

There are, however, two differences between the Patterson and reciprocal-space results. The first is the treatment of multiple vectors at a single grid point of the Patterson map. The Patterson approach suggests that the value at each Patterson grid point should be used only once in the search function sum. The reciprocal lattice approach states that the Patterson map must be sampled at each vector regardless of other vectors in the same position. It will, however, be seen that as the chosen grid interval is reduced the Patterson and reciprocal-space criteria converge. The second difference is that the reciprocal-space search function in no way suggests a positive contribution from the unknown terms corresponding to the cross-vectors between virus particles. Differences between these criteria are to be expected as the chosen functions are themselves different.

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The Determination of the Heavy-Atom Substitution Sites in the Satellite Tobacco Necrosis Virus

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The heavy-atom binding sites in two derivatives of crystalline satellite tobacco necrosis virus were found by a vector search method [Argos & Rossman, *Acta Cryst.* (1976), **B32**, 2975–2979]. For each derivative there is a single major site in each of the 60 icosahedrally related subunits in the virus coat. The derivative scale factor, the occupancy, and positional and thermal parameters of the sites were refined by structure factor calculations. Single isomorphous phases calculated from either refined derivative were sufficiently accurate to locate the substitution site in the difference Fourier map of the other derivative. The refinement statistics indicate that the major sites have been found.

Introduction

The satellite tobacco necrosis virus (STNV) is one of the smallest viruses known, so small that it lacks some functions necessary for replication and must co-infect

with the larger, fully competent tobacco necrosis virus in order to propagate (Kassanis & Nixon, 1961). The virus particle is an aggregate of 60 copies of the single coat-protein molecule, M.W. 23 000, arranged in a $T=1$ icosahedral surface lattice (Åkervall *et al.*, 1971; Klug, 1971) and a single-stranded ribonucleic acid molecule, M.W. 400 000, which codes for that coat protein (Reichmann, 1964).

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Purified STNV crystallizes in space group $C2$ with $a=319$, $b=304$, $c=185.5$ Å, $\beta=94.37^\circ$, $Z=4$ with a single particle of M.W. 2×10^6 in the crystallographic asymmetric unit (Fridborg *et al.*, 1965). Because of the relatively small size of the virus, the high order of the crystals, and the unusual occurrence of an entire particle as the asymmetric unit, an effort is being made to determine the structure, ultimately at high resolution, of this intact, infectious virion.

The structure can be determined by the conventional multiple isomorphous replacement method (MIR; Green, Ingram & Perutz, 1954) using heavy-atom derivatives of the coat protein for phase determination because, although the number of sites is expected to be very large, the set of sites should retain the icosahedral point symmetry of the coat. Thus, the region that must be explored for heavy-atom binding sites is only a single subunit and is small enough to be completely explored systematically. The search requires the directions of the non-crystallographic symmetry axes, *i.e.* the particle orientation, to generate the symmetry-related sites. Further, the refinement of sites found by the search requires the particle-centre location in order to assign cell coordinates to the sites for structure factor calculation. The orientation has been determined by fitting an icosahedral axis set to the peaks observed in the rotation function (Rossmann & Blow, 1962) calculated with 10 Å STNV data (Lentz & Strandberg, 1974) with an estimated error of $\pm 0.7^\circ$ in each of the three angles describing that orientation. The approximate particle-centre location was determined by comparison of the observed low-resolution ($d > 25$ Å) data with structure factors calculated for a uniformly dense spherical shell placed in all possible locations in the cell (Rossmann, Åkervall, Lentz & Strandberg, 1973).

We report here the successful application of the vector search method and programs of Argos & Rossmann (1976) to the solution of two STNV derivatives at 10 Å resolution and the preliminary refinement of those heavy-atom sites, such refinement being a clear demonstration of the correctness of the solution and of the applicability and practicality of the MIR technique to virus structure determination.

Experimental

The 'platinum' derivative was prepared by replacing roughly half (*ca* 50 μ l) of the mother liquor (1.2% virus in 0.001M $MgSO_4$) surrounding a crystal wedged in a glass capillary (Åkervall & Strandberg, 1971) with a solution containing 1.5% virus and 0.005M K_2PtCl_4 . Three such replacements were made on successive days giving a final concentration of about 0.0044M K_2PtCl_4 . After soaking for a day at the final concentration, the capillary was drained and sealed. Although data collection was usually begun immediately, these crystals were stable for many weeks. However, if the capillaries were not drained, the crystals generally deteriorated within a few days.

