Internal structural anisotropy of spherical viruses studied with magnetic birefringence

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Communicated by Sir John Randall

Received on 16 November 1982

Six so called spherical viruses (four plant and two animal) are shown to exhibit magnetically induced birefringence in solution. They must therefore be magnetically and optically anisotropic. This is attributed to static structural anisotropy of the interiors as neither natural shape nor field-induced deformations are likely causes. Thus at least part of these virus cores have a symmetry differing from that of their capsids. An estimate of the average orientation of the RNA bases is given for the plant viruses: turnip yellow mosaic, bromegrass mosaic, tomato bushy stunt and turnip crinkle. The packing geometry of the nucleic acid/protein cores of adenovirus and probably influenza virus are anisotropic but to an extent that cannot be quantified.

Key words: adenovirus/influenza virus/magnetic birefringence/plant virus/virus internal structure

Introduction

Magnetically induced birefringence has previously been used to investigate the solution behaviour and structure of two filamentous bacteriophages Pf1 and fd (Maret *et al.*, 1979; Torbet and Maret, 1981). Consequently, highly oriented fibres, which gave improved X-ray diffraction patterns, were formed by partial drying in a magnetic field (Torbet and Maret, 1979). This technique was subsequently developed and exploited for detailed structural studies (Nave *et al.*, 1979, 1981; Banner *et al.*, 1981). This paper is an extension of that work to the study of the spherical viruses listed in Table I.

The four plant viruses (Table I) each contain single-stranded RNA encapsidated in a $T = 3$, 180 protein subunit icosahedron. Electron microscopy has revealed little about the RNA structure in small plant viruses. This is also true of X-ray crystallography which, however, has detailed the structure of much of the protein of three viruses (Harrison *et al.*, 1978; Abad-Zapatero *et al.*, 1980; Unge *et al.*, 1980). Most of the information which is summarized below on the disposition of protein and nucleic acid has been obtained from neutron scattering (reviewed by Jacrot, 1981). In turnip yellow mosaic virus (TYMV) the protein forms a very dense shell and is hardly penetrated by the RNA which is distributed throughout the interior (Jacrot *et al.*, 1977). The protein of bromegrass mosaic virus (BMV) capsid is not very densely packed and is interpenetrated to a limited extent by RNA which is localized into a narrow sheet (Jacrot *et al.*, 1977; Chauvin *et al.*, 1978a). In tomato bushy stunt virus (TBSV) the protein subunit has two domains, one is closely packed on the surface, the other extends inwards into the RNA shell and there is a central cavity which is smaller than that in BMV (Chauvin *et al.*, 1978b; Harrison *et al.*, 1978). Both the N-terminal part of the protein subunits and the RNA are believed to be spatially disordered because they do not give

rise to clear features in high resolution electron density maps (Harrison *et al.*, 1978). No neutron scattering results have been published on the structure of turnip crinkle virus (TCV) but it is expected to be very like TBSV (Munowitz *et al.*, 1980). Thus apart from the latter two these plant viruses have significant differences in their protein-RNA interactions and distribution.

Measurements are also reported on two spherical animal viruses, adenovirus and influenza virus. The former has an icosahedron ($T = 25$) capsid which is composed of three major structural proteins. The core contains double-stranded DNA in association with four species of protein. The nucleoprotein may be in the form of folded rods (Nermut, 1979) with a structure akin to chromatin (Corden *et al.*, 1976; Mirza and Weber, 1982). The outer envelope of influenza virus, recently shown to be spherical in solution (Mellema *et al.*, 1981), is a lipid bilayer from which project two types of glycoprotein. It has a segmented RNA genome and the eight segments are believed to interact with the protein in the core to form a regular double-helical structure (Compans *et al.*, 1972). In general, because it has proved to be a difficult problem to approach experimentally, little is known about the packing geometry of the cores in most spherical viruses. Here it is demonstrated that the internal symmetry of spherical viruses can differ from that of their exteriors.

