SHORT COMMUNICATIONS

Acta Cryst. (1994). D**50**, 105-109

Crystallization and preliminary X-ray diffraction studies of the bacteriophage Qβ.

By KARIN VALEGÅRD, KERSTIN FRIDBORG AND LARS LILJAS, *Department of Molecular Biology, University of Uppsala, BMC, Box* 590, *S*-751 24 *Uppsala, Sweden*

(*Received* 22 *June* 1993; *accepted* 26 *August* 1993)

Abstract

Crystals of bacteriophage Qβ have been obtained by the vapor-diffusion technique. The crystals diffract to at least 3.5 Å resolution. The crystal space group is $C222_1$ with the unit-cell parameters a = 478, b = 296, c = 477 Å, $\alpha = \beta = \gamma = 90°$. The unit cell contains four virus particles. A pattern of systematic extinctions has been used to deduce the packing of the particles in the cell. A limited data set to 3.9 Å resolution has been collected, and the predicted position has been confirmed by the self-rotation and the Patterson functions.

Introduction

Qβ is a member of group III RNA coliphages (Fiers, 1979). The icosahedral protein shell is composed of three different proteins, the coat protein, the A1 protein and the A2 protein. The main component is the coat protein, which comprises 132 amino acids. One of the two minor proteins, the A1 protein, is a read-through transcript. It has the same sequence as the coat protein in the N terminus but it contains an additional 196 amino acids. There are about five copies of the A1 protein per virion, most likely replacing some of the coat protein subunits (Horiuchi, Webster & Matsuhashi, 1971; Weiner & Weber, 1971). Only one copy of the A2 protein appears to be present per virion. The location of the A2 protein in the particle is not clear. The total number of protein subunits in the shell is 180, and the shell has a T = 3 quasi-symmetry. Both the A1 and the A2 protein seem to play a role in the attachment of the bacterial F-pilus (Hofstetter, Monstein & Weissmann, 1974).

The protein shell encloses one RNA molecule. Qβ-RNA is a positive-sense, single-stranded, 4220-nucleotide-long molecule. The complete nucleotide sequence was determined by Mekler (1981).

The structure of the related bacteriophage MS2 (group I RNA coliphage) has been solved and refined to 2.8 Å resolution (Golmohammadi, Valegård, Fridborg & Liljas, 1993; Valegård, Liljas, Fridborg & Unge, 1990). This was the first structure of a bacterial virus and it shows no structural homology to other virus coat protein structures determined so far. There is about 25% sequence identity between MS2 and Qβ. The conserved residues might be involved in RNA interaction and/or play an important role in the assembly and disassembly processes. In this paper we present the crystallization procedure of the bacteriophage Qβ and also some preliminary results from the examination of these crystals.

Crystallization and X-ray analysis

Bacteriophage Qβ was propagated in *Escherichia coli* strain M27 and purified according to Valegård, Unge, Montelius, Strandberg & Fiers (1986). Purified phage was stored at approximately 10 mg ml^{-1} in 0.05 *M* Tris/HCl buffer, pH 7.4, 0.2 *M* NaCl, 10^{-4} *M* $MgSO_4$, 10^{-5} *M* EDTA and 0.02%(*w*/*v*) NaN_3 at 277 K.

The crystallization experiments were performed in hanging drops by the vapor-diffusion technique at room temperature, 293 K. The solution in the crystallization well was prepared by mixing 12 µl of virus solution, 10 mg ml^{-1}, with 8 µl of 2% PEG 6000 in 0.05 *M* Tris/HCl pH 7.4, 0.2 *M* NaCl, 0.1 m*M* $MgSO_4$, 0.01 m*M* EDTA and 0.02%(*w*/*v*) NaN_3. The droplets were equilibrated against 0.4 *M* NaCl.

Data were collected both on film and image plate using synchrotron radiation at the protein crystallographic station 9.6 at the SERC Synchrotron Radiation Source in Daresbury, England. The synchrotron was running at a energy of 2 GeV, with an average current of 200 mA and the Wiggler magnetic field strength was 5 T. The wavelength of the X-ray beam was 0.89 Å. Film data were collected with a crystal-to-film distance of 175 mm using a Enraf-Nonius Arndt-Wonacott camera. The collimator size was 0.2 mm. Image-plate data were collected on an R-AXIS II C image-plate system (Rigaku) with a crystal-to-detector distance of 290 mm.

