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LETTER TO THE EDITOR

Lipid and Protein Organization in Sindbis Virus

The radial electron density distribution in the Sindbis virus particle has been determined to a resolution of 28 Å from measurements of spherically averaged X-ray diffraction. The most striking feature of the density profile is a deep minimum at $r = 232$ Å, from which we infer that the lipids of Sindbis virus are organized in a bilayer at about that radius. Comparison of these results with electron micrographs of intact virus and of isolated cores shows that the inner polar groups of the bilayer interact directly with the core protein, and the outer polar groups with the external glycoprotein. Protein probably does not form a bridge across the bilayer.

A number of animal viruses acquire a lipoprotein envelope by a process of "budding" out through the surface of an infected cell (Morgan, Howe & Rose, 1961; Compans, Holmes, Dales & Choppin, 1966; Acheson & Tamm, 1967). A direct relation can thus be observed between the envelope and the cell membrane. Sindbis is a particularly simple virus of this sort, well suited for biochemical study. There are only two proteins in the particle—a glycoprotein of molecular weight 53,000, which can be released by detergent treatment, and a smaller protein of molecular weight 30,000 associated with the RNA in the "core" or "nucleocapsid" (Strauss, Burge, Pfefferkorn & Darnell, 1968). The lipids, which are released by detergent treatment from association with any protein, constitute about 26% of the dry weight of the virus particle. They are largely phospholipids and cholesterol, in a ratio by weight of about 3 : 1 (Pfefferkorn & Hunter, 1963).

Electron micrographs of negatively stained Sindbis virus preparations show a spherical particle, about 350 Å in radius (Plate I(a); cf. Simpson & Hauser, 1968). In most images there is a region, about 280 to 300 Å in radius, that excludes stain, and a peripheral fringe of surface projections (spikes). Occasionally, particles are seen penetrated by stain, outlining the region between radii of 200 and 250 Å (Plate I(a); cf. Compans, 1971). These two sorts of images are also characteristic of the lipid-containing bacteriophage PM2 (Silbert, Salditt & Franklin, 1969). A recent X-ray diffraction study of PM2 (Harrison, Caspar, Camerini-Otero & Franklin, 1971) has shown that the particles of this phage are sufficiently regular to allow determination of its radial density profile at 25 Å resolution. We describe here the results of a similar investigation of Sindbis virus.

Sindbis virus was grown in baby hamster kidney cells and purified by the method of David (manuscript in preparation). For preparing X-ray diffraction specimens, 2 mg of virus in Tris–NaCl–EDTA buffer (0·05 M-Tris (pH 7·4), 0·1 M-NaCl, 0·001 M-EDTA) were sedimented in a swinging bucket rotor for 2 hours at 30,000 rev./min. The clear, gel-like pellet, essentially a very concentrated solution of virus, was introduced into a quartz capillary, and the sample was sealed with a drop of buffer in the capillary to prevent drying. Sindbis virus cores were prepared by the method of Strauss *et al.* (1968), using 0·2% NP-40 instead of deoxycholate.

A solution of particles with approximate spherical symmetry will diffract a beam of X-rays into a series of concentric circular fringes (Finch & Holmes, 1967). The

separation and relative intensity of these fringes are related to the variation of electron density as a function of radius within the particle. A limiting resolution for observing these fringes is set by the regularity and rigidity of the structure and by its deviation from spherical symmetry. The diffraction pattern from Sindbis virus (Plate I(c)) shows fringes out to a spacing of about 28 Å, indicating that at this level of detail the particles are regular and identical. The fringes in the pattern from PM2 also extend to about 25 Å (Harrison *et al.*, 1971). A similar marked fluctuation in density must therefore occur both in Sindbis and in PM2, with lipid hydrocarbon, localized over a relatively narrow range of radii, contrasting strongly with the much denser lipid polar groups, protein and RNA.

The spherically averaged Sindbis–virus transform, shown in Figure 1, has been obtained from photographs such as the one in Plate I(c). Background corrections and scaling were carried out as in similar work on bushy-stunt virus (Harrison, 1969) and PM2 (Harrison *et al.*, 1971). The Figure includes a rough absolute scale, obtained by setting $F(0)$ equal to the calculated total number of electrons in the virus particle minus the number of solvent electrons excluded from the occupied volume. The uncertainties in extrapolating the transform to the origin and in computing the net electron density could lead to errors as great as $\pm 50\%$ in the absolute scale factor. The allocation of signs to the fringes is based on the principle of minimum wavelength (Bragg & Perutz, 1952). The single ambiguity occurs near the origin, where the amplitude maximum near 0·004 $Å^{-1}$ could be either positive or negative; successive fringes must then alternate in sign. Fourier syntheses calculated with both choices show that only the assignment indicated in Figure 1 leads to a structurally plausible density profile.