Results and Discussion

The magnetically induced birefringence given by each virus solution varied linearly with the square of the magnetic field strength (Figure 1); no saturation was observed, therefore the orientation is weak and the measurements are within the range of validity of the classical Cotton-Mouton (CM) effect (the magnetic analogue of the Kerr electro-optic effect). The observation of a signal demonstrates that these viruses have a degree of anisotropy in their diamagnetic and optical properties and must therefore deviate from spherical symmetry in some way. There are *a priori* three possible sources of this effect: (a) field-induced anisotropy; (b) naturally occurring shape fluctuations; and (c) static structural anisotropy. These will be discussed in turn. Strong fields have been observed to influence the structure of the long ($2\ \mu\text{m}$; mol. wt. = 37.5×10^6) bacteriophage Pf1 within the span of a temperature-mediated structural transition (Torbet and Maret, 1981). However, the effect is small and this phage has highly anisotropic and near-parallel aligned subunits. The viruses investigated here are spherical. In stable conditions the protein subunits in the plant viruses, which are 4–8 times smaller than Pf1, are unlikely to be very anisotropic. For example, in TBSV the protein is composed of two globular domains and a disordered N-terminal region (Harrison *et al.*, 1978). In the former, β -pleated sheets are well represented but as they are only weakly anisotropic (Worcester, 1978) and have little overall preferred direction, the total diamagnetic anisotropy is unlikely to be significant. Thus there is little likelihood of field-induced deformation of the capsid. The natural shape fluctuations are unlikely to be large or prolong-

Fig. 1. Continuously measured magnetically induced birefringence normalized to concentration, $\Delta n/c$, of solutions of TYMV, BMV, adenovirus and influenza virus. The plots for TBSV and TCV fall near to that of TYMV and BMV. The small contribution from the cell containing buffer only is subtracted. BMV and TBSV were in their contracted forms. As BMV has a divided genome, samples were a mixture of three species of virion each with about the same RNA content. No concentration effects were observed with the possible exception of influenza. The error in $\Delta n/c$ is $\pm 10\%$ in all but influenza for which it is nearer $\pm 20\%$. The sample optical path length varied from 0.2 to 3 cm. The CM constant per unit concentration is given by $CM/c = \Delta n/c \lambda H^2$ ($\lambda = 6328 \text{ \AA}$). Birefringence was measured using a combined photoelastic modulation and compensation technique (Maret and Dransfeld, 1977).

ed enough to be significant for the following reasons. First, high resolution X-ray crystallography has been successful (Harrison *et al.*, 1978; Abad-Zapatero *et al.*, 1980; Unge *et al.*, 1980) second, neutron solution scattering can be analyzed in terms of spherical structures (Jacrot *et al.*, 1977; Chauvin *et al.*, 1978a, 1978b; Jacrot, 1981) and third, n.m.r. studies of solutions of intact virus confirm that the components are rather immobile (Munowitz *et al.*, 1980; McCain *et al.*, 1982; Vriend *et al.*, 1982). The absence of any measurable magnetically induced birefringence from empty capsids of TYMV reinforces the above arguments (M. Katouzian-Safadi, in preparation). Even with all the TYMV nucleic acid bases stacked to form a stiff rod the maximum torque is $< 0.05 kT$. As any internal base-stacked helical stems are much smaller, $\sim 20 \text{ bp}$ (Fiers *et al.*, 1976), the deforming forces on the RNA are bound to be weak relative to structural constraining forces. Therefore the signal from the intact viruses probably originates from static structural anisotropy and, as the capsids have spherical symmetry, this property

must be associated with the virus core.

The results on the four plant viruses will be discussed first. As concluded above, the magnetic birefringence is due to the structure of the interior which is RNA in the plant viruses. The values of $B_n f$ and f in Table I are obtained as described in Materials and methods. The value of $B_n f$ corresponds to the minimum number of bases that would give rise to the measured signal. The average orientation factor, f , of the bases gives a qualitative measure of the deviation from spherical symmetry ($1 \geq f \geq -0.5$; $f = 0$ for random or spherical packing). The values of f for BMV, TBSV and TCV are similar, while that of TYMV is significantly smaller. None of them are very large, indicating that although the viruses are anisotropic they are only weakly so. If all the bases had their symmetry axes parallel the CM' values would be $1/f^2$ (> 100) times greater. A structure similar to that (i.e., with all the base planes nearly perpendicular to the symmetry axis) of the concentrically coiled model for the DNA in bacteriophage T4 (Earnshaw *et al.*, 1978) would give rise to an f value of about -0.4 using the method of calculation of Maestre and Kilkson (1965). Thus, the small values of f reported in Table I could result from many different structures. Furthermore, f is obtained assuming an axis of rotational symmetry for which there is no evidence. For example, the tRNA molecule behaves as a rotationally symmetric particle which has 36 (Table I) base planes parallel, the remainder being randomly arranged. This interpretation is clearly at odds with the crystal structure (Moras *et al.*, 1980) which resembles a bent rod with arms of approximately equal length separated by an angle of $\sim 100^\circ$ and with the base planes at an angle $\leq 20^\circ$ to the long axis of the arm. Using a recently derived equation (Mellado and García de la Torre, 1982), this model is calculated to have an f value close to that measured (see Materials and methods). This encourages belief in the estimates presented for the plant viruses and shows that they are related to RNA conformation but also makes it clear that detailed interpretation is not possible.