Oscillation films were auto-indexed using the program *OSC*123 (Kim, 1989) and processed using the program *OSC* (Rossmann, 1979). Image-plate data were processed using the R-AXIS software. Data were scaled using the *PROTEIN* package (Steigemann, 1974). The self-rotation function was calculated using the program *GLRF* (Rossmann & Blow, 1962; Tong & Rossmann, 1990). Other calculations were performed using *CCP*4 (SERC Daresbury Laboratory, 1979).

Results and discussion

Crystallization

Crystals normally appear after one week but the number of crystals in each well was usually too large. A smaller number of crystals was obtained when the droplets were first equilibrated against 0.4 *M* NaCl for a relatively long period (in practice several months), after which the outer solution was changed to 0.6 *M* NaCl. After a couple of months the crystals had a size of 0.3-0.6 mm in the longest dimension (Fig. 1).

X-ray diffraction pattern and packing in the crystal

A typical oscillation photograph is shown in Fig. 2. The crystals belong to the space group $C222$ or $C222_1$, with cell dimensions a = 478, b = 296, c = 477 Å, $\alpha = \beta = \gamma = 90°$. Diffraction is observed to at least 3.5 Å resolution, but most crystals gave useful data to about 4 Å. Preliminary data from the processing and scaling of oscillation films and image-plate data show that they can be indexed and scaled with relatively good merging statistics. A partial data set was obtained from 20 oscillation films. Using only full reflections, these films

gave 46 000 independent reflections to 3.9 Å resolution, with a scaling R value of 18.0%. The merging R value is defined as

$$R_{\text{merge}} = \Sigma_h \Sigma_i |I_h - I_{hi}| / \Sigma_h \Sigma_i I_{hi},$$

where I_h is the mean of intensity observations I_{hi} of reflection h. The data set is roughly 20% complete between 10 and 4.5 Å resolution.

The diffraction pattern has some unusual features. In the c^* direction the distance between the spots is very short (not shown on Fig. 2*a*, which was obtained from a crystal oriented with the c^* axis roughly parallel to the beam). However, it is possible to resolve these reflections using the well collimated synchrotron radiation. On many rows of reflections along the c^* direction, each second reflection is systematically absent. The pattern of absences is such that reflections with an odd l index are absent on some rows and reflections with an even l index are absent on others (Fig. 2*b*). On further rows there are no systematic extinctions. A comparison of lines with increasing h index showed that the pattern of extinctions in the c^* direction repeats with a period of about 3.2 in the h index. There have been some reports in the literature of similar patterns of extinctions. Pickersgill (1987) has described a crystal form of ribulose bisphosphate carboxylase (rubisco) with the apparent space group $C222_1$, showing a pattern of systematic absences along the c^* direction with a period of 39 in the h index. The systematic absences could be explained by an arrangement where four rubisco complexes were positioned in the cell with their molecular center on a special position on the a axis. The crystal is built up by layers stacked along **c**, where each layer has $C2$ symmetry. The rubisco complex has 222 symmetry, and a non-crystallographic twofold axis will be aligned parallel to the twofold b axis and generate an extinction pattern. From the periodicity of the extinction pattern, an approximate coordinate of the molecular center along the a axis could be estimated.

