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The structure of a spherical plant virus (bromegrass mosaic virus) established by neutron diffraction

By B. Jacrot†, P. Pfeiffer‡ and J. Witz‡
† *Institut Laue-Langevin, B.P. no.* 156, 38042-*Grenoble Cédex, France, and*
‡ *Institut de biologie moléculaire et cellulaire du CNRS, Laboratoire des virus de plantes,* 15, *rue Desartes,* 67000-*Strasbourg, France*

The localization of the RNA in a spherical plant virus is established by neutron diffraction. For BMV, this localization is different at low pH than it is at high pH (swollen virus).

The localization of the RNA even in the simplest spherical viruses is difficult to determine by X-rays diffraction. The X-ray method has been used almost to the extreme of its capacity by Klug, Longley & Leberman (1966) for turnip yellow mosaic virus. The method uses diffraction by the virus in very high salt concentration, in order to match the protein scattering. The method is certainly not general, as high ionic strength may induce conformational changes in the virion. For instance bromegrass mosaic virus (BMV) incubated in 1 M NaCl, will release its RNA with a possible collapse of the protein shell, depending upon the pH (Pfeiffer & Hirth 1974), so it is doubtful if one can achieve matching of the protein for X-ray diffraction. Neutron diffraction provides an easy and very general method of solving the structure of viruses at low resolution. The method takes advantage of the possibility of matching the neutron scattering of protein or nucleic acid by an appropriate mixture of ordinary and heavy water (Schoenborn & Nunes (1972), Jacrot (1973)). For instance one may calculate that for BMV, taking into account the amino acid composition of the protein and the number of exchangeable protons, the scattering power of the protein will be matched on the average by a mixture of 40 % D_2O and 60 % H_2O. Similarly a mixture with 70 % D_2O will match the nucleic acid of this virus. So it is possible to get a diffraction pattern from a virus in solution with only the protein or the nucleic acid moiety contributing. This is valid up to the level of resolution where the protein no longer scatters as a body of homogenous scattering power.

We have applied this technique to locate the RNA in BMV. This virus is subject to a pH and temperature induced swelling (Incardona & Kaesberg 1964; Incardona, McKee & Flanegan 1973) and it is of special interest to see if this swelling is accompanied by a reorganization of the RNA.

The virus was prepared as already described (Pfeiffer & Hirth 1974). The pH was adjusted by dialysis against appropriate buffer (acetate at pH 4.85, tris at pH 7.3). Then the deuteration of the solution was achieved by dialysis against the appropriate D_2O/H_2O mixture in which the H_2O component was in fact the above mentioned buffer. At pH 7.3 the dialysis was done either in the presence of EDTA or of Mg^{2+} (10 mM), as the latter ion is known to inhibit the temperature dependent part of the swelling of BMV or the closely related cowpea chlorotic mottle virus (CCMV) (Incardona *et al.* 1973; K. Adolph 1975). Tests were done in an analytical centrifuge to check that the virus swelling is not affected by the presence of D_2O.

Diffraction was observed on the small angle scattering instrument of the I.L.L. (Schmatz, Springer, Schelten & Ibel 1974). This instrument has a two dimensional detector (Allemand *et al.* 1975) of a very high stability, which allows easy observation of peaks which are less than 1 % above the background. Data were recorded with wavelengths between 0.6 and 1 nm ($\Delta\lambda/\lambda$ of 8 %) and sample to detector distance varied between 2.40 and 10 m. Virus concentrations used were about 0.5 % for the radius of gyration and 1–2 % for the diffraction pattern. A detailed account of these experiments will be given elsewhere.

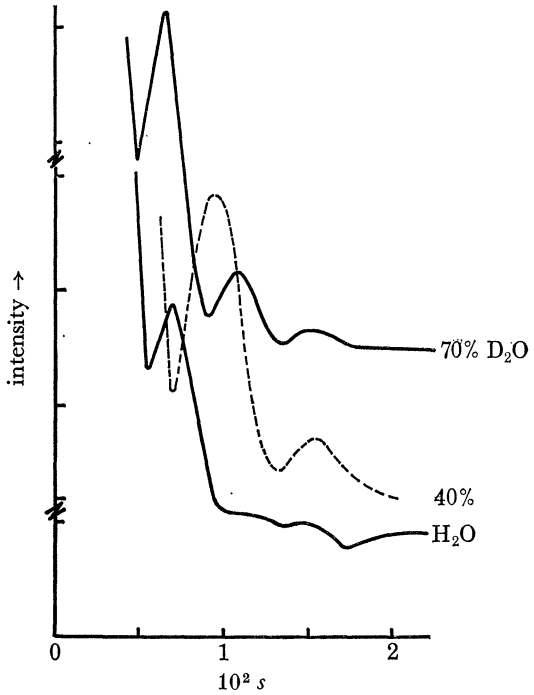

FIGURE 1. Modification of the diffraction pattern, of BMV solution at various D_2O contents. In 40 % D_2O only nucleic acid contributes to the low angle scattering and in 70 % D_2O only the protein. ($s = 2 \sin\theta/\lambda$.) pH 5; $\lambda = 0.612$ nm. Data have also been measured with better resolution.