Adenovirus gives the strongest signal (Figure 1) and has the largest CM' of the viruses investigated (Table I). This demonstration of internal anisotropy rules out models which ascribe icosahedral symmetry to all of the core (Brown *et al.*, 1975; Corden *et al.*, 1976). As the latter is composed of DNA and protein it is not known whether both or only one are responsible for the anisotropy. Therefore, the fact that the value of f estimated assuming the protein to be isotropic (Table I in parenthesis) is very similar to that found for three of the plant viruses must be considered as coincidental. For similar reasons the estimate of the f value for ribosomes is not meaningful. However, it is worth noting that ribosomes are magnetically anisotropic and to a degree probably not very different from that of small plant viruses.

Influenza virus is quite different from the others in that it has a lipid bilayer envelope which is likely to be more susceptible to deformation than are purely protein capsids. However, neutron solution scattering experiments indicate that if there is a deviation from spherical symmetry it is very small (Mellema *et al.*, 1981). Although magnetic orientation of very large egg lecithin vesicles has been observed, no magnetic deformation was evident (Boroske and Helfrich, 1978). Thus, it is again probable that shape variations are not important, although they cannot be completely excluded. Unlike the other viruses in Table I, influenza virus gives a negatively induced birefringence. The short cylindrical alfalfa mosaic virus bottom component, which has no lipid, also

Table 1. Cotton-Mouton constants

	$M_t \times 10^6$	$M_{tNA} \times 10^6$	Range in c mg/ml	$CM/c \times 10^{-7}$ $T^{-2} \text{ cm}^2 \text{ mg}^{-1}$	$CM' \times 10^{-22}$ $T^{-2} \text{ cm}^2 \text{ particle}^{-1}$	$\pm B_n f$	$\pm f$
TYMV	5.53	1.9	21–10	1.10	10.1	420	0.070
BMV	4.7	1.05	94–16	0.83	6.5	335	0.100
TBSV	8.7	1.5	54–13	0.77	11.1	440	0.093
TCV	8.8	1.5	44–7	0.85	12.4	460	0.099
Adenovirus type 2	175	25	4–1.3	12.5	3630	(7925)	(0.098)
Influenza Texas A	200	5	100–10	–0.47	–156		
Yeast tRNA ^{asp}	0.024	0.024	15–7	1.9	0.076	36.2	0.48
Ribosome 80S wheat germ	3.9	2.1	40–5	1.15	7.5	(360)	(0.055)

CM = Cotton-Mouton constant; $CM' = CM/N$ where N = number of viruses per unit volume; c = concentration; M_t = total mol. wt.; M_{tNA} = mol. wt. of nucleic acid; B_n = number of nucleic acid bases; f = average orientation factor of the bases; k = Boltzmann constant; T = absolute temperature; H = magnetic field strength; λ = wavelength of light; $\Delta\alpha$ = particle optical anisotropy; $\Delta\chi$ = particle diamagnetic anisotropy; Δn = magnetic birefringence; K = a constant. For weakly orienting samples such as are of interest here $CM = \Delta n/\lambda H^2$ where $CM = NK \Delta\chi \Delta\alpha/15\lambda kT$. If the anisotropy is solely due to nucleic acid then $CM' = 5.78 \times 10^{-22} (B_n f)^2$ at room temperature. These equations are derived in Materials and methods.

gives a negative signal, $CM' \sim -1.8 \times 10^{-22} T^{-2} \text{ cm}^2$ (S. Cusack, personal communication). As both the diamagnetic ($\Delta\chi_B$) and optical ($\Delta\alpha_B$) anisotropies of a nucleic acid base are negative, their product is necessarily positive. Therefore as the CM' of influenza is negative the contribution from the protein in the core must dominate and there may or may not be a component from the RNA. This suggests that there is some alignment of the nucleoprotein complex in influenza virus that is unquantifiable.