Crystals of the phage MS2, which is related to Qβ, have the space group $R32$ with the cell dimensions a_{rhomb} = 274.0 Å and α = 63.4°. Space group $C2$ is a subgroup of $R32$, and the corresponding $C2$ dimensions for the MS2 crystals are a = 465.9, b = 287.8, c = 274.0 Å, β = 121.72°. The particles pack through interactions along their fivefold axes, which are aligned along the rhombohedral axes (which corresponds to the c axis and ab diagonals in the $C2$ cell). As a consequence of the packing, a non-crystallographic twofold axis is aligned along the a axis of the $C2$ cell (Figs. 3*a* and 3*b*). The centered layer in MS2 has the dimensions 465.9 × 287.8 Å. The centered layer of the Qβ crystals has similar proportions (a = 478, b = 296 Å). This suggests that the packing in this layer is similar to the packing in the MS2 crystals. Our interpretation of the packing in the $C222_1$ cell is that the virus particles are sitting with their centers on special positions on the twofold a axis. There are four virus particles per unit cell with molecular centers at x, 0.0, 0.0; x + 0.5, 0.5, 0.0; $-x$, 0.0, 0.5; $-x$ + 0.5, 0.5, 0.5, and half a particle in the asymmetric unit. This arrangement leads to the packing of centered layers with the first layer shifted by x and the second layer (at z = 1/2) shifted by $-x$ along the a axis (Fig. 3*c*). The arrangement of the Qβ particles in the centered layer is the same as in the MS2 crystals (Fig. 3*a*). The twofold b axis of the $C2$ cell is a particle twofold axis, but in the $C222_1$ cell the twofold b axis will relate two virus particles, and a particle twofold will be parallel to the b axis. A consequence of this arrangement is that the particles centered on x, 0.0, 0.0 and $-x$, 0.0, 0.5 will be related by a pure translation.

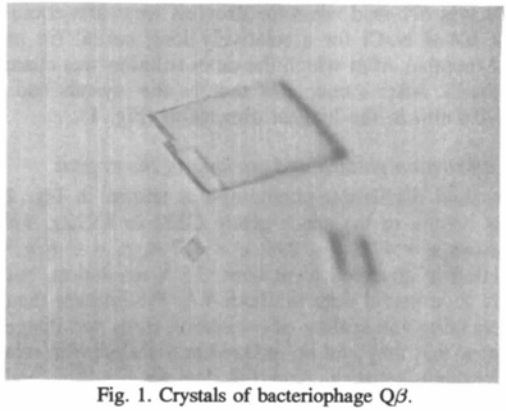
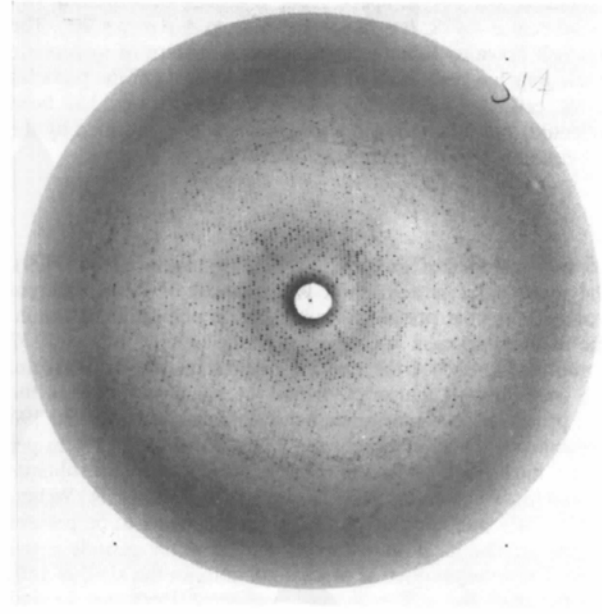
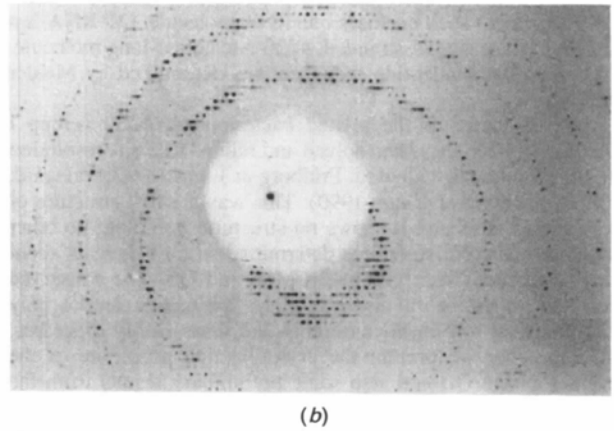


Fig. 1. Crystals of bacteriophage Qβ.



(*a*)



(*b*)

Fig. 2. (*a*) A 0.5° rotation photograph of a Qβ crystal collected at the synchrotron station in Daresbury, England. The crystal-to-film distance was 175 mm giving a resolution limit of 2.7 Å at the edge of the photograph. The exposure time was 33 min. (*b*) Central part of another rotation photograph, showing parts of the h, $-h$, l layer and a few upper layers.