The 'iodine' derivative was prepared at room temperature by reacting a 0.1% STNV solution in 0.05M borate buffer at pH 9 with an I_3^- solution (final concentration 4.8 I_3^- ions per subunit). After 15 min the reaction was stopped by dialysis against 0.001M $MgSO_4$. The modified STNV was concentrated by ammonium sulphate precipitation and subsequent dialysis against 0.001M $MgSO_4$ for at least 5d. Iodination caused a significant increase in absorbance in the UV region 305 to 325 nm (Edelhoch, 1962); however, at this stage we have not determined which residues are modified. Neutron activation analysis indicates about three I atoms bound per protein subunit.

The 'iodine' derivative was crystallized with STNV native seed crystals. The seeds were transferred to a capillary containing 1.5% iodinated STNV solution, and the solution was replaced every third day. Usually the crystals had grown large enough for data collection within about two weeks. The part of the crystal grown from the modified virus was isomorphous with the native.

Three-dimensional data sets to 10 Å resolution were collected for the native virus crystals and the two derivatives by single-layer, screened precession photography with large crystals wedged in sealed, flattened glass capillaries (Åkervall & Strandberg, 1971). The photographs were measured on an automated microdensitometer constructed by Dr V. Klimecki of the Chemistry Department, Uppsala University in collaboration with our group. The data reduction was accomplished with the program system described by Järup, Kannan, Liljas & Strandberg (1970). Each set contained approximately 8100 measured reflexions of the 10 000 possible in the 10 Å Cu sphere. The data sets were scaled together by the method of Kraut, Sieker, High & Freer (1962), and the derivative structure factors were then arbitrarily multiplied by 1.05 in an attempt to compensate for the anticipated greater scattering of substituted crystals.

Vector search

The vector search procedure and associated computer programs have been described by Argos & Rossmann

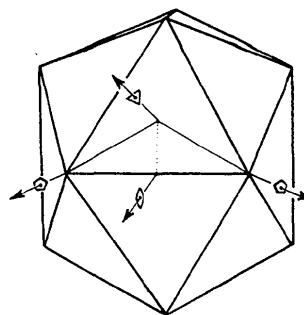


Fig. 1. Icosahedron showing the location of symmetry axes and asymmetric volume boundaries described in the text.

(1976). Only a small change in the mode of stored map access was required to convert the programs for use on an IBM 370/155.

The heavy-atom difference Patterson maps were calculated at 3 Å intervals ($a/100, b/100, c/50$) with all data between 50 and 10 Å resolution. The maps were calculated with unmodified coefficients but all points within 10 Å of the origin were set to zero when the map was stored in the search program.

The asymmetric volume of the icosahedral virus particle may be chosen to be bounded by one threefold axis and two neighbouring fivefold axes (Fig. 1). There is a twofold axis midway between those fivefold axes. The particle has 222 symmetry, a subset of the 532 icosahedral symmetry, and therefore the vector set has $2/m, 2/m, 2/m$ symmetry. Because the search function uses only the vectors relating atoms within, but not between, particles, the function has the same symmetry

as that self-vector set and there is a mirror plane connecting the threefold and twofold axes of the asymmetric volume. Thus, the two halves of the asymmetric volume are equivalent and a peak observed in the search corresponds to either of two sites in real space, here called the different 'hands' of a site.

The difference Patterson syntheses for the two derivatives were searched with vector sets for all icosahedrally distributed heavy-atom positions in constant radius shells from 49 to 100 Å from the particle centre in 3 Å intervals. At each radius, points with the same polar coordinates were used: the points were chosen to be 3 Å apart at 90 Å radius; elsewhere, the interval is proportional to the ratio of the radii. The coordinates of the boundary symmetry axes were determined from the rotation-function best fit (Lentz & Strandberg, 1974) and are listed in Table 1.

Table 1. Polar coordinates of axes used to generate icosahedrally related heavy-atom sites and to define asymmetric volume

Axis	ψ	ϕ	Boundary of search triangle
3	62.628°	11.646°	x
2	41.857	8.610	x
5	99.851	15.140	
2	84.955	104.265	
5	46.968	53.593	x