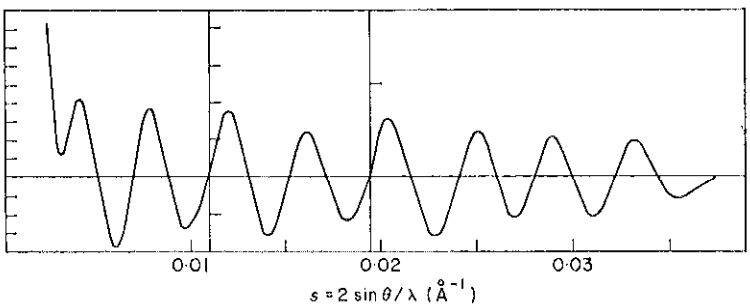

FIG. 1. The transform of Sindbis virus in 0·1 M-NaCl, plotted as a function of $s = 2 \sin \theta/\lambda$. Diffracted intensities were measured from photographs, such as the one shown in Plate I(c), using a rotating-drum two-dimensional scanning microdensitometer (Optronics International). The scan was averaged around the circular fringes to improve accuracy. The outer part of the central maximum was also measured photographically, and the curve was extrapolated to the origin using a Gaussian approximation (cf. Guinier, 1963). Changes of scale in the ordinate are indicated by vertical lines: in all cases, one division equals 5×10^4 electrons, on the approximate absolute scale obtained as described in the text.

The radial distribution of electron density in the Sindbis virus particle, computed by spherical Fourier inversion of the transform, is shown in Figure 2. A striking feature of the profile is the deep minimum at a radius of about 232 Å. The electron density at this point is about 0·065 e/Å^3 less than the solvent density, on the rough absolute scale established as described above. Since the buffer has a density of 0·335 e/Å^3, the actual value at the minimum is about $0{\cdot}27 \pm 0{\cdot}03$ e/Å^3. This value is in the