In this model, the particles are positioned on the twofold axes in the a direction, and therefore the $0kl$ layer has to show extinctions of reflections with odd l indices. This is confirmed by the observed data. The extinctions generated by the additional translation in the b plane will depend on the position of the particles in relation to the b axis. Constructive interference will occur (Pickersgill, 1987) when

$$2\pi(h\Delta + 1/2) = 2\pi n$$

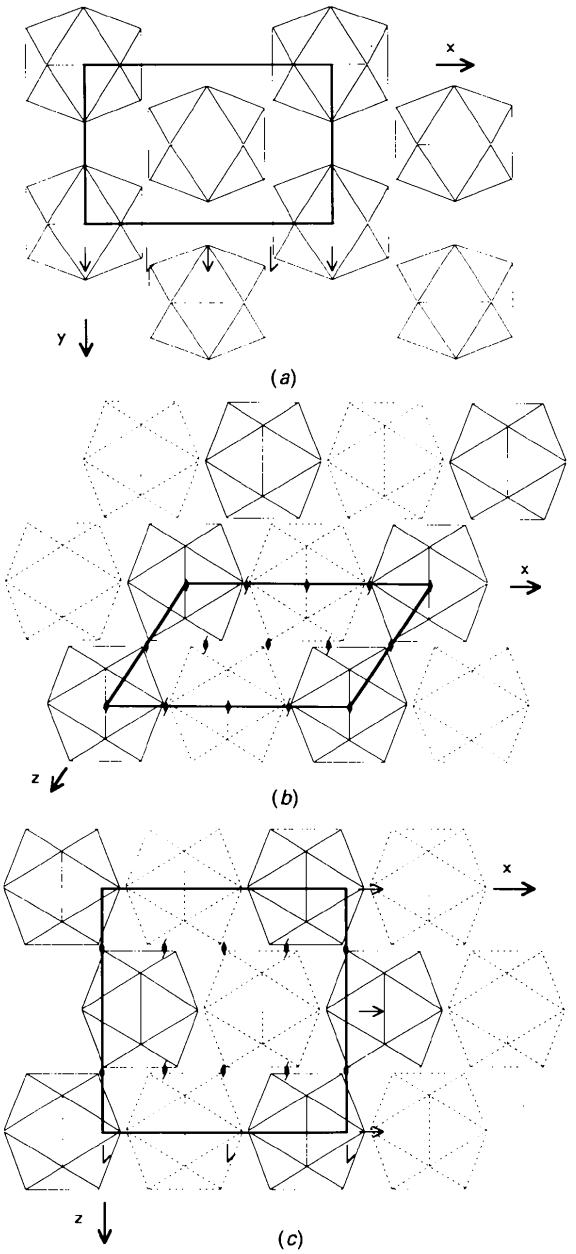


Fig. 3. Comparison of the packing of particles in the MS2 and Qβ crystals. (*a*) The centered *ab* layer of the MS2 ($C2/R32$) and Qβ ($C222_1$) crystals. The symmetry elements of the $C2$ cell are indicated. (*b*) The *ac* plane of the $C2$ ($R32$) crystal. Particles at b = 1/2 are shown with broken lines. (*c*) The *ac* plane of the $C222_1$ crystals.

where Δ is the shift in the a direction between successive layers. If Δ is 1/3.2 we will obtain the observed pattern of extinctions. If we choose the origin as in Fig. 3(*c*), the x coordinate of one particle is -1/6.4 or -0.1562. A more accurate value of the particle position was obtained from a Patterson function using the limited data set to 3.9 Å resolution. The only significant peak in the Patterson map (42% of the value of the origin peak) was found at u = 0.3083, v = 0.0, w = 0.5000. This corresponds to a particle center of x = -0.1542, y = 0.0, z = 0.0. With a particle in this position, the contact between symmetry-related particles in two adjacent c layers will be roughly along the fivefold axis. The diagram shows that the particle position on the a axis can be estimated from icosahedral geometry, if identical interactions along the fivefold axes are assumed. The ideal position would correspond to a particle center at x = -0.1545, which is only 0.14 Å from the position obtained from the Patterson function. For an a axis of 477.6 Å the particle center will be at x = -73.6 Å and the shift in the a direction between successive layers in the c direction is 147.3 Å. The packing of two successive layers is very similar to the packing in MS2 crystals (Figs. 3*b* and 3*c*). The difference is that the third layer in the $C222_1$ cell is shifted by x = -147.3 Å. The packing between layers will again be along fivefold particle axes. The packing along the c direction will thus be similar to the packing in the a direction, as seen by the similar lengths of the a and c cell edges.