Figure 1 shows the low angle diffraction pattern measured with BMV in H_2O, 40 % D_2O and 70 % D_2O. The differences between these spectra reflect the difference in the location of the protein and RNA in the virus. Figure 2 shows a complete diffraction pattern obtained by combining data collected with various distances and wavelengths after subtraction of a flat background, but otherwise uncorrected.

Analysis was done partly by Fourier inversion of the data, using the spherical approximation (Harrison 1969; Mateu *et al.* 1972) and partly by model fitting. The outside diameter of the protein shell and RNA bunches is obtained with an accuracy of 1 %. The accuracy for inside diameters is clearly not as good.

Figure 3 summarizes the structural features deduced from this analysis. The size of the virus deduced from X-ray diffraction by Anderegg, Wright & Kaesberg (1963) and Incardona & Kaesberg (1964) is confirmed, as well as the existence of a hole at the centre of the virus in the low pH state. In addition our data give accurate information on the location of the RNA and its penetration into the protein shell. One can see that the swelling is accompanied by an important increase of the penetration of the RNA into the protein shell. This effect is inhibited

by magnesium ions, thus explaining the sensitivity of the virus to ribonuclease at neutral pH in the absence of these ions. It also explains why the hyprochromicity in CCMV (assuming that the RNA behaves in a similar way) varies with pH in the absence of magnesium, but not in its presence (Jacrot 1975).

It is difficult to be certain of the number of RNA bunches. From symmetry arguments, it is likely that there are 32 bunches in the centres of the hexamers and pentamers. This suggestion is compatible with our model and the length of the RNA. A measurement of the length of RNA fragments after digestion of the swollen virus with ribonuclease will be done to confirm this hypothesis.

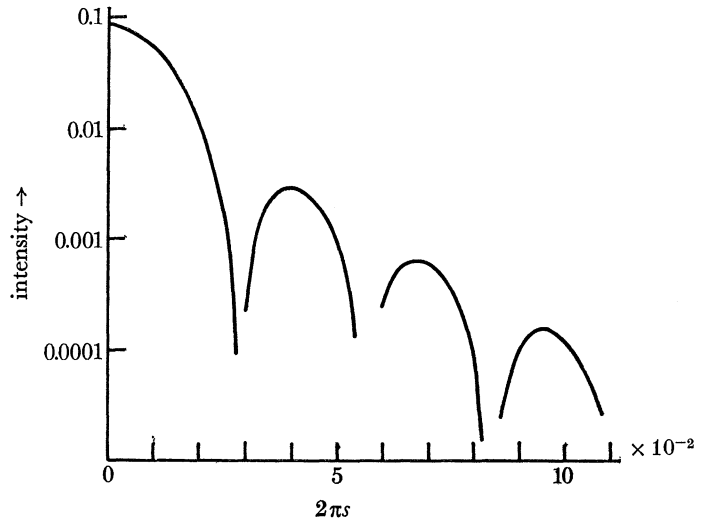

FIGURE 2. Averaged radial distribution of the intensity scattered by a BMV solution at pH 5, with 70% D_2O. No correction has been applied, except the subtraction of a flat background.

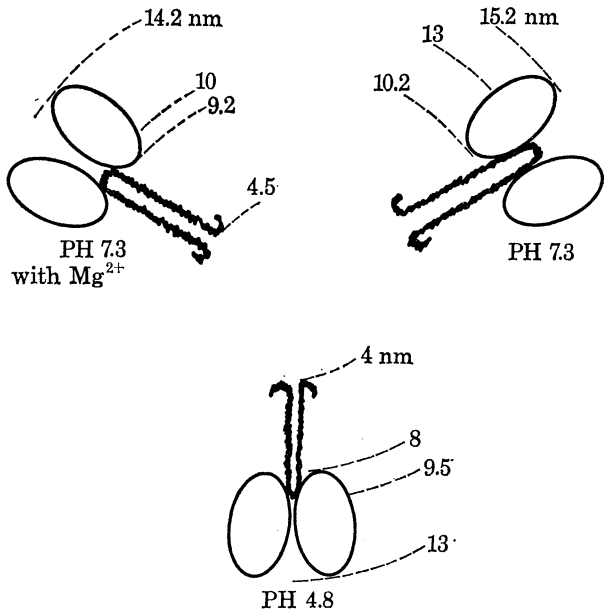

FIGURE 3. Summary of the results obtained for the various states of the virus. The penetration of the nucleic acid into the protein shell is deduced from the diffraction data, but the folding of the RNA is hypothetical.

An extensive investigation is now being carried out to compare the location of the RNA and its penetration into the protein shell for various spherical viruses.

Note added in proof (*June* 1976). Experiments on several spherical plant viruses (TYMV, CMV, SBMV) indicate in all cases an organization very similar to that of BMV at low pH, namely with a limited penetration of the RNA in the protein shell.

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