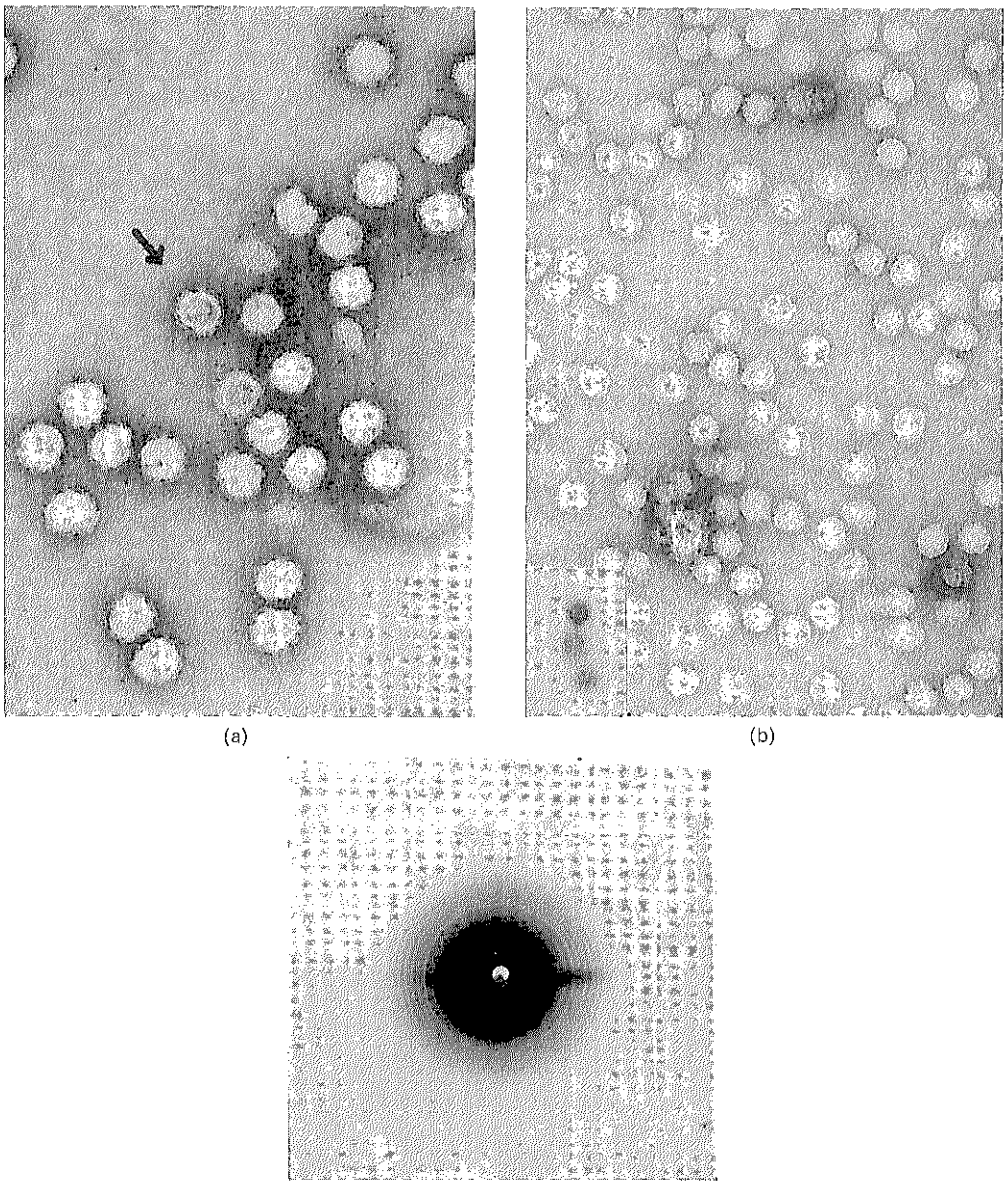

PLATE I. (a) Electron micrograph of Sindbis virus, negatively stained with uranyl acetate (pH 7). The particle indicated by an arrow has been partially penetrated by stain, revealing a shell with a mean radius of about 250 Å. × 100,000.

(b) Electron micrograph of Sindbis cores, negatively stained with uranyl acetate (pH 7). In weakly stained fields, particles with positively stained centers (insert) are sometimes observed. × 100,000.

(c) X-ray diffraction photograph of Sindbis virus in Tris–NaCl–EDTA buffer. The photograph was taken with quartz-monochromatized $CuK\alpha_1$ radiation from a rotating-anode microfocus X-ray tube. A helium-filled tube was interposed between the sample and the film, to reduce scatter and absorption. The original specimen-to-film distance was 9 cm; the scale on the print is 1 cm=0·024 $Å^{-1}$. The system of fringes (overexposed in the center) extends to a limiting resolution of about 28 Å. The horizontal streak is parasitic scatter from the monochromator.

[*facing p. 524*

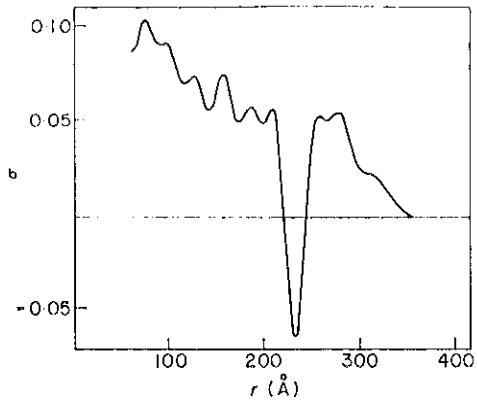

FIG. 2. The spherically averaged electron density of Sindbis virus, plotted as a function of radius, with the solvent density taken as zero. The approximate absolute density scale is in electrons/Å^3.

range characteristic of hydrocarbons, such as the fatty acid portion of phospholipids (Rand & Luzzati, 1968); all other components of the virus particle, protein, sugars, RNA and lipid polar groups, are much denser. We therefore conclude that most or all of the volume near the radius of 232 Å is occupied by the hydrocarbon chains of lipid molecules. At both smaller and larger radii, the density rises above the level of that of the solvent, as expected for protein or RNA. The particularly high density for $r<120$ Å may indicate concentration of RNA near the center of the particle, although it may be merely an artifact arising from experimental errors in measuring and scaling the scattering at very small angles. Localization of RNA near the center is also suggested by the tendency of the internal part of the core to stain positively with uranyl acetate (Plate I(b); cf. Horzinek & Mussgay, 1969). The viral RNA, of molecular weight 4×10^6 (Dobos & Faulkner, 1970), could be accommodated in a 120 Å sphere, giving a mean electron density similar to the calculated value in Figure 2.

The diagram in Figure 3 shows a molecular interpretation of the electron density profile and of images seen in the electron microscope. There are three principal structural domains: the lipid, the core and the outer protein.

(1) *Lipid.* We can infer directly from the density profile that the lipids of Sindbis virus are organized in a bilayer: no other arrangement could account for the deep minimum at 232 Å. The polar groups are localized near 210 and 258 Å, as indicated by the density maxima at these radii. The interpolar-group distance of 48 Å is typical of phospholipid–cholesterol bilayers, e.g. in myelin (Caspar & Kirschner, 1971) or in hydrated lecithin–cholesterol mixtures (Rand & Luzzati, 1968; Levine & Wilkins, 1971).