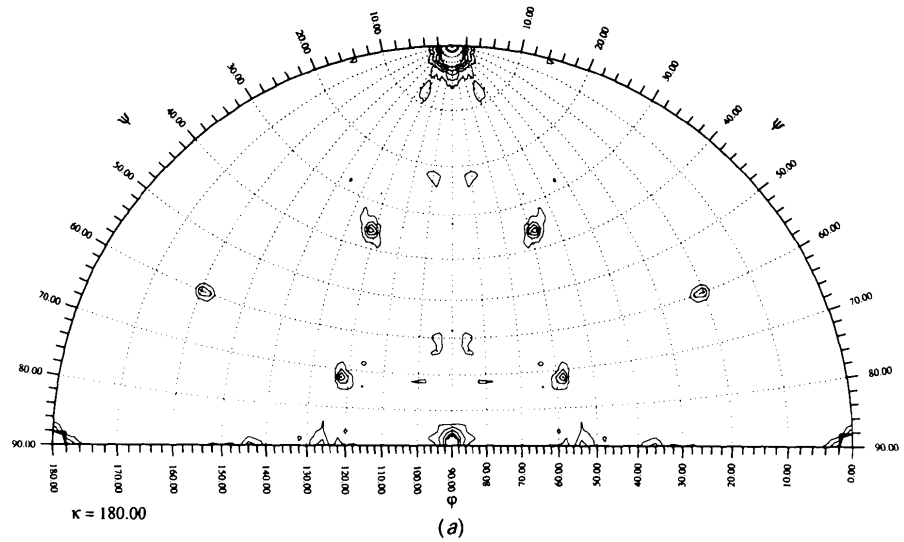
A self-rotation function calculated with the limited data set confirmed the suggested orientation. The rotation function showed strong peaks at all the expected positions for fivefold, threefold and twofold peaks (Fig. 4).

A very similar type of packing has been suggested for one crystal form of southern bean mosaic virus, SBMV (Akimoto, Wagner, Johnson & Rossmann, 1975). This virus can be crystallized in space group $R32$, with contacts along icosahedral fivefold axes, coinciding with the rhombohedral axes of the $R32$ cell like in the MS2 crystals described above. A $C222_1$ crystal form, obtained in the same crystallization experiment as the $R32$ crystals, showed cell dimensions and extinctions on the $0kl$ layer indicating the same type of packing contacts as in Fig. 3.

