introduction of known triplet phases from three-beam interference measurements should be regarded as complementary to other means of providing *a priori* phase information. The method does not require the presence of heavy atoms in the structure. The limitations of methods based on physically estimated triplet phases lie primarily in the requirements of the experiment: the crystal mosaicity must be moderate, FWHM of ω rocking curves should not exceed about 0.05°, and the lifetime in the beam must be sufficient to allow orientation of the crystal and some measurements.

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