The mean surface area per molecule has been measured in a number of lamellar lipid model systems (Luzzati, 1968). Using these figures and the known lipid composition of Sindbis virus, we calculate a total polar group surface area of $13{\cdot}2\times10^5$ Å^2. The sum of the areas of spherical surfaces with radii of 210 Å and 258 Å is $13{\cdot}9\times10^5$ Å^2. The two figures agree within the uncertainty of about 10% in the estimate based on polar-group areas and compositional data. We conclude that the lipid bilayer extends completely around the core and that less than 10% of the area at radii between 210 and 258 Å is occupied by components other than lipid. Note that the total surface area at the outside of the bilayer ($r=258$ Å) is 50% greater than at the

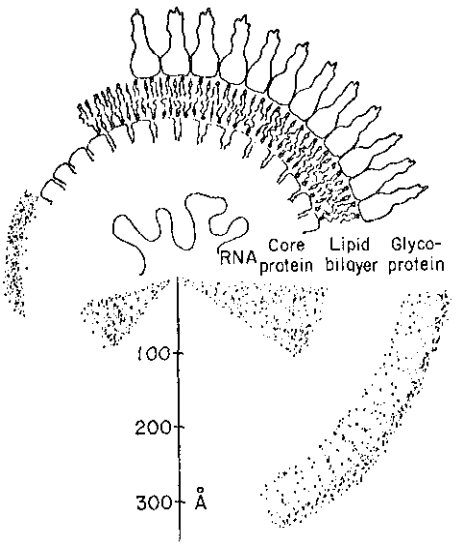

FIG. 3. Diagram indicating the relation of the proposed structure of Sindbis virus to the pattern of uranyl acetate staining in electron micrographs of virus particles and of cores. The upper part shows the organization of protein, lipid and RNA: the elongated glycoprotein subunits, the core protein subunits, and the lipid components of the bilayer are depicted schematically, and the apparent concentration of RNA near the particle center is indicated by the wavy line. The lower right-hand part shows the deposition of negative stain, both in normal images of intact virus (below) and in stain-filled particles (above). The left-hand part shows the staining of cores: the usual negatively stained image (above) and the positively stained image (below). The scale at the bottom shows radial distances in Å.

inside ($r = 210$ Å). The lipid molecules on either side are presumably apportioned accordingly, since the electron density is equal in the two polar-group regions. The present resolution of about 28 Å is insufficient to show whether there is an asymmetric distribution of cholesterol, as in myelin (Caspar & Kirschner, 1971).

(2) *Core.* Electron micrographs (Plate I(b)) show that the outer radius of the core is about 205 Å. Its surface must therefore make direct contact with the inner polar groups of the lipid bilayer: the nucleocapsid protein is in this sense also a "membrane protein". No projections that might penetrate the lipid bilayer are evident in electron micrographs of cores, and the isolated cores in concentrated fields seem to approach each other with a packing diameter comparable to the diameter measured from individual images.

There are approximately 400 protein subunits of molecular weight 30,000 in the core (Strauss, Burge & Darnell, 1969). If these were packed without any gaps, they would extend inward to a radius of about 140 Å. In fact, there is probably some tapering and clustering of the subunits and thus a smaller inner radius. This inner boundary has not been included in Figure 3, but RNA has been indicated schematically at small radii.

(3) *Outer protein.* This protein is represented in the electron density profile by the peak and shoulder extending from about 260 Å to the outer margin of the particle at 350 Å. The shoulder corresponds in radial position to the spikes seen in electron micrographs. Just as the spiky appearance indicates a high degree of penetration by

negative stain, so the relatively low electron density in the shoulder shows that there is considerable solvent interpenetrating the protein or carbohydrate in this region. It is generally assumed that carbohydrate will be found on the outside of the virus particle, but we cannot at present identify any feature of the profile as a distinct contribution of this component.

The question of whether outer protein forms a bridge across the bilayer, to interact directly with the core, is of importance for considering assembly of Sindbis virus particles at the cell surface. The conclusion, stated above, that more than 90% of the bilayer region must be occupied by lipid, indicates that there is little or no direct contact of outer protein with the core. This view is consistent with the striking observations of Compans (1971), who found that selective removal of outer protein could be effected by brief exposure of Sindbis virus to the proteolytic enzyme bromelain. Examination of treated virus in the electron microscope showed round, spikeless particles with no surface detail. It thus appears that the outer protein can be taken away without disrupting the remainder of the structure.

During maturation of Sindbis virus, the core must recognize those regions of infected cell membrane that contain viral outer protein, since no host-cell proteins are included in the mature particle (Pfefferkorn & Clifford, 1964). If protein does not form a bridge across the bilayer, the specificity of this interaction must be mediated by the lipid components. David (manuscript in preparation) has detected differences between the lipid composition of Sindbis virus grown in chick fibroblasts and that of the fibroblast plasma membrane. These differences could reflect the requirement for specific interaction between the two proteins and the intervening bilayer.

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