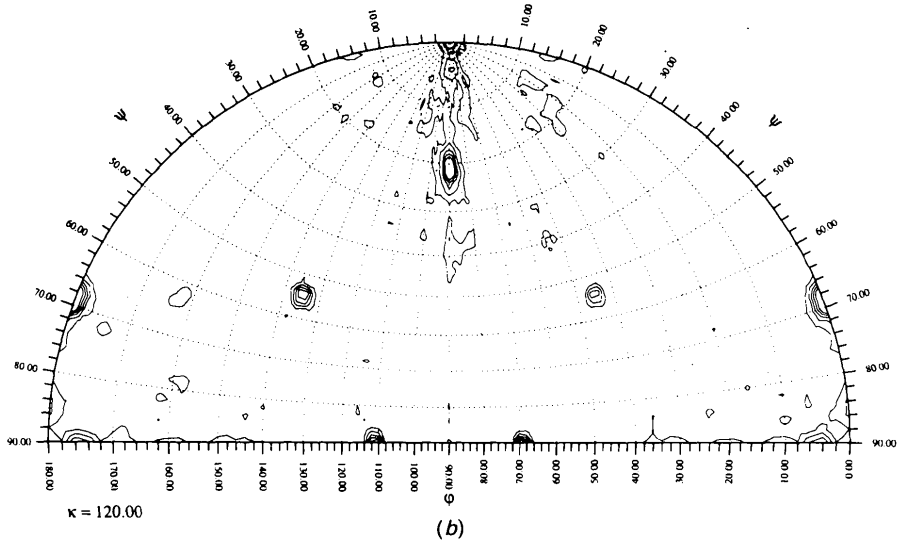
Crystal disorder

The type of packing described here has in some cases been connected with disorder. In one crystal form of methemoglobin with an apparent space group of $C222_1$ the diffraction pattern along the c^* direction showed well defined rows with systematic absences alternating with diffuse rows (Bragg & Howells, 1954; Cochran & Howells, 1954). The cell dimensions of the crystals indicated that they contained layers of molecules with a similar packing to that in monoclinic met-hemoglobin. The interpretation was that these layers were packed in the c direction, each with a shift in the a direction, but with random sign. This type of disorder was shown to produce a diffraction pattern, periodically alternating along $\mathbf{a}^*$, of well defined reflections and diffuse streaks in the c^* direction. In this interpretation, the space group $C222_1$ was the result of the packing disorder only.

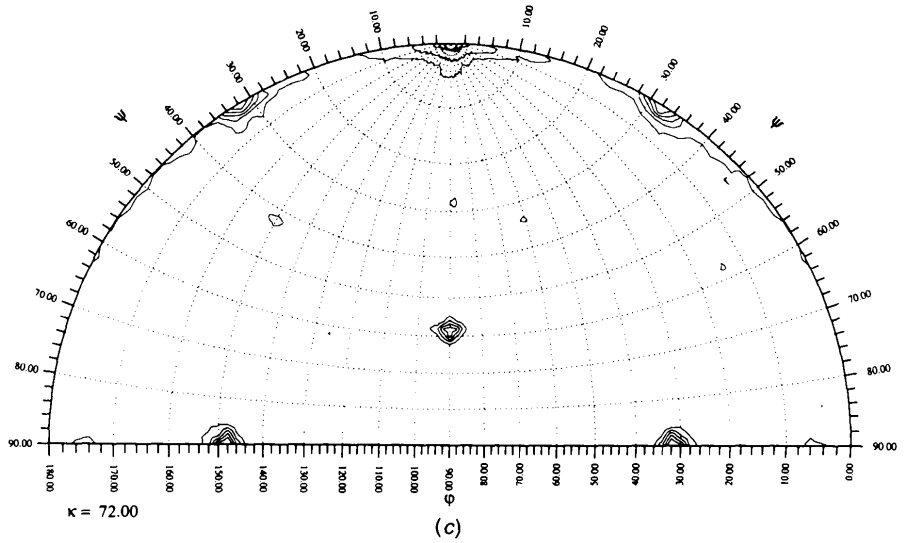
Also in the crystals of rubisco described above, the rows of reflections in the c^* direction were diffuse for those h values where no extinctions occurred. With a very low beam divergence, satellite reflections could be resolved. In this case, the packing of the molecules was compatible with the observed space group ($C222_1$). However, because of the abnormal diffraction pattern, the apparent space group $C222_1$ was suggested to be a statistical average of the diffraction of



(*a*)



(*b*)



(*c*)

Fig. 4. Self-rotation function calculated with 13 611 reflections between 11 and 6 Å resolution. The radius was 50 Å, and the Patterson origin was removed. The search was performed with two intervals. The rotation function has been contoured at intervals of 1σ, starting at the 1σ level. Twofold peaks (κ = 180.0°) are expected at ψ = 0.0, φ = 0.0; ψ = 90.0, φ = 0.0; ψ = 90.0, φ = 90.0; ψ = 36.0, φ = 121.7; ψ = 36.0, φ = 58.3; ψ = 72.0, φ = 121.7; ψ = 72.0, φ = 58.3°. Threefold peaks (κ = 120.0°) are expected at ψ = 20.9, φ = 90.0; ψ = 90.0, φ = 69.1; ψ = 90.0, φ = 110.9; ψ = 69.1, φ = 0.0; ψ = 54.7, φ = 45; ψ = 54.7, φ = 135.0°. Fivefold peaks (κ = 72.0°) are expected at ψ = 58.3, φ = 90.0; ψ = 90.0, φ = 31.7; ψ = 90.0, φ = 148.3; ψ = 31.7, φ = 0.0°.