It has been shown that six so-called spherical viruses have anisotropic cores. The most likely explanation of why this is so is that they arise as a by-product of packaging constraints such as the requirement of the plant viruses to compact long RNA molecules while incorporating some stems and loops of secondary structure and possibly tertiary features. Anisotropic optical polarizability suggests anisotropy in the long range electrodynamic van der Waal's attractive forces, so the immediate environment of these spherical viruses may experience them as weakly anisotropic, but this is unlikely to be significant in most circumstances (Parsegian and Brenner, 1976).

It has already been suggested (Torbet and Maret, 1979) that crystal growth of magnetically anisotropic (dia- or paramagnetic) particles may be assisted in a strong field. The particles suffer a weak torque which acts to bring their axis of smallest diamagnetism nearer to the magnetic field direction. At the site of crystal growth the particles must perform interact and this could be in such a way as to enhance the magnetic torque, as for example, the concerted behaviour of Pf1 and fd in solution lead to the production of highly oriented fibres (Maret *et al.*, 1979; Torbet and Maret, 1979, 1981). The success of magnetic crystal growth is dependent on the relationship between the space group and the magnetic properties. Although rotational cooperativity is not an obvious property of icosahedral viruses, crystal growth in a strong magnetic field is worth investigating as it may give rise to oriented cores. Greater success is likely to be achieved with the preparation of extracted nucleic acid/protein cores (or ribosomes) which probably have shape in addition to diamagnetic anisotropy. Finally, the magnetic anisotropy of spherical viruses emphasises that this is a rather general property.

Materials and methods

Theory of magnetically induced birefringence

Consider a dilute monodisperse solution of non-interacting anisotropic particles which have an axis of rotational symmetry in their diamagnetic and optical properties. The following are defined: the diamagnetic, $\Delta\chi$, and optical, $\Delta\alpha$, anisotropies per particle are given by the difference between their respective values parallel and perpendicular to the axis of symmetry; N = number of particles per unit volume; k = Boltzmann constant; T = absolute temperature; H = magnetic field strength; λ = wavelength of light; n_0 = refractive index of the solution (1.33); CM = Cotton-Mouton constant; Δn = magnetic birefringence; i.e., the difference in the refractive indices of light linearly polarized parallel and perpendicular to the applied magnetic field direction; θ = angle between particle symmetry axis and the field direction; $K = 2\pi(n_0^2 + 2)^2/9n_0$ using the Lorentz-Lorenz formula to account for the local optical electric field which may be approximate as it does not have a firm theoretical basis for anisotropic particles in solution.

In a magnetic field, these particles have an orienting energy $\Delta\chi H^2 \cos^2 \theta/2$ and suffer a torque which is opposed by the randomizing effect of Brownian motion. As the magnetic orientation of a diamagnetically anisotropic particle is analogous to induced electric dipole orientation the theory of the latter given by O'Konski *et al.* (1959) and Shah (1963) can be easily transposed as follows. For the solution defined above, the orientation factor, $\Phi = (1.5 \cos^2 \theta - 0.5)$, gives the degree to which the particles are aligned. $\Phi = \int_0^\pi (1.5 \cos^2 \theta - 0.5) f(\theta) 2\pi \sin \theta d\theta$ where $f(\theta)$ is the angular distribution which is equal to the Boltzmann factor normalized so that the total probability of finding a particle in any direction is unity. Thus $\Phi = 1.5 \int_0^\pi x^2 \exp x^2 dx/a^2 \int_0^\pi \exp x^2 dx - 0.5$, when $\Delta\chi$ is positive and $a^2 = |\Delta\chi| H^2/2 kT$ and $x^2 = a^2 \cos^2 \theta$. When $\Delta\chi$ is negative, x^2 and a^2 are replaced by $-x^2$ and $-a^2$, respectively. The above equation is not an exact integral but can be easily computed. Φ (Figure 2) is a function of the ratio $|\Delta\chi| H^2/2 kT$ and has a limiting value of 1 or -0.5 ($1 \geq \Phi \geq -0.5$) depending on whether $\Delta\chi$ is positive, orientation parallel to the field, or negative, orientation in a plane perpendicular to the field. There are significant differences in the shape of Φ for positive and negative $\Delta\chi$ in the range $2 \leq |\Delta\chi| H^2/2 kT \leq 10$ but in conditions of poor orientation such as are of interest here ($|\Phi| \leq 0.05$ and $|\Delta\chi| H^2 \geq 0.6 kT$) $\Phi = \Delta\chi H^2/15 kT$ (O'Konski *et al.*, 1959; Shah, 1963).