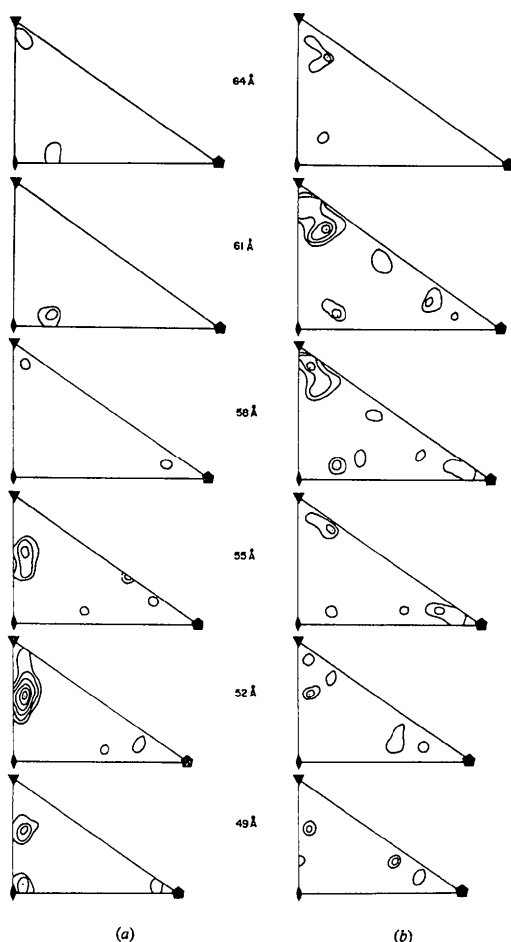


Fig. 2. Constant radius sections through the vector search function from 49 to 64 Å radius. The contours are in estimated standard deviations above the mean of the complete function beginning with 2σ . (a) Pt, highest peak at $\psi = 53.8^\circ$, $\phi = 7.36^\circ$, $r = 52$ Å. (b) I, highest peak at $\psi = 57.7^\circ$, $\phi = 5.34^\circ$, $r = 61$ Å. Polar coordinates of triangle boundaries are given in Table 1.

The sections from 49 to 64 Å, which contain all the larger peaks, are shown in Fig. 2. The highest Pt peak is 7.5σ (estimated standard deviation) above the mean; the highest I peak, 5.0σ . For both searches the next highest peaks are around 3.5σ . The major I peak extends over several sections and has subsidiary maxima. If the iodinated residue were tyrosine, the result of the search would be consistent with a mixture of the two 3-monoiodotyrosines and possibly also 3,5-diiodotyrosine or with more than one reactive residue in the vicinity.

None of the major sites are on the non-crystallographic symmetry axes or on the planes connecting those axes. While this is, hopefully, because of the genuine lack of substitution there, it may also result from the greatly reduced number of vectors included in the summations at these points, because the program eliminates excess duplicated vectors. The lack here of 'radial streaks' as observed by Argos & Rossmann (1976) at the known sites in their model calculations may be the result of the sharpening of our Patterson maps due to the exclusion of the data to 50 Å resolution.

Refinement

The preliminary refinement of the major site for each derivative was by structure factor calculations holding all parameters but one constant and searching for a minimum in the residual, R , calculated with respect to

observed ΔF , for each parameter and assuming exact icosahedral equivalence of the 60 sites. Initially, the site coordinates were fixed at the vector search maxima and the derivative scale factor and occupancy were refined to approximate the magnitude of the observed differences. Once the scale and occupancy stabilized, the coordinates of the particle centre were varied. The centre had been poorly located initially and shifted from $x=0.23$, $z=0.26$ to $x=0.249$, $z=0.255$, or about 6 Å, before a minimum in R was reached. Significantly, each derivative taken independently gave a large shift to the same centre location.

As each site in the vector search may be either of two sites in real space, to use the derivative data to calculate double isomorphous phases it is necessary to make a 'hand' choice for each site such that the two derivative sites are self-consistent. A single cycle of double isomorphous phase calculation was run for each of the four possible combinations of the I and Pt major sites, and that combination giving the lowest R was chosen. Then the x , y , and z site coordinates, the derivative scale, occupancy, and temperature factor were individually adjusted to their residual minima. This rather tedious optimization was performed initially with only the centrosymmetric data; however, only small shifts were obtained when the procedure was repeated with three-dimensional data. Once the heavy-atom parameters had been reoptimized with the full data, difference Fourier maps were calculated for each derivative using only single isomorphous replacement (SIR, Blow & Rossmann, 1961) phases calculated from the other derivative. Sections from these icosahedrally averaged difference Fourier maps are shown in Fig. 3. These maps confirm the locations of the major sites found by the vector search procedure as well as indicate that a self-consistent 'hand' combination had been chosen earlier, *i.e.* the sites indicate a hand preference and it is that chosen by R minimization.

Double isomorphous phases were then calculated. Various statistics for the derivatives and the phase set are given in Table 2. For both derivatives the lack-of-closure error is well below the calculated heavy-atom contribution. That heavy-atom contribution is greater than observed differences for the Pt derivative and nearly equal to observed differences for the I derivative, as compared to an ideal ratio of $\sqrt{2}$ for non-centrosymmetric data (Adams *et al.*, 1969). New heavy-atom

Table 2. Preliminary heavy-atom refinement statistics

4553 F 's with significant measured heavy-atom intensity differences, Z occupancy in relative 'electrons', not on absolute scale, B conventional temperature factor, E root-mean-squared lack-of-closure error, f root-mean-squared calculated heavy-atom contribution, ΔF root-mean-squared observed heavy-atom difference, R_{mod} sum over all reflexions of the lack-of-closure error divided by the sum of the calculated heavy-atom contribution as defined by Adams *et al.* (1969).