layers of $C2$ symmetry. The pattern suggests that the disorder is in the sign of the shift along **a** between successive layers in the c direction. The amplitude of the scattered X-rays along **c*** is

$$A(w) = FG(w),$$

where F is the structure factor of the layer, w is the fractional reciprocal index along **c***, and $G(w)$ is the Laue function. For a shift of random sign along **a** for each layer, the Laue function will be (Glauser & Rossmann, 1966; Pickersgill, 1987)

$$G(w) = [1 - K^N\exp(\pi iNw)]/[1 - K\exp(\pi iw)],$$

where N is the number of layers and

$$K = \cos[(2\pi h)/\Delta].$$

According to this formula, rows with h values of $1/\Delta$ will have normal reflections for even values of l, and rows with h values of $1/2\Delta$ will have normal reflections for odd values of l. When h is $1/4\Delta$, $G(w)$ will be 1, and a continuous diffraction along **c*** will be observed. Pickersgill (Pickersgill, 1987) has also discussed other models of disorder to explain the appearance of satellite reflections.

In our case, there are some signs of disorder. The crystal mosaicity seems to be relatively high in the c direction, and there is some streaking of reflections. However, distinct reflections can be observed also on lines with maximal streaking. It is difficult to estimate the degree of disorder. Clearly the packing interactions between the centered ab layers will be very similar for both signs of the shift in the a direction, since the two interacting surfaces will be related by the particle twofold axis.

We are presently processing and scaling the data. The structure will be solved by molecular replacement using the MS2 structure as the starting model.

This work was supported by a grant from the Swedish Natural Science Research Council. We thank the staff at the SERC synchrotron in Daresbury for their help with the data collection.

References

AKIMOTO, T., WAGNER, M. A., JOHNSON, J. E. & ROSSMANN, M. G. (1975). *J. Ultrastruct. Res.* **53**, 306-318.

BRAGG, W. L. & HOWELLS, E. R. (1954). *Acta Cryst.* **7**, 409-411.

COCHRAN, W. & HOWELLS, E. R. (1954). *Acta Cryst.* **7**, 412-415.

FIERS, W. (1979). *Comprehensive Virology*, edited by H. FRAENKEL-CONRAT & R. R. WAGNER, pp. 69-204. New York: Plenum.

GLAUSER, S. & ROSSMANN, M. G. (1966). *Acta Cryst.* **21**, 175-177.

GOLMOHAMMADI, R., VALEGÅRD, K., FRIDBORG, K. & LILJAS, L. (1993). *J. Mol. Biol.* In the press.

HOFSTETTER, H., MONSTEIN, H. & WEISSMANN, C. (1974). *Biochim. Biophys. Acta*, **374**, 238-251.

HORIUCHI, K., WEBSTER, R. E. & MATSUHASHI, S. (1971). *Virology*, **45**, 429-439.

KIM, S. (1989). *J. Appl. Cryst.* **22**, 53-60.

MEKLER, P. (1981). PhD thesis, Univ. of Zurich, Switzerland.

PICKERSGILL, R. W. (1987). *Acta Cryst.* A**43**, 502-506.

ROSSMANN, M. G. (1979). *J. Appl. Cryst.* **12**, 225-238.

ROSSMANN, M. G. & BLOW, D. M. (1962). *Acta Cryst.* **15**, 24-31.

STEIGEMANN, W. (1974). PhD thesis, Technische Univ. Munchen, Germany.

SERC Daresbury Laboratory (1979). *CCP4 Program Suite*. SERC Daresbury Laboratory, Warrington, England.

TONG, L. & ROSSMANN, M. G. (1990). *Acta Cryst.* A**46**, 783-792.

VALEGÅRD, K., LILJAS, L., FRIDBORG, K. & UNGE, T. (1990). *Nature (London)*, **345**, 36-41.

VALEGÅRD, K., UNGE, T., MONTELIUS, I., STRANDBERG, B. & FIERS, W. (1986). *J. Mol. Biol.* **190**, 587-591.

WEINER, A. M. & WEBER, K. (1971). *Nature (London) New Biol.* **234**, 206.