When the magnetically induced birefringence is used to monitor orientation of a solution, $\Delta n = \Delta n_s \Phi$, where Δn_s is the birefringence when $\Phi = 1$. Using the Lorentz-Lorenz formula $\Delta n_s = KN\Delta\alpha$ so $\Delta n = KN\Delta\chi\Delta\alpha H^2/15 kT$ and a plot of Δn against H^2 will yield a straight line and its slope divided by the wavelength gives the classical Cotton-Mouton constant, $CM = \Delta n/\lambda H^2$ or $CM = NK \Delta\chi\Delta\alpha/15\lambda kT$. If anisotropy is solely due to nucleic acid, $\Delta\chi$ and $\Delta\alpha$ can be replaced by $B_n f \Delta\chi_B$ and $B_n f \Delta\alpha_B$, respectively. Where $\Delta\alpha_B$ and $\Delta\chi_B$ are average anisotropies for a nucleic acid base; B_n = number of bases; $f_i = 1.5 \cos^2 \beta_i - 0.5$, orientation factor of base i ; β_i = angle between the symmetry axis of a base (perpendicular to the base plane) and that of the particle; $f = \Sigma f_i/B_n$, average orientation factor of the bases ($1 \geq f \geq -0.5$). If the bases are randomly or spherically arranged or in the unlikely event that $\beta = 54.7^\circ$, for all bases, $f = 0$. The average values of the anisotropies are approximately $\Delta\chi_B = -4.8 \times 10^{-28} JT^{-2}$ (Veillard *et al.*, 1961) and $\Delta\alpha_B = -6.5 \text{ \AA}^3$ (Cairney and Harrington, 1982). Thus $CM' = 5.78 \times 10^{-22} (B_n f)^2$ at room temperature. This is an approximation because of the problem of

Fig. 2. The variation of the orientation factor, Φ , as a function of a^2 ($|\Delta\chi|H^2/2kT$) for positive and negative $\Delta\chi$. In order to facilitate comparison the negative $\Delta\chi$ curve is also drawn (dashed line) with its sign changed and value doubled. For weak orientation $\Phi = \Delta\chi H^2/15kT$ ($|\Phi| \leq 0.05$) which is shown by the straight lines for which the a^2 scale has been expanded 20 times.

accurately estimating the internal optical field strength and shape factors (i.e., K and $\Delta\alpha_p$) and a possible contribution from the sugars (agarose solutions exhibit magnetic birefringence) was not taken into account in the calculation for $\Delta\chi$ (Veillard *et al.*, 1961).

If the nucleic acid is in the form of a rigid bent-rod then f^2 is replaced by f'^2 $\{1 + 12(\gamma^2 - \gamma)\cos^2\phi/2\sin^2\phi/2\}$ where γ = fractional length of shortest arm; ϕ = angle between the arms (Mellado and García de la Torre, 1982) and f' = average orientation factor for the bases relative to the arm of the long axis and is taken to be the same in both arms.

Solution conditions

TYMV: 10 mM Tris/HCl pH 7.5; BMV: 50 mM sodium acetate, 8 mM $MgCl_2$, pH 4.5; TBSV: 10 mM Tris/HCl, pH 7.0 or H_2O ; TCV: 50 mM Tris, 50 mM maleic acid, pH 6.5 adjusted with maleic acid; adenovirus: 50% glycerol, 1.3 M CsCl; influenza: 10 mM phosphate, 0.75 M KCl, pH 7.5; yeast tRNA^{asp}: 10 mM sodium cacodylate, pH 7.0, NaCl varied 20–350 mM; ribosomes 80S wheat germ: 10 mM Tris/HCl, 3 mM magnesium acetate, 50 mM potassium acetate, 1 mM dithiothreitol.

Acknowledgements

I thank the following people for the supply of samples, their interest and involvement: M. Katouzian-Safadi, J. Chroboczek, M. Cuillel, S. Cusack, C. Devaux, J. Kruse, Li, Z.Q., J.E. Mellema and P. Timmins. I thank T.A. Jones for seminal remarks and M. Mautner for suggestions. I am grateful to G. Maret who assisted in this work.

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