Compound	Z	B	E	f	ΔF	R_{mod}
Pt	55	120	476	1032	844	0.316
I	32	85	466	612	664	0.601

difference Fourier maps based on those phases showed no new minor substitution sites in averaged maps.

Conclusion

Our results agree with the model calculations of Argos & Rossmann (1976) in confirming the ability of the vector search procedure to locate correctly heavy-atom sites related by a high-order non-crystallographic symmetry. The phasing statistics indicate that the major heavy-atom scatterers have been located; the difference Fourier maps, both SIR and MIR, confirm the vector search result that there are no other large sites. We will report elsewhere (Strandberg *et al.*, manuscript in preparation) the generation of a completely independent 10 Å phase set by the molecular replacement method (Rossmann & Blow, 1962; Bricogne, 1974; Argos, Ford & Rossmann, 1975), but it is relevant here that those phases also give the proper sites in averaged difference Fourier maps for both derivatives.

We have reported a preliminary 10 Å electron density map of the virus based on a combination of the derivatives and the molecular replacement phases (Strandberg *et al.*, 1975), and are continuing refinement of these derivatives, in particular, searching for secondary sites of lower or no non-crystallographic symmetry with the aim of generating an improved 10 Å map.

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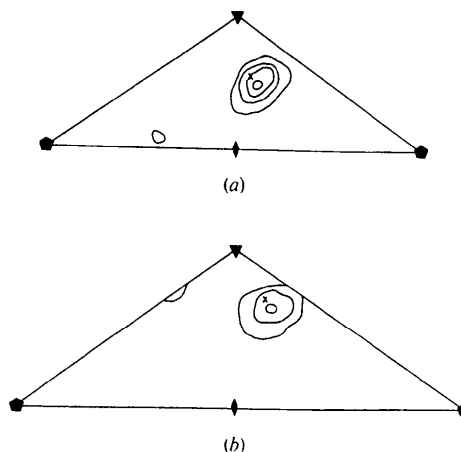


Fig. 3. Sections through the SIR heavy-atom difference Fourier maps based on partially refined one-site derivatives. (a) Pt phased on I only, $r=51.75$ Å, (b) I phased on Pt only, $r=62.25$ Å. X marks the site located by vector search.

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A Platinum Zigzag Chain in Bis(ethylenediamine)copper(II) Bis(oxalato)platinate(II): the Crystal Structure of $[\text{Cu}(\text{C}_2\text{N}_2\text{H}_8)_2][\text{Pt}(\text{C}_2\text{O}_4)_2]$

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The title compound, obtained by mixing aqueous solutions of $\text{K}_2[\text{Pt}(\text{C}_2\text{O}_4)_2]$ and $[\text{Cu}(\text{C}_2\text{N}_2\text{H}_8)_2]\text{Cl}_2$ in equimolar amounts, is triclinic, space group $P\bar{1}$, with $a=6.978$ (4), $b=9.418$ (6), $c=12.351$ (8) Å, $\alpha=73.14$ (5), $\beta=100.59$ (4), $\gamma=111.73$ (4)°, $Z=2$. The structure was solved by Patterson and Fourier methods and refined by least squares to $R=0.079$ for 4035 observed reflexions. The structure is built up of zigzag chains of $[\text{Pt}(\text{C}_2\text{O}_4)_2]^{2-}$ anions running in channels formed by $[\text{Cu}(\text{C}_2\text{N}_2\text{H}_8)_2]^{2+}$ cations.

Introduction

Much of the recent interest in linear-chain, transition metal compounds is connected with investigations of the direct metal-to-metal contacts (Interrante, 1974; Keller, 1975; Miller & Epstein, 1975). Pt compounds of this type with very weak intermolecular interactions can be prepared, as well as others which show evidence of strong exchange, leading finally to quasi one-dimensional metallic behaviour. Metal interactions in crystals of the former group of complexes, e.g. in the Magnus

green salt (MGS) type materials, can be studied by ESR after doping the diamagnetic lattice with sterically very similar but paramagnetic Cu^{II} ions, e.g. $[\text{Cu}(\text{NH}_3)_4]^{2+}$ (Fritz & Keller, 1965; Soos, Huang, Valentine & Hughes, 1973).

When we tried to dope linear-chain bis(oxalato)platinate(II) with paramagnetic tetraamminecopper(II) cations we found a red-brown 1:1 compound of bis(ethylenediamine)copper(II) and bis(oxalato)platinate(II) with a new type of structure, which